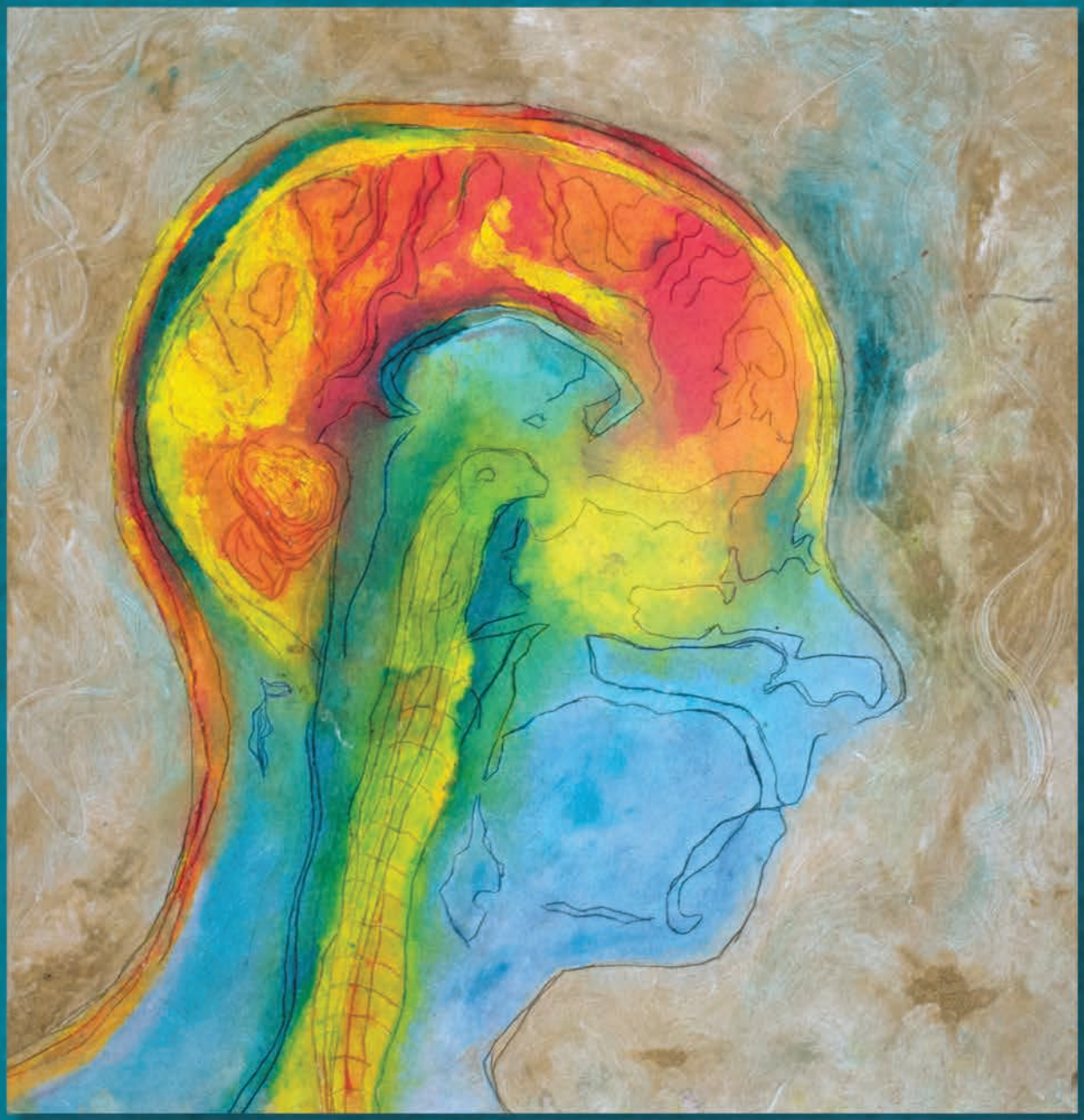
Psychopharmacology

Drugs, the Brain, and Behavior

THIRD EDITION



Jerrold S. Meyer ▪ Linda F. Quenzer

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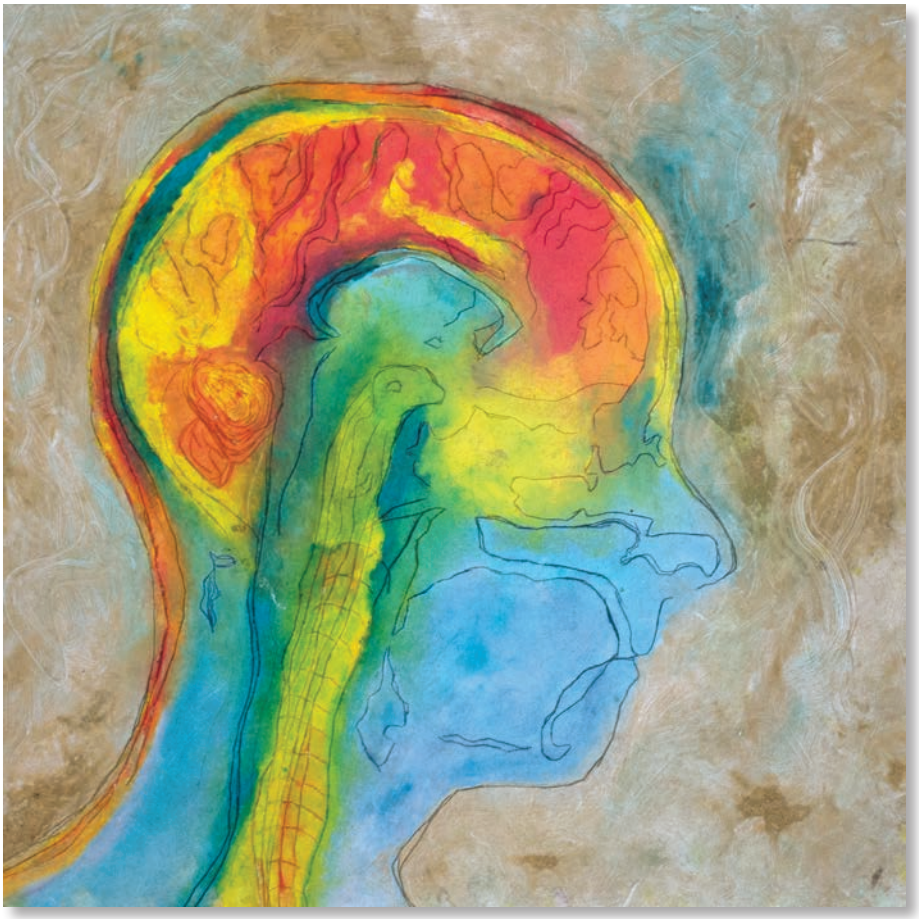

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Chapter 20, Neurodegenerative Diseases
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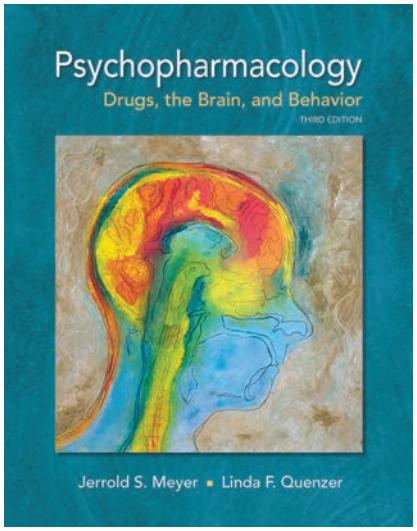

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To our students, who challenge and inspire us,
and to the many outstanding researchers
whose work is central to this book's contents.

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Preface

When we wrote the preface to the Second Edition of *Psychopharmacology: Drugs, the Brain, and Behavior*, we were struck by the many exciting developments in the field and the remarkable rate of progress elucidating the underlying neurobiological mechanisms of psychoactive drug action. This has not changed over the 5 years since the publication of that edition. The entire field of neuroscience, including neuropsychopharmacology, continues to be driven by technical advances. Using optogenetics, neurobiologists can activate or suppress anatomically and molecularly defined populations of nerve cells with amazing temporal precision. Neuropharmacologists can visualize the 3-dimensional structure of neurotransmitter receptors, enabling synthetic chemists to design novel agonist or antagonist drugs with much greater selectivity than could have been possible before. And huge projects like the Human Connectome Project (www.humanconnectomeproject.org) are using the most advanced neuroimaging techniques to map the detailed circuitry of the living human brain. Because of these technical innovations, we continue to add new information to Chapter 4, on *Methods of Research in Psychopharmacology*. Readers may choose to go through the chapter in its entirety to familiarize themselves with all of the neuropharmacological and behavioral methods reviewed, or they may choose to use the chapter as a reference source when they encounter an unfamiliar method in one of the book's later chapters.

Development and introduction of new pharmaceutical compounds continues as well, although the emphasis has somewhat shifted away from the large pharmaceutical companies to a greater reliance on drug discovery efforts by researchers at universities and medical centers. Statistics show that development of new drugs for CNS disorders (e.g., schizophrenia, depression, and Alzheimer's disease) costs more than for other kinds of disorders, and the failure rate is significantly higher. These data have caused many of the large companies to downsize their CNS drug discovery programs. As you read the chapters on drug addiction, mental disorders, and neurodegenerative disorders, it will become apparent that new medications for these disorders are being introduced at a slower rate than expected, despite ongoing research that continues to identify potential new molecular targets for pharmacotherapy. For this reason, we must admit that exciting advances in understanding the basic structure and function of the nervous system have not yet led to similar progress in treating, much less "curing," disorders of this system. We came to the same conclusion when writing the preface to the Second Edition, so it's disappointing that the hoped-for surge in medication development failed to occur during the intervening period.

As before, this new edition of the text is completely updated to incorporate the latest research findings, methodological advances, and novel drugs of abuse. Regarding the latter, illicit drug labs in the United States and abroad are working hard to turn out massive amounts of recreational drugs, whether already known compounds such as cocaine or fentanyl, or novel synthetic compounds that can only be identified by submitting drug seizures to advanced forensic laboratories for chemical analysis. The national drug epidemic involving fentanyl, heroin, and other opioid compounds is discussed in Chapter 11. New and, in some cases, highly dangerous stimulant and cannabinoid drugs are introduced in Chapters 12, 14, and 15 respectively. Most chapters have new opening vignettes and breakout boxes, and new photographs, drawings, and graphs have been added to bring attention both to updated material and to completely new topic areas for discussion.

Importantly, in preparing this next edition of the book we have maintained our conviction that a deep understanding of the relationship between drugs and behavior requires basic knowledge of how the nervous system works and how different types of drugs interact with nervous system function (i.e., mechanisms of drug action). We have also continued to present the methods and findings from behavioral pharmacological studies using animal models alongside key studies from the human clinical research literature. Pharmacologists must depend on in vitro preparations and laboratory

animal studies for determining mechanisms of drug action, for screening new compounds for potential therapeutic activity, and, of course, for basic toxicology and safety testing. In cases in which clinical trials have already been performed based on promising preclinical results, both sets of findings are presented. In other instances in which clinical trials had not yet been undertaken at the time of our writing, we have striven to point you toward new directions of drug development so that you can seek out the latest information using your own research efforts.

A new point of emphasis in the text concerns neural circuits as mediators of behavior and as targets of drug action. As implied above in referring to the Human Connectome Project, focusing on circuits instead of cells as the nervous system's functional units is the contemporary way to think about how our brains control our actions, and how drugs, whether recreational or medicinal, alter our subjective awareness and behavior.

The Third Edition of *Psychopharmacology: Drugs, the Brain, and Behavior* retains the same four-section organization as the previous editions. Chapters 1 through 4 provide extensive foundation materials, including the basic principles of pharmacology, neurophysiology and neuroanatomy, cell signaling (primarily synaptic transmission), and current methods in behavioral assessment and neuropharmacology. An increased use of clinical examples demonstrates the relevance of the material to real-life issues. Chapters 5 through 8 describe key features of major neurotransmitter systems, including the catecholamines, serotonin, acetylcholine, glutamate, and GABA. These are the neurotransmitter systems most often associated with psychoactive drug effects, and presentation of their neurochemistry, anatomy, and function lays the groundwork for the chapters that follow. Chapters 9 through 16 cover theories and mechanisms of drug addiction and all the major substances of abuse. Finally, Chapters 17 through 20 consider the neurobiology of neuropsychiatric and neurodegenerative disorders and the drugs used to treat these disorders. Among the neuropsychiatric disorders, special emphasis is placed on affective disorders such as major depression and bipolar disorder, various anxiety disorders, and schizophrenia. Bulleted interim summaries highlight the key points made in each part of the chapter. New to this edition, study questions are provided at the end of each chapter to assist students in reviewing the most important material. Finally, a dedicated website for the book (oup-arc.com/access/meyer-3e) is available that offers Web Boxes (advanced topics for interested readers), study resources such as flashcards, web links, and animations that visually illustrate key neurophysiological and neurochemical processes important for psychopharmacology.

It has been our privilege in the first two editions of *Psychopharmacology: Drugs, the Brain, and Behavior* to introduce so many students to the study of drugs and behavior. With this new and updated edition, we hope to continue this tradition and perhaps inspire some of you to continue your studies in graduate school and join the thousands of researchers worldwide who are working to better understand and ultimately defeat illnesses like addiction, depression, schizophrenia, and Alzheimer's disease.

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Psychopharmacology

Drugs, the Brain, and Behavior

THIRD EDITION

CHAPTER 1

Maggot therapy can be used to clean wounds and prevent infection. (PA Images/Alamy Stock Photo.)

Principles of Pharmacology

WILLIAM S. BAER (1872–1931) WAS AN ORTHOPEDIC SURGEON at Johns Hopkins University, where he established the orthopedic department and led it for most of his life, training many of the outstanding orthopedists of the day. During World War I Baer observed that soldiers who had severe and deep flesh wounds did not have the fever associated with infection and showed little of the expected necrotic (dead) tissue damage if there was a significant presence of maggots (fly larvae) in the wounds. Although it had been believed that early peoples (Australian aborigines and Mayan Indian tribes) and others throughout history had used maggots to clean wounds, it was Baer who once again recognized their importance, especially in tense battlefield conditions where infection was especially hard to treat. Apparently the maggots ingested the dying tissue but left healthy tissue intact. Baer, upon doing further "pharmacological" experiments, showed that his hospitalized patients with severe and chronic bone infections showed remarkable recovery after being treated with maggots—the inflamed and dying tissue was ingested, leaving wounds clean and healthy, and new tissue formed. As long as the maggots were sterilized, secondary infections were avoided. After his research, "maggot therapy" became popular and was used throughout the 1930s and 1940s until penicillin was established as an easier treatment for infection. However, it has been suggested that in modern times, maggot therapy will be reintroduced to treat those wounds infected with antibiotic-resistant bacteria. Presently in the European Union, Japan, and Canada, maggots are considered "medicinal drugs," and in 2005 the U.S. Food and Drug Administration approved the use of maggots as a medical "device."

What actually causes the amazing healing process is not entirely clear, but pharmacologists are beginning to understand that maggot secretions suppress the immune system and reduce inflammation, and they may also enhance cell growth and increase oxygen concentration in the wound. This is certainly not the first time pharmacology has returned to earlier forms of therapeutics, but the science now can isolate and identify those components that lead to healing. ■

Pharmacology: The Science of Drug Action

Pharmacology is the scientific study of the actions of drugs and their effects on a living organism. Until the beginning of the last century, pharmacologists studied drugs that were almost all naturally occurring substances. The importance of plants in the lives of ancient humans is well documented. Writings from as early as 1500 BCE describe plant-based medicines used in Egypt and in India. The Ebers Papyrus describes the preparation and use of more than 700 remedies for ailments as varied as crocodile bites, baldness, constipation, headache, and heart disease. Of course, many of these treatments included elements of magic and incantation, but there are also references to some modern drugs such as castor oil and opium. The Chinese also have a very long and extensive tradition in the use of herbal remedies that continues today. World Health Organization estimates suggest that in modern times, as many as 80% of the people in developing countries are totally dependent on herbs or plant-derived medicinals. And in 1999, in the United States, modern herbal medicines and drugs based on natural products represented half of the top 20 drugs on the market (Hollinger, 2008). Many Americans are enamored with herbal medications despite limited clinical support for their effectiveness, because they believe these treatments are more "natural." Nevertheless, serious dangers have been associated with some of them. **Web Box 1.1** discusses the benefits and dangers of herbal remedies.

When placed in historical context, it can be seen that drug development in the United States is in its infancy. The rapid introduction of many new drugs by the pharmaceutical industry has forced the development of several specialized areas of pharmacology. Two of these areas are of particular interest to us. **Neuropharmacology** is concerned with drug-induced changes in the functioning of cells in the nervous system, and **psychopharmacology** emphasizes drug-induced changes in mood, thinking, and behavior. In combination, the goal of **neuropsychopharmacology** is to identify chemical substances that act on the nervous system to alter behavior that is disturbed because of injury, disease, or environmental factors. Additionally, neuropsychopharmacologists are interested in using chemical agents as probes to gain an understanding of the neurobiology of behavior.

When we speak of **drug action**, we are referring to the specific molecular changes produced by a drug when it binds to a particular target site or receptor. These molecular changes lead to more widespread alterations in physiological or psychological functions, which we consider **drug effects**. The site of drug action may be very different from the site of drug effect. For example, atropine is a drug used in ophthalmology to dilate the pupil of the eye before eye examinations. Atropine has a site of action (the eye muscles of the iris) that is close to the site of its ultimate effect (widening the pupil), so it is administered directly to the eye. In comparison, morphine applied to the eye itself has no effect. Yet when it is taken internally, the drug's action on the brain leads to "pinpoint" pupils. Clearly, for morphine, the site of effect is far distant from the site of its initial action.

Keep in mind that because drugs act at a variety of target sites, they always have multiple effects. Some may be **therapeutic effects**, meaning that the drug–receptor interaction produces desired physical or behavioral changes. All other effects produced are referred to as **side effects**, and they vary in severity from mildly annoying to distressing and dangerous. For example, amphetamine-like drugs produce alertness and insomnia, increased heart rate, and decreased appetite. Drugs in this class reduce the occurrence of spontaneous sleep episodes characteristic of the disorder called *narcolepsy*, but they produce anorexia (loss of appetite) as the primary side effect. In contrast, the same drug may be used as a prescription diet control in weight-reduction programs. In such cases, insomnia and hyperactivity are frequently disturbing side effects. Thus therapeutic and side effects can change, depending on the desired outcome.

It is important to keep in mind that there are no "good" or "bad" drugs, because all drugs are just chemicals. It is the way a drug is procured and used that determines its character. Society tends to think of "good" drugs as those purchased at a pharmacy and taken at the appropriate dosage for a particular medicinal purpose, and "bad" drugs as those acquired in an illicit fashion and taken recreationally to achieve a desired psychological state. Even with this categorization, the differences are blurred because many people consider alcohol to be "bad" even though it is purchased legally. Morphine and cocaine have legitimate medicinal uses, making them "good drugs" under some conditions, although they can, when misused, lead to dangerous consequences and addiction, making the same drugs "bad." Finally, many "good" prescription drugs are acquired illicitly or are misused by increasing the dose, prolonging use, or sharing the drug with other individuals, leading to "bad" outcomes. As you will read in later chapters, the ideas of Americans about appropriate drug use have changed dramatically over time (see the sections on the history of the use of narcotics in Chapter 11 and cocaine in Chapter 12).

Many of the drug effects we have described so far have been **specific drug effects**, defined as those based on the physical and biochemical interactions of a drug with a target site in living tissue. In contrast, **nonspecific drug effects** are those that are based not on the chemical activity of a drug–receptor interaction,

but on certain unique characteristics of the individual. It is clear that an individual's background (e.g., drug-taking experience), present mood, expectations of drug effect, perceptions of the drug-taking situation, attitude toward the person administering the drug, and other factors influence the outcome of drug use. Nonspecific drug effects help to explain why the same individual self-administering the same amount of ethyl alcohol may experience a sense of being lighthearted and gregarious on one occasion, and depressed and melancholy on another. The basis for such a phenomenon may well be the varied neurochemical states existing within the individual at different times, over which specific drug effects are superimposed.

Placebo effect

Common examples of nonspecific effects are the multiple outcomes that result from taking a **placebo**. Many of you automatically think of a placebo as a "fake" pill. A placebo *is* in fact a pharmacologically inert compound administered to an individual; however, in many instances it has not only therapeutic effects, but side effects as well. Just as many of the symptoms of illness may have psychogenic or emotional origins, belief in a drug may produce real physiological effects despite the lack of chemical activity. These effects are not limited to the individual's subjective evaluation of relief, but include measurable physiological changes such as altered gastric acid secretion, blood vessel dilation, hormonal changes, and so forth.

In a classic study, two groups of patients with ulcers were given a placebo. In the first group, the medication was provided by a physician, who assured the patients that the drug would provide relief. The second group also received the placebo, but it was administered by a nurse, who described it as experimental in nature. In group 1, 70% of the patients found significant relief, but in group 2, only 25% were helped by the "drug" (Levine, 1973). Based on these results, it is clear that a sugar pill is not a drug that can heal ulcers, but rather its effectiveness depends on the ritual of the therapeutic treatment that can have both neurobiological and behavioral effects that influence the outcome. It is a perfect example of mind–body interaction, and there has been increasing interest in understanding the mechanism responsible for the placebo effect as a means to enhance the therapeutic effectiveness of drug treatments. Although some consider deliberate prescription of placebos to patients unethical because of the deception involved, other physicians and ethicists have identified appropriate uses for placebos that represent an inexpensive treatment that avoids interactions with other medications.

Placebo effects may in part be explained by Pavlovian conditioning in which symptom improvement in the past has been associated with particular characteristics of a medication, for example, its taste, color, shape, and size; a particular recommending clinician, with her white coat, reassuring tone of voice, or attitude; or aspects of the medical facility. Since a placebo effect has been demonstrated many times in animal models, cues in the environment are apparently sufficient, and verbal reassurances are not necessary. In fact, patients have been shown to benefit even if they are told that the medication is a placebo, so deception is apparently not a necessity; however, verbal suggestion interacts with conditioning (see Colagiuri et al., 2015). There have been suggestions that patients might be trained to respond to a placebo by alternating days of placebo treatment with days of active drug treatment. That would allow the reduction of the use of the active agent, potentially minimizing side effects and reducing the cost of treatment.

A second possible explanation for the placebo effect is that of conscious, explicit expectation of outcomes. For example, those individuals who anticipate relief, that is, individuals with an optimistic outlook, may show an enhanced placebo response. Of great interest are the placebo-induced neurobiological effects within the brain. Research has shown that when placebos effectively reduce pain, those individuals who are responders have significantly higher levels of natural pain-relieving opioid neuropeptides in their cerebrospinal fluid than those individuals who do not show a response to the placebo. Further, the subjects who anticipate pain relief show reduced neural activity in pain-related brain regions (see Benedetti et al., 2011).

There is every reason to believe that Pavlovian conditioning and conscious expectation both contribute to the placebo effect, but other factors may also have a part (see Carlino et al., 2016; Murray and Stoessl, 2013). Placebo effects may involve social learning. That is, observing another individual anticipating a positive outcome can be a more powerful inducer of the placebo effect than direct conditioning or verbal suggestions. Others have found that anticipating a successful outcome reduces anxiety and activates reward networks in the brain. Finally, a number of genetic variants have been found that influence the placebo effect. Understanding more about which genes identify patients who will respond to placebo could allow treatment to be adjusted to maximize outcome (Colagiuri et al., 2015). This is one step toward personalized medicine (see the last section of this chapter). A model of these psychosocial–psychobiological factors is shown in **FIGURE 1.1**.

In contrast to placebos, negative expectations may increase the level of anxiety experienced, which may also influence outcome of treatment. Expecting treatment failure when an inert substance is given along with verbal suggestions of negative outcome, such as increased pain or another aversive event, would increase anxiety as well as causing an accompanying change in

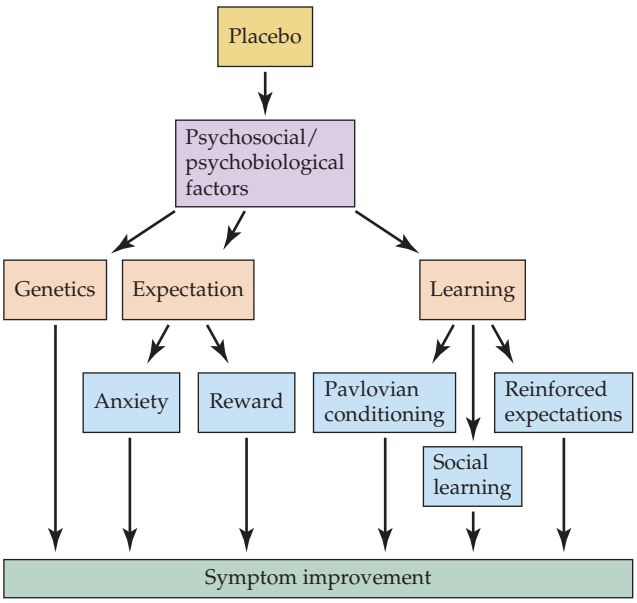

FIGURE 1.1 Placebo effects A model of psychosocial–psychobiological factors that influence clinical improvement following placebo administration. (After Benedetti et al., 2011.)

neural mechanisms, including increases in stress hormones. This is the **nocebo** effect, and both the nocebo-induced increase in pain reported and the hormonal stress response can be reduced by treatment with an antianxiety drug, demonstrating that expectation-induced anxiety plays a part in the nocebo effect. Nocebos are important to study because warnings about potential side effects can lead to greater side effect occurrence. Unfortunately, because drug companies are required by law to provide a comprehensive listing of all possible side effects, many individuals have negative expectations, leading to increased side effects.

In pharmacology, the placebo is essential in the design of experiments conducted to evaluate the effectiveness of new medications, because it eliminates the influence of expectation on the part of the experiment's participants. The control group is identical to the experimental group in all ways and is unaware of the substitution of an inactive substance (e.g., sugar pill, saline injection) for the test medication. Comparison of the two groups provides information on the effectiveness of the drug beyond the expectations of the participants. Of course, drugs with strong subjective effects or prominent side effects make placebo testing more challenging because the experimental group will be aware of the effects while those experiencing no effects will conclude they are the control group. To avoid that problem, some researchers may use an "active" placebo, which is a drug (unrelated to the drug being tested) that produces some side effects that suggest to the control participants that they are getting the active agent. In other cases clinical researchers may feel that it is unethical to leave the placebo group untreated if there is an effective agent available. In that case the control group will be given the older drug, and

BOX 1.1 Pharmacology in Action

Naming Drugs

Drug names can be a confusing issue for many people because drugs that are sold commercially, by prescription or over the counter, usually have four or more different kinds of names. All drugs have a chemical name that is a complete chemical description suitable for synthesizing by an organic chemist. Chemical names are rather clumsy and are rarely used except in a laboratory setting. In contrast, generic names (also called nonproprietary names) are official names of drugs that are listed in the United States Pharmacopeia. The generic name is a much shorter form of the chemical name but is still unique to that drug. For example, one popular antianxiety drug has the chemical name 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one and the generic name diazepam. The brand name, or trade name, of that drug (Valium) specifies a particular manufacturer and a formulation. A brand name is trademarked and copyrighted by an individual company, which means that the company has an exclusive right to advertise and sell that drug.

Slang or street names of commonly abused drugs are another way to identify a particular chemical. Unfortunately, these names change over time and vary with geographic location and particular groups of people. In addition, there is no way to know the chemical characteristics of the substance in question. Some terms are used in popular films or television and become more generally familiar, such as "crack" or "ice," but most disappear as quickly as they appear. The National Institute on Drug Abuse (NIDA) has compiled a list of more than 150 street names for marijuana and more than 75 for cocaine, including coke, big C, nose candy, snow, mighty white, Foo-foo dust, Peruvian lady, dream, doing the line, and many others.

effectiveness of the new drug will be compared with it rather than with a placebo.

The large contribution of nonspecific factors and the high and variable incidence of placebo responders make the **double-blind experiment** highly desirable. In these experiments, neither the patient nor the observer knows what treatment the participant has received. Such precautions ensure that the results of any given treatment will not be colored by overt or covert prejudices on the part of the participant or the observer. If you would like to read more about the use of placebos in both clinical research and therapeutics and the associated ethical dilemmas, refer to the articles by Brown (1998) and Louhiala (2009).

Throughout this chapter, we present examples that include both therapeutic and recreational drugs that affect mood and behavior. Since there are usually several names for the same substance, it may be helpful for you to understand how drugs are named (**BOX 1.1**).

Pharmacokinetic Factors Determining Drug Action

Although it is safe to assume that the chemical structure of a drug determines its action, it quickly becomes clear that additional factors are also powerful contributors. The dose of the drug administered is clearly important, but more important is the amount of drug in the blood that is free to bind at specific target sites (**bioavailability**) to elicit drug action. The following sections of this chapter describe in detail the dynamic factors that contribute to bioavailability. Collectively, these factors constitute the **pharmacokinetic** component of drug action; they are listed below and illustrated in **FIGURE 1.2**.

1. *Routes of administration*. How and where a drug is administered determines how quickly and how completely the drug is absorbed into the blood.
2. *Absorption and distribution*. Because a drug rarely acts where it initially contacts the body, it must pass through a variety of cell membranes and enter the blood plasma, which transports the drug to virtually all of the cells in the body.
3. *Binding*. Once in the blood plasma, some drug molecules move to tissues to bind to active target sites (receptors). While in the blood, a drug may also bind (**depot binding**) to plasma proteins or may be stored temporarily in bone or fat, where it is inactive.
4. *Inactivation*. Drug inactivation, or **biotransformation**, occurs primarily as a result of metabolic

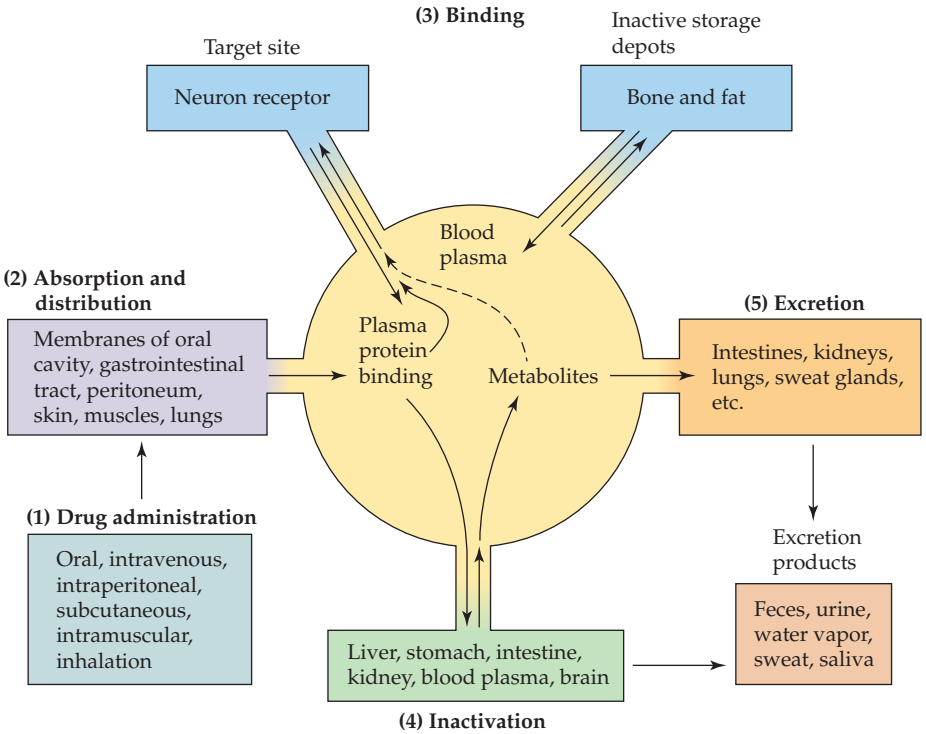

FIGURE 1.2 Pharmacokinetic factors that determine bioavailability of drugs From the site of administration (1), the drug moves through cell membranes to be absorbed into the blood (2), where it circulates to all cells in the body. Some of the drug molecules may bind to inactive sites such as plasma proteins or storage depots (3), and others may bind to receptors in target tissue. Blood-borne drug molecules also enter the liver (4), where they may be transformed into metabolites and travel to the kidneys and other discharge sites for ultimate excretion (5) from the body.

processes in the liver as well as other organs and tissues. The amount of drug in the body at any one time is dependent on the dynamic balance between absorption and inactivation. Therefore, inactivation influences both the intensity and the duration of drug effects.

5. *Excretion*. The liver metabolites are eliminated from the body with the urine or feces. Some drugs are excreted in an unaltered form by the kidneys.

Although these topics are discussed sequentially in the following pages, keep in mind that in the living organism, these factors are at work simultaneously. In addition to bioavailability, the drug effect experienced will also depend on how rapidly the drug reaches its target, the frequency and history of prior drug use (see the discussion on tolerance later in the chapter), and nonspecific factors that are characteristics of individuals and their environments.

Methods of drug administration influence the onset of drug action

The route of administration of a drug determines how much drug reaches its site of action and how quickly the drug effect occurs. There are two major categories of administration methods. **Enteral** methods of administration use the gastrointestinal (GI) tract (*enteron* is the Greek word for "gut"); agents administered by these methods are generally slow in onset and produce highly variable blood levels of drug. The most common enteral method of administration is oral, but rectal administration with the use of suppositories is another enteral route. All other routes of administration are **parenteral** and include those that do not use the alimentary canal, such as injection, pulmonary, and topical administration.

Oral administration (**PO**) is the most popular route for taking drugs, because it is safe, self-administered, and economical, and it avoids the complications and discomfort of injection methods. Drugs that are taken orally come in the form of capsules, pills, tablets, or liquid, but to be effective, the drug must dissolve in stomach fluids and pass through the stomach wall to reach blood capillaries. In addition, the drug must be resistant to destruction by stomach acid and stomach enzymes that are important for normal digestion. Insulin is one drug that can be destroyed by digestive processes, and for this reason it currently cannot be administered orally. However, several pharmaceutical companies have been actively testing various forms of insulin, believing an oral drug would make insulin therapy for diabetes less complicated and unpleasant and lead to better compliance with the treatment regimen. Although there is no oral insulin, there are some oral medications available that may be effective for some diabetic patients because they inhibit the digestion of starches or increase the amount of glucose excreted in the urine.

Movement of the drug from the site of administration to the blood circulation is called **absorption**. Although some drugs are absorbed from the stomach, most drugs are not fully absorbed until they reach the small intestine. Many factors influence how quickly the stomach empties its contents into the small intestine and hence determine the ultimate rate of absorption. For example, food in the stomach, particularly if it is fatty, slows the movement of the drug into the intestine, thereby delaying absorption into the blood. The amount of food consumed, the level of physical activity of the individual, and many other factors make it difficult to predict how quickly the drug will reach the intestine. In addition, many drugs undergo extensive first-pass metabolism. **First-pass metabolism** is an evolutionarily beneficial function because potentially harmful chemicals and toxins that are ingested pass via the portal vein to the liver, where they are chemically altered by a variety of enzymes before passing to the heart for circulation throughout the body (**FIGURE 1.3**). Unfortunately, some therapeutic drugs taken orally may undergo extensive metabolism (more than 90%), reducing their bioavailability. Drugs that show extensive first-pass effects must be administered at higher doses or in an alternative manner, such as by injection. Because of these many factors, oral administration produces drug plasma levels that are more irregular and unpredictable and rise more slowly than those produced by other methods of administration.

Rectal administration requires the placement of a drug-filled suppository in the rectum, where the suppository coating gradually melts or dissolves, releasing the drug, which will be absorbed into the blood. Depending on the placement of the suppository, the drug may avoid some first-pass metabolism. Drug absorbed from the lower rectum into the hemorrhoidal vein bypasses the liver. However, deeper placement means that the drug is absorbed by veins that drain into the portal vein, going to the liver before the general circulation. Bioavailability of drugs administered in this way is difficult to predict, because absorption is irregular. Although rectal administration is not used as commonly as oral administration, it is an effective route in infants and in individuals who are vomiting, unconscious, or unable to take medication orally.

Intravenous (**IV**) injection is the most rapid and accurate method of drug administration in that a precise quantity of the agent is placed directly into the blood and passage through cell membranes such as the stomach wall is eliminated (see Figure 1.3). However, the quick onset of drug effect with IV injection is also a potential hazard. An overdose or a dangerous allergic reaction to the drug leaves little time for corrective measures, and the drug cannot be removed from the

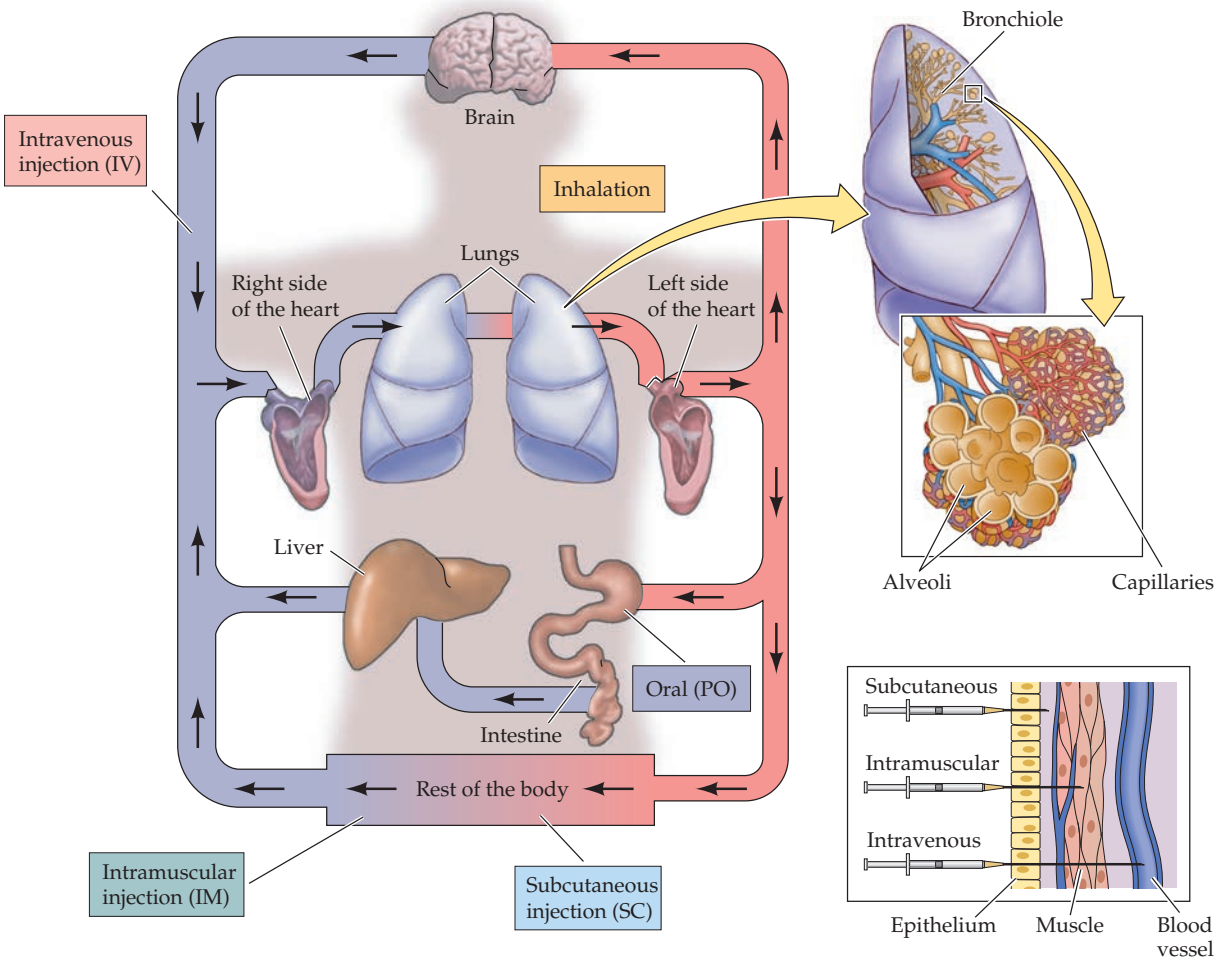

FIGURE 1.3 Routes of drug administration First-pass effect. Drugs administered orally are absorbed into the blood and must pass through the liver before reaching the general circulation. Some drug molecules may be destroyed in the liver before they can reach target tissues. The arrows indicate the direction of blood flow in the arteries (red) and veins (blue). (Top inset) Pulmonary absorption through capillaries in the alveoli. Rapid absorption occurs after inhalation because the large surface area of the lungs and the rich capillary networks provide efficient exchange of gases to and from the blood. (Bottom inset) Methods of administration by injection. The speed of absorption of drug molecules from administration sites depends on the amount of blood circulating to that area.

body as it can be removed from the stomach by stomach pumping.

For drug abusers, IV administration provides a more dramatic subjective drug experience than self-administration in other ways, because the drug reaches the brain almost instantly. Drug users report that intravenous injection of a cocaine solution usually produces an intense "rush" or "flash" of pure pleasure that lasts for approximately 10 minutes. This experience rarely occurs when cocaine is taken orally or is taken into the nostrils (snorting; see the discussion on topical administration). However, intravenous use of street drugs poses several special hazards. First, drugs that are impure or of unknown quality provide uncertain doses, and toxic reactions are common. Second, lack of sterile injection equipment and aseptic technique can lead to infections such as hepatitis, human immunodeficiency virus (HIV), and endocarditis (inflammation of the lining of the heart). Fortunately, many cities have implemented free needle programs, which significantly reduce the probability of cross infection. Third, many drug abusers attempt to dissolve drugs that have insoluble filler materials, which, when injected, may become trapped in the small blood vessels in the lungs, leading to reduced respiratory capacity or death.

An alternative to the IV procedure is **intramuscular** (**IM**) injection, which provides the advantage of slower, more even absorption over a period of time. Drugs administered by this method are usually absorbed within 10 to 30 minutes. Absorption can be slowed down by combining the drug with a second drug that constricts blood vessels, because the rate of drug absorption is dependent on the rate of blood flow to the muscle (see Figure 1.3). To provide slower, sustained action, the

drug may be injected as a suspension in vegetable oil. For example, IM injection of medroxyprogesterone acetate (Depo-Provera) provides effective contraception for 3 to 6 months without the need to take daily pills. One disadvantage of IM administration is that in some cases, the injection solution can be highly irritating, causing significant muscle discomfort.

Intraperitoneal (**IP**) injection is rarely used with humans, but it is the most common route of administration for small laboratory animals. The drug is injected through the abdominal wall into the peritoneal cavity—the space that surrounds the abdominal organs. IP injection produces rapid effects, but not as rapid as those produced by IV injection. Variability in absorption occurs, depending on where (within the peritoneum) the drug is placed.

In **subcutaneous** (**SC**) administration, the drug is injected just below the skin (see Figure 1.3) and is absorbed at a rate that is dependent on blood flow to the site. Absorption is usually fairly slow and steady, but there can be considerable variability. Rubbing the skin to dilate blood vessels in the immediate area increases the rate of absorption. Injection of a drug in a nonaqueous solution (such as peanut oil) or implantation of a drug pellet or delivery device further slows the rate of absorption. Subcutaneous implantation of drug-containing pellets is most often used to administer hormones. Implanon and Nexplanon are two contraceptive implants now available in the United States. The hormones are contained in a single small rod about 40 mm (1.5 inches) long that is implanted through a small incision just under the skin of the upper arm. A woman is protected from pregnancy for a 3-year period unless the device is removed.

Inhalation of drugs, such as those used to treat asthma attacks, allows drugs to be absorbed into the blood by passing through the lungs. Absorption is very rapid because the area of the pulmonary absorbing surfaces is large and rich with capillaries (see Figure 1.3). The effect on the brain is very rapid because blood from the capillaries of the lungs travels only a short distance back to the heart before it is pumped quickly to the brain via the carotid artery, which carries oxygenated blood to the head and neck. The psychoactive effects of inhaled substances can occur within a matter of seconds.

Inhalation is the method preferred for self-administration in cases when oral absorption is too slow and much of the active drug would be destroyed in the GI tract before it reached the brain. Nicotine released from the tobacco of a cigarette by heat into the smoke produces a very rapid rise in blood level and rapid central nervous system (CNS) effects, which peak in a matter of minutes. Tetrahydrocannabinol (THC), an active ingredient of marijuana, and crack cocaine are also rapidly absorbed after smoking. In addition to the inherent dangers of the drugs themselves, disadvantages of inhalation include irritation of the nasal passages and damage to the lungs caused by small particles that may be included in the inhaled material.

Topical application of drugs to mucous membranes, such as the conjunctiva of the eye, the oral cavity, nasopharynx, vagina, colon, and urethra, generally provides local drug effects. However, some topically administered drugs can be readily absorbed into the general circulation, leading to widespread effects. One such delivery method is **sublingual administration**, which involves placing the drug under the tongue, where it contacts the mucous membrane and is absorbed rapidly into a rich capillary network. Sublingual administration has several advantages over oral administration, because it is not broken down by gastric acid or gastric enzymes. Further, its absorption is faster because it is absorbed directly into the blood and is not dependent on those factors that determine how quickly the stomach empties its contents into the small intestine. Additionally, since the drug is not absorbed from the GI tract, it avoids first-pass metabolism. **Intranasal administration** is of special interest because it causes local effects such as relieving nasal congestion and treating allergies, but it can also have systemic effects, in which case the drug moves very rapidly across a single epithelial cell layer into the bloodstream, avoiding first-pass liver metabolism and producing higher bioavailability than if given orally. The approach is noninvasive, painless, and easy to use, hence it enhances compliance. Even more important is the fact that intranasal administration allows the blood–brain barrier to be bypassed, perhaps by achieving direct access to the fluid that surrounds the brain (**cerebrospinal fluid** [**CSF**]) and moving from there to extracellular fluid found in the intercellular spaces between neurons. A large number of drugs, hormones, steroids, proteins, peptides, and other large molecules are available in nasal spray preparations for intranasal delivery, although not all drugs can be atomized. Hence neuropeptides such as the hormone oxytocin can be administered by intranasal sprays to achieve significant concentrations in the brain. **Web Box 1.2** describes a study that evaluated the effects of intranasal oxytocin administration on social behavior in autistic adults.

Intranasal absorption can also be achieved without dissolving the drug. Direct application of finely powdered cocaine to the nasal mucosa by sniffing leads to rapid absorption, which produces profound effects on the CNS that peak in about 15 to 30 minutes. One side effect of "snorting" cocaine is the formation of perforations in the nasal septum, the cartilage that separates the two nostrils. This damage occurs because cocaine is a potent vasoconstrictor. Reducing blood flow deprives the underlying cartilage of oxygen, leading to necrosis. Additionally, contaminants in the cocaine act

as chemical irritants, causing tissue inflammation. Cocaine addicts whose nasal mucosa has been damaged by chronic cocaine "snorting" may resort to application of the drug to the rectum, vagina, or penis.

Although the skin provides an effective barrier to the diffusion of water-soluble drugs, certain lipid-soluble substances (i.e., those that dissolve in fat) are capable of penetrating slowly. Accidental absorption of industrial and agricultural chemicals such as tetraethyl lead, organophosphate insecticides, and carbon tetrachloride through the skin produces toxic effects on the nervous system and on other organ systems. (For greater detail on environmental toxins, see the online chapter **Environmental Neurotoxicants and Endocrine Disruptors** on the Companion Website.) **Transdermal** (i.e., through the skin) drug administration with skin patches provides controlled and sustained delivery of drug at a preprogrammed rate. The method is convenient because the individual does not have to remember to take a pill, and it is painless without the need for injection. It also provides the advantage of avoiding the first-pass effect. In cases of mass vaccination campaigns, such as those undertaken during pandemics, transdermal delivery is much quicker than other methods, and it reduces the dangers of accidental needle sticks of health care workers and unsafe disposal of used needles. Patches consist of a polymer matrix embedded with the drug in high concentration. Transdermal delivery is now a common way to prevent motion sickness with scopolamine, reduce cigarette craving with nicotine, relieve angina pectoris with nitroglycerin, and provide hormones after menopause or for contraceptive purposes. The major disadvantage of transdermal delivery is that because skin is designed to prevent materials from entering the body, a limited number of drugs are able to penetrate. However, techniques are continuing to be developed to increase skin permeability through a variety of methods. For instance, handheld ultrasound devices that send low-intensity sound energy waves through surrounding fluid in the tissue temporarily increase the size of the pores in the skin, allowing absorption of large molecules from a skin patch. Other "active" patch systems that help to move large molecules through the skin use iontophoresis, which involves applying a small electrical current with tiny batteries to the reservoir or the patch. The electrical charge repels drug molecules with a similar charge and forces them through the skin at a predetermined rate. If the amount and duration of current are changed, drug delivery can be restricted to the skin for local effects or can be forced more deeply into the blood. This process is also capable of pulling molecules out through the skin for monitoring. Such monitoring might be used by diabetic individuals to more frequently and painlessly evaluate levels of blood glucose. An additional approach uses mechanical disruption of the skin. Small arrays of microneedles about 1 μm in diameter and 100 μm long and coated with drug or vaccine are placed on the skin. The needles penetrate the superficial layer of the skin—the stratum corneum—where the drug is delivered without stimulating underlying pain receptors. This method provides the opportunity for painless vaccinations and drug injections that can be self-administered. These and other developing techniques have been described by Langer (2003) and Banga (2009).

Special injection methods must be used for some drugs that act on nerve cells, because a cellular barrier, the blood–brain barrier (discussed later in the chapter), prevents or slows passage of these drugs from the blood into neural tissue. For example, **epidural** injection is used when spinal anesthetics are administered directly into the cerebrospinal fluid surrounding the spinal cord of a mother during childbirth, bypassing the blood–brain barrier. In animal experiments, a microsyringe or a cannula enables precise drug injection into discrete areas of brain tissue (**intracranial**) or into the cerebrospinal fluid–filled chambers, the ventricles (**intracerebroventricular**). In this way, experimenters can study the electrophysiological, biochemical, or behavioral effects of drugs on particular nerve cell groups. This method is described in Chapter 4. Animal research has evolved into potentially important treatment methods for human conditions such as cerebral meningitis (inflammation of one of the protective membranes covering the brain). An **infusion pump** implanted under the skin of the scalp can be programmed to deliver a constant dose of antibiotic into the cerebral ventricles; this device permits treatment of brain infection and is useful because antibiotics are normally prevented from passing the blood–brain barrier. These infusion pumps have important uses in delivering drugs systemically as well. With appropriate software, it is possible to provide pulsed administration of an agent that mimics the normal biological rhythm, for example, of hormones. An exciting development has been the addition of feedback regulation of these pumps, which includes a sensor element that monitors a substance such as blood glucose in a diabetic individual and responds with an appropriate infusion of insulin delivered from an implantable pump that acts much like an artificial pancreas. The downside to these pumps is the risk of infection and frequent clogging, which reduces their usefulness in maintaining stable drug concentrations over prolonged periods.

Many disorders of the CNS are characterized by abnormal changes in gene activity, which alter the manufacture of an essential protein such as an enzyme or a receptor. **Gene therapy** refers to the application of deoxyribonucleic acid (DNA), which encodes a specific protein, to a particular target site. DNA can be used to increase or block expression of the gene product to correct the clinical condition. One significant difficulty in the application of gene therapy involves creating the

appropriate gene delivery system. Such a delivery system, which is called a *vector*, is needed to carry the gene into the nuclei of target cells to alter protein synthesis. Administering gene therapy is clearly more challenging when disorders of the CNS, rather than disorders of any other part of the body, are treated. Vectors are usually injected directly into the brain region targeted for modification. **Viral vectors** are frequently considered for this delivery system because of the special ability of viruses to bind to and enter cells and their nuclei, where they insert themselves into the chromosomes to alter DNA. Because viruses vary in terms of binding, cell entry proteins, and other properties, a variety of viruses are being evaluated.

Lim and colleagues (2010) provide a review of viral vector delivery as an approach to treating diseases of the CNS. Human trials have been increasing in number, but much research remains to be done before the safety and usefulness of gene therapy are fully demonstrated. Concerns expressed by researchers include the following: that an immune response may be initiated by the introduction of foreign material, that the viral vector may recover its ability to cause disease once it is placed in the human cell, and that inserting the vector in the wrong place may induce tumor growth. Nevertheless, many animal studies are highly encouraging, and gene therapy is believed to have enormous potential for the treatment of debilitating disorders of the nervous system such as stroke-induced damage, spinal cord injury, chronic pain conditions, and neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and Huntington's disease.

IMPACT ON BIOAVAILABILITY Because the route of administration significantly alters the rate of absorption, blood levels of the same dose of a drug administered by different routes vary significantly. **FIGURE 1.4** compares drug concentrations in blood over time for various routes of administration. Keep in mind that the peak level for each method reflects not only differences in absorption rate, but also the fact that slow absorption provides the opportunity for liver metabolism to act on some of the drug molecules before absorption is complete. Advantages and disadvantages of selected methods of administration are summarized in **TABLE 1.1**.

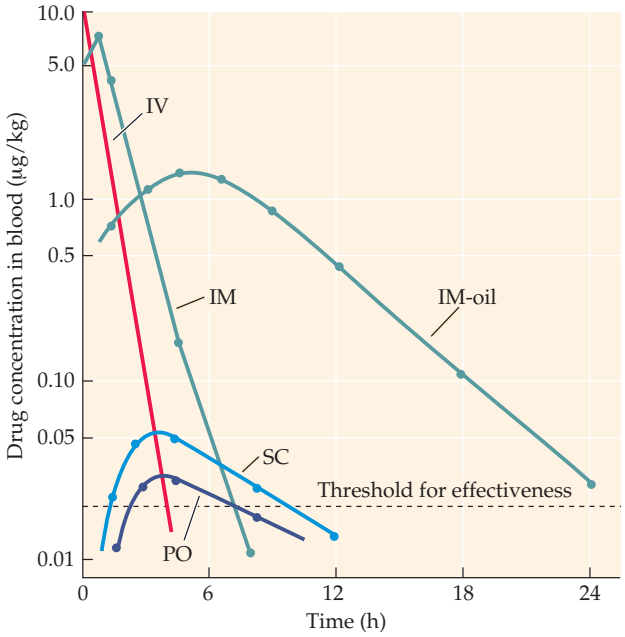

FIGURE 1.4 The time course of drug blood level depends on route of administration The blood level of the same amount of drug administered by different procedures to the same individual varies significantly. Intravenous (IV) administration produces an instantaneous peak when the drug is placed in the blood, followed by a rapid decline. Intramuscular (IM) administration produces rapid absorption and rapid decline, although IM administration in oil (IM-oil) shows slower absorption and gradual decline. Slow absorption after subcutaneous (SC) administration means that some of the drug is metabolized before absorption is complete. For this reason, no sharp peak occurs, and overall blood levels are lower. Oral (PO) administration produces the lowest blood levels and a relatively short time over threshold for effectiveness in this instance. (After Levine, 1973 and Marsh, 1951.)

Multiple factors modify drug absorption

Once the drug has been administered, it is absorbed from the site of administration into the blood to be circulated throughout the body and ultimately to the brain, which is the primary target site for **psychoactive drugs** (i.e., those drugs that have an effect on thinking, mood, and behavior). We have already shown that the rate of absorption is dependent on several factors. Clearly, the route of administration alters absorption because it determines the area of the absorbing surface, the number of cell layers between the site of administration and the blood, the amount of drug destroyed by metabolism or digestive processes, and the extent of binding to food or inert complexes. Absorption is also dependent on drug concentration, which is determined in part by individual differences in age, sex, and body size. Finally, absorption is dependent on the solubility and ionization of the drug.

TRANSPORT ACROSS MEMBRANES Perhaps the single most important factor in determining plasma drug levels is the rate of passage of the drug through the various cell layers (and their respective membranes) between the site of administration and the blood. To understand this process, we need to look more carefully at cell membranes.

Cell membranes are made up primarily of complex lipid (fat) molecules called **phospholipids**, which have a negatively charged phosphate region (the head) at one end and two uncharged lipid tails (**FIGURE 1.5A**).

TABLE 1.1 Advantages and Disadvantages of Selected Routes of Drug Administration

Route of administration	Advantages	Disadvantages
Oral (PO)	Safe; self-administered; economical; no needle-related complications	Slow and highly variable absorption; subject to first-pass metabolism; less-predictable blood levels
Intravenous (IV)	Most rapid; most accurate blood concentration	Overdose danger; cannot be readily reversed; requires sterile needles and medical technique
Intramuscular (IM)	Slow and even absorption	Localized irritation at site of injection; needs sterile equipment
Subcutaneous (SC)	Slow and prolonged absorption	Variable absorption depending on blood flow
Inhalation	Large absorption surface; very rapid onset; no injection equipment needed	Irritation of nasal passages; inhaled small particles may damage lungs
Topical	Localized action and effects; easy to self-administer	May be absorbed into general circulation
Transdermal	Controlled and prolonged absorption	Local irritation; useful only for lipid-soluble drugs
Epidural	Bypasses blood–brain barrier; very rapid effect on CNS	Not reversible; needs trained anesthesiologist; possible nerve damage
Intranasal	Ease of use; local or systemic effects; very rapid; no first-pass metabolism; bypasses blood–brain barrier	Not all drugs can be atomized; potential irritation of nasal mucosa

These molecules are arranged in a bilayer, with their phosphate ends forming two almost continuous sheets filled with fatty material (**FIGURE 1.5B**). This configuration occurs because the polar heads are attracted to the polar water molecules. Hence the charged heads are in contact with both the aqueous intracellular fluid and the aqueous extracellular fluid. Proteins that are found inserted into the phospholipid bilayer have functions that will be described later (see Chapter 3). The molecular characteristics of the cell membrane prevent most molecules from passing through unless they are soluble in fat.

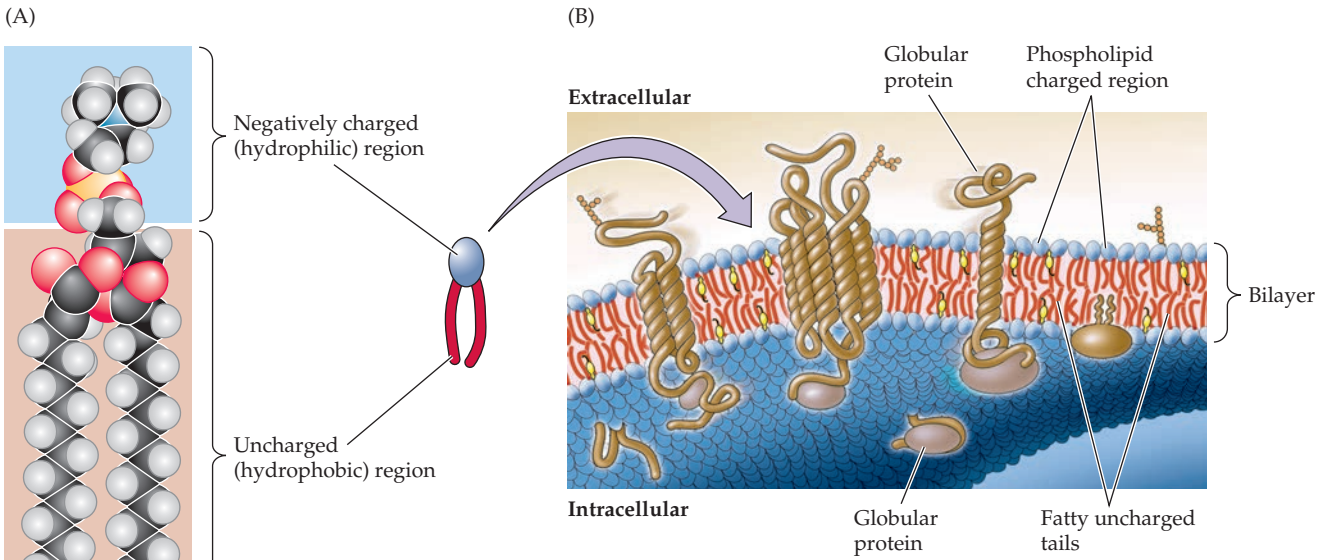

FIGURE 1.5 Cell membranes (A) Example of a phospholipid molecule with a negatively charged group (PO_4^-) at one end (hydrophilic) and two fatty uncharged tails (hydrophobic). (B) The arrangement of individual phospholipid molecules forms a bilayer, with negatively charged heads attracted to the water molecules of both intracellular and extracellular fluids. The fatty tails of the molecules are tucked within the two charged layers and have no contact with aqueous fluid. Embedded in the bilayer are protein molecules that serve as receptors or channels.

LIPID-SOLUBLE DRUGS Drugs with high lipid solubility move through cell membranes by **passive diffusion**, leaving the water in the blood or stomach juices and entering the lipid layers of membranes. Movement across the membranes is always in a direction from higher to lower concentration. The larger the concentration difference on each side of the membrane (called the **concentration gradient**), the more rapid is the diffusion. Lipid solubility increases the absorption of drug into the blood and determines how readily a drug will pass the lipid barriers to enter the brain. For example, the narcotic drug heroin is a simple modification of the parent compound morphine. Heroin, or diacetylmorphine, is more soluble in lipid than is morphine, and it penetrates into brain tissue more readily, thus having a quicker onset of action and more potent reinforcing properties. This occurs despite the fact that before the psychotropic drug effects occur, the heroin must be converted to morphine by esterase enzymes in the brain. That property makes heroin a prodrug, that is, one that is dependent on metabolism to convert an inactive drug to an active one, a process called **bioactivation**. This strategy is one used by pharmaceutical companies that develop prodrugs that cross the blood–brain barrier (see later section) if the active drug cannot penetrate easily.

IONIZED DRUGS Most drugs are not readily lipid soluble, because they are weak acids or weak bases that can become ionized when dissolved in water. Just as common table salt (NaCl) produces positively charged ions (Na^+) and negatively charged ions (Cl^-) when dissolved in water, many drugs form two charged (ionized) particles when placed in water. Although NaCl is a strong electrolyte, which causes it to almost entirely dissociate in water, most drugs are only partially ionized when dissolved in water. The extent of **ionization** depends on two factors: the relative acidity/alkalinity (pH) of the solution, and an intrinsic property of the molecule (pK_a).

Acidity or alkalinity is expressed as pH, which is described on a scale of 1 to 14, with 7 being neutral. Acidic solutions have a lower pH, and alkaline (basic) solutions have a pH greater than 7.0. Drugs are dissolved in body fluids that differ in pH (**TABLE 1.2**), and these differences play a role in drug ionization and movement from one body fluid compartment to another, for example, from the stomach to the bloodstream, or from the bloodstream into the kidney urine.

The second factor determining ionization is a characteristic of the drug molecule. The pK_a of a drug represents the pH of the aqueous solution in which that drug would be 50% ionized and 50% non-ionized. In general, drugs that are weak acids ionize more readily in an alkaline environment and become less ionized in an acidic environment. The reverse is true of drugs that are weak bases. If we put the weak acid aspirin (acetylsalicylic acid) into stomach acid, it will remain primarily in a non-ionized form (**FIGURE 1.6**). The lack of electrical charge makes the drug more lipid soluble and hence readily absorbed from the stomach to the blood. In the intestine, where the pH is around 5.0 to 6.0, ionization increases and absorption through that membrane is reduced compared with that of the stomach.

TABLE **1.2** pH of Body Fluids

Fluid	pH
Stomach	1.0–3.0
Small intestine	5.0–6.6
Blood	7.35–7.45
Kidney urine	4.5–7.5
Saliva	6.2–7.2
Cerebrospinal fluid (CSF)	7.3–7.4

This raises the question of why aspirin molecules do not move from the stomach to the blood and back to the stomach again. In our example, aspirin in the acidic gastric fluid is primarily in non-ionized form and thus passes through the stomach wall into the blood. In blood (pH 7.4), however, aspirin becomes more ionized; it is said to be "trapped" within the blood and does not return to the stomach. Meanwhile, the circulation moves the aspirin molecules away from their concentrated site at the stomach to maintain a concentration gradient that favors drug absorption. Hence, although passive diffusion would normally cease when drug concentration approached a 50:50 equilibrium, the combination of ion trapping and blood circulation of the drug away from the absorbing surface means that absorption from oral administration can be quite high. Keep in mind that although the acidic stomach favors absorption of weak acids, much of the aspirin is absorbed in the small intestine because absorption is also determined by the length of time the drug is in contact with the absorptive membrane.

Drugs that are highly charged in both acidic and basic environments are very poorly absorbed from the gastrointestinal tract and cannot be administered orally. This explains why South American hunters readily eat the flesh of game killed with curare-poisoned arrows. Curare is highly ionized in both the acidic stomach and the alkaline intestine, so the drug does not leave the digestive system to enter their blood.

OTHER FACTORS Factors other than ionization have a significant influence on absorption as well. For instance, the much larger surface area of the small intestine and the slower movement of material through the intestine, as compared with the stomach, provide a

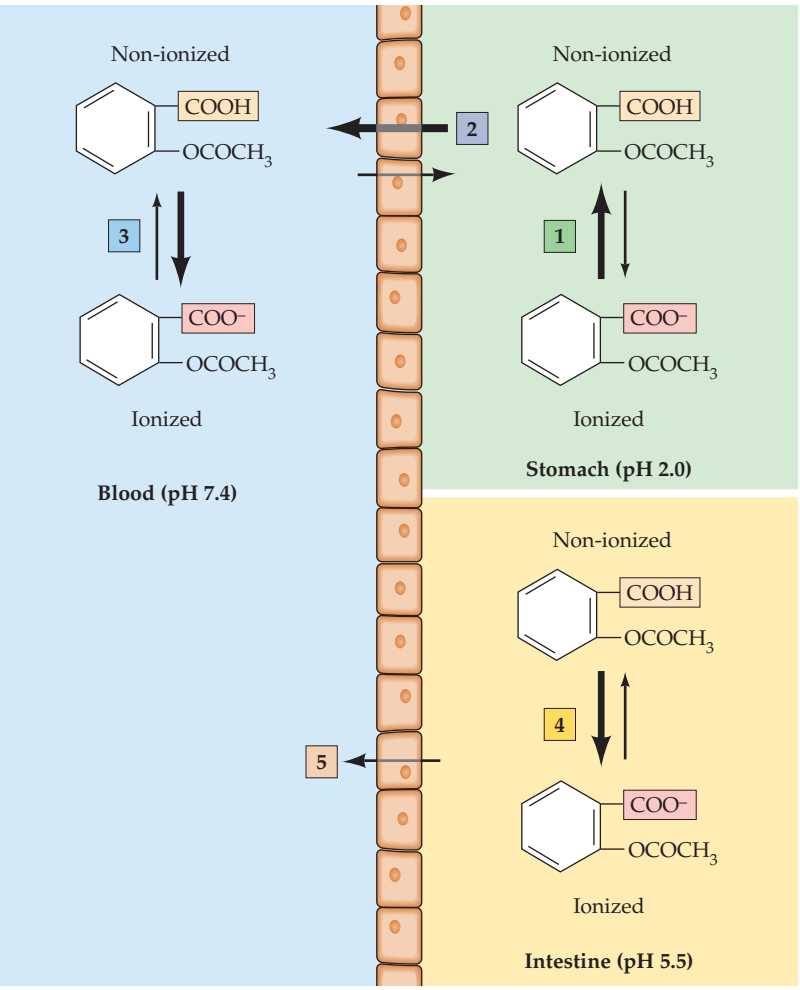

FIGURE 1.6 Effect of ionization on drug absorption On the right side of the cell barrier in stomach acid (pH 2.0), aspirin molecules tend to remain in the non-ionized form (1), which promotes the passage of the drug through the cell walls (2) to the blood. Once the intact aspirin molecules reach the blood (pH 7.4), they ionize (3) and are "trapped" in the blood to be circulated throughout the body. In the lower portion of the figure, when the aspirin has reached the intestine, it tends to dissociate to a greater extent (4) in the more basic pH. Its more ionized form reduces passage (5) through the cells to the blood, so absorption from the intestine is slower than from the stomach.

much greater opportunity for absorption of all drugs. Therefore, the rate at which the stomach empties into the intestine very often is the significant rate-limiting factor. For this reason, medication is often prescribed to be taken before meals and with sufficient fluid to move the drug through the stomach and into the intestine.

Since drug absorption is closely related to the concentration of the drug in body fluids (e.g., stomach), it should certainly be no surprise to you that the drug dosage required to achieve a desired effect is directly related to the size of the individual. In general, the larger the individual, the more diluted the drug will be in the larger fluid volume, and less drug will reach target sites within a given unit of time. The average dose of a drug is typically based on the response of individuals between the ages of 18 and 65 who weigh 150 pounds. However, for people who are very lean or obese, the average dose may be inappropriate because of variations in the ratio of fat to water in the body. For these individuals, body surface area, which reflects both size and weight, may serve as a better basis for determining drug dose. The sex of the individual also plays a part in determining plasma drug level: in women, adipose tissue, relative to water, represents a larger proportion of the total body weight. Overall, the total fluid volume, which contains the drug, is relatively smaller in women than in men, producing a higher drug concentration at the target site in women. It should be obvious also that in the smaller fluid volume of a child, a standard dose of a drug will be more concentrated and therefore will produce a greater drug effect.

Drug distribution is limited by selective barriers

Regardless of the route of administration, once the drug has entered the blood, it is carried throughout the body within 1 or 2 minutes and can have an action at any number of receptor sites. In general, those parts of the body in which blood flow is greatest will have the highest concentration of drug. Since blood capillaries have numerous pores, most drugs can move from blood and enter body tissues regardless of lipid solubility, unless they are bound to protein (see the discussion on depot binding later in this chapter). Quite rapidly, high concentrations of drugs will be found in the heart, brain, kidneys, and liver. Other tissues with less vasculature will more slowly continue to absorb the drug from the plasma, causing plasma levels to fall gradually. As plasma levels fall, the concentration of drug in the highly vascularized organs will be greater than that in the blood, so the drug will move from those organs back into the plasma to maintain equilibrium. Hence those organs will have an initial high concentration of drug, and then **drug redistribution** will reduce drug concentration there. Ultimately drug concentration will be in equilibrium in all tissues. Drug redistribution may be responsible for terminating the action of a drug, as in the case of the rapid-acting CNS depressant thiopental.

Thiopental, a barbiturate used for intravenous anesthesia, is highly lipid soluble; therefore, rapid onset of sedation is caused by entry of the drug into the brain. Deep sedation does not last very long, because the blood level falls rapidly as a result of redistribution of the drug to other tissues, causing thiopental to move from the brain to the blood to maintain equilibrium. High levels of thiopental can be found in the brain 30 seconds after IV infusion. However, within 5 minutes, brain levels of the drug drop to threshold anesthetic concentrations. In this way, thiopental induces sleep almost instantaneously but is effective for only about 5 minutes, followed by rapid recovery.

Because the brain receives about 20% of the blood that leaves the heart, lipid-soluble drugs are readily distributed to brain tissue. However, the blood–brain barrier limits the movement of ionized molecules from the blood to the brain.

BLOOD–BRAIN BARRIER Blood plasma is supplied by a dense network of blood vessels that permeate the entire brain. This system supplies brain cells with oxygen, glucose, and amino acids, and it carries away carbon dioxide and other waste products. Despite the vital role that blood circulation plays in cerebral function, many substances found in blood fluctuate significantly and would have disruptive effects on brain cell activity if materials were transferred freely between blood and brain (and the brain's associated cerebrospinal fluid).

Cerebrospinal fluid (CSF) is a clear, colorless liquid that fills the subarachnoid space that surrounds the entire bulk of the brain and spinal cord and also fills the hollow spaces (ventricles) and their interconnecting channels (aqueducts), as well as the centrally located cavity that runs longitudinally through the length of the spinal cord (central canal) (**FIGURE 1.7A**). CSF is manufactured by cells of the choroid plexus, which line the cerebral ventricles. In contrast to the wide fluctuations that occur in the blood plasma, the contents of the CSF remain quite stable. Many substances that diffuse out of the blood and affect other organs in the body do not seem to enter the CSF, nor do they affect brain tissue. This separation between brain capillaries and the brain/CSF constitutes what we call the blood–brain barrier. **FIGURE 1.7B** shows an enlargement of the relationship between the cerebral blood vessels and the CSF.

The principal component of the blood–brain barrier is the distinct morphology of brain capillaries. Figure 1.8 shows a comparison between typical capillaries found throughout the body (**FIGURE 1.8A**) and capillaries that serve the CNS (**FIGURE 1.8B**). Because the job of blood vessels is to deliver nutrients to cells while removing waste, the walls of typical capillaries

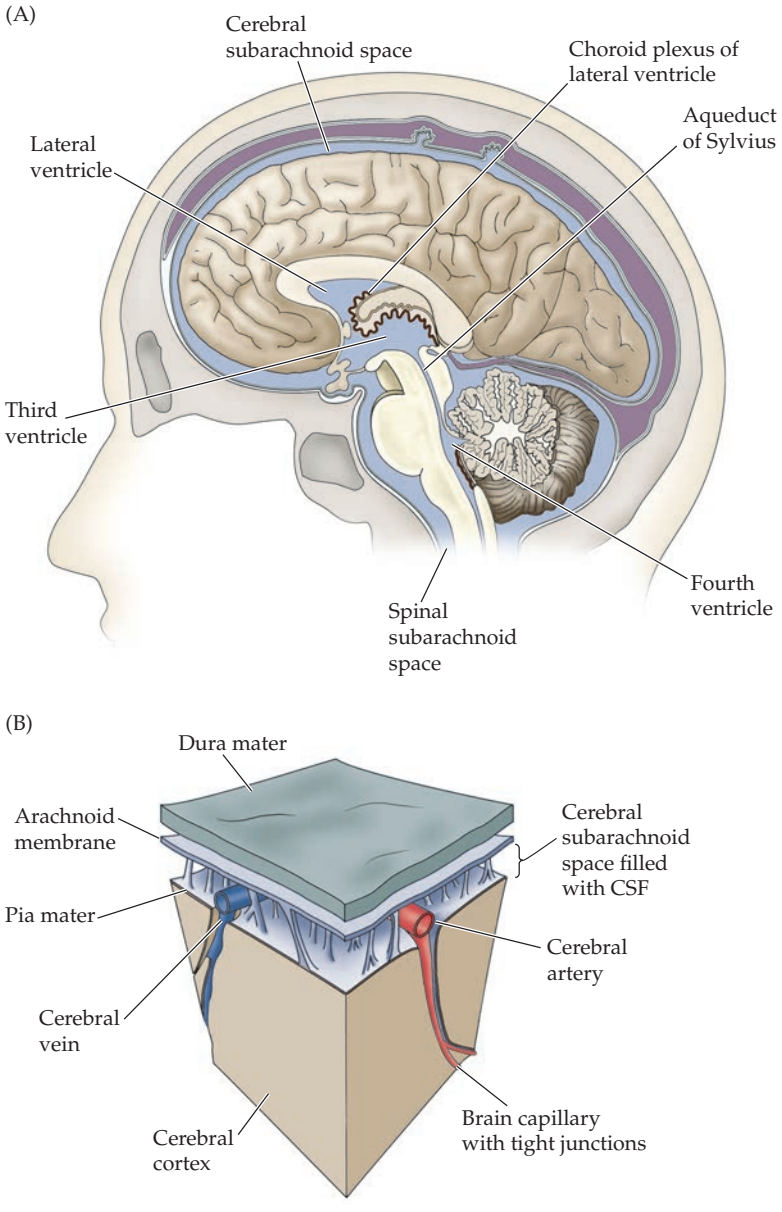

FIGURE 1.7 Distribution of cerebrospinal fluid (A) Cerebrospinal fluid (CSF; blue) is manufactured by the choroid plexus within the cerebral ventricles. In addition to filling the ventricles and their connecting aqueducts, CSF fills the space between the arachnoid membrane and the pia mater (subarachnoid space) to cushion the brain against trauma. (B) The enlarged diagram shows detail of CSF-filled subarachnoid space and its relationship to cerebral blood vessels. Note how the blood vessels penetrate the brain tissue.

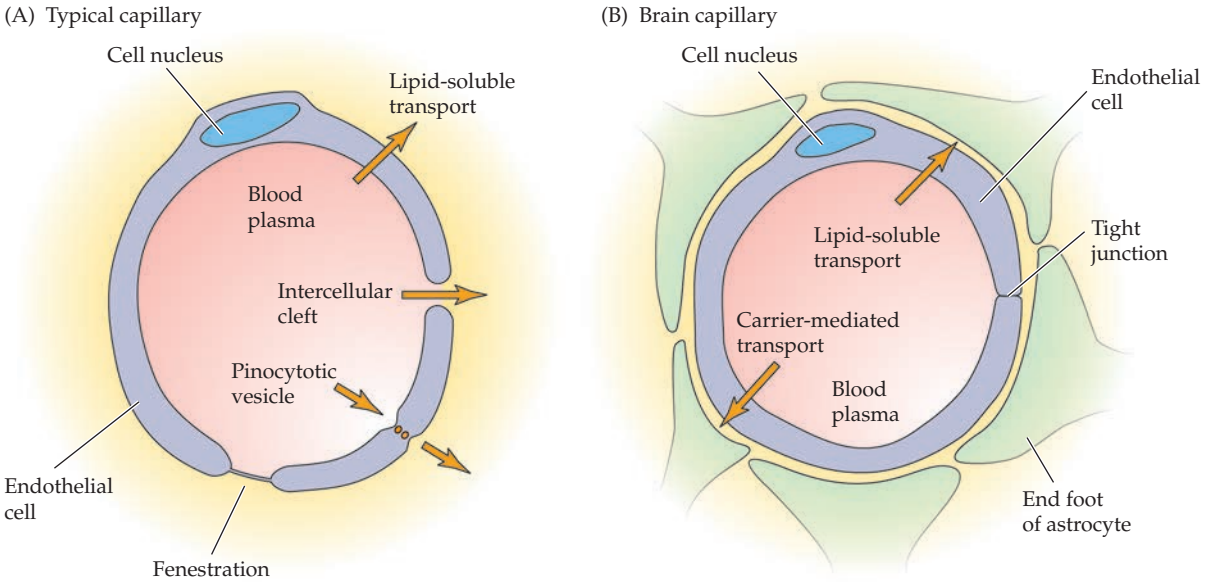

FIGURE 1.8 Cross section of typical capillaries and brain capillaries (A) Capillaries found throughout the body have characteristics that encourage movement of materials between the blood and surrounding cells. (B) Brain capillaries minimize movement of water-soluble molecules through the blood vessel wall because there are essentially no large or small clefts or pinocytotic sites. (After Oldendorf, 1977.)

are made up of endothelial cells that have both small gaps (**intercellular clefts**) and larger openings (**fenestrations**) through which molecules can pass. In addition, general capillaries have **pinocytotic vesicles** that envelop and transport larger molecules through the capillary wall. In contrast, in brain capillaries, the intercellular clefts are closed because adjoining edges of the endothelial cells are fused, forming **tight junctions**. Also, fenestrations are absent and pinocytotic vesicles are rare. Although lipid-soluble materials can pass through the walls of the blood vessels, most materials are moved from the blood of brain capillaries by special transporters. Surrounding brain capillaries are numerous glial feet—extensions of the glial cells called **astrocytes** or **astroglia**. It is becoming apparent that these astrocytic glial feet contribute to both postnatal formation and maintenance of the blood–brain barrier throughout adulthood. It has been shown that blood–brain barrier characteristics depend on the CNS environment, because if the endothelial cells are removed and cultured without astroglia, they lose their barrier function. Conversely blood–brain barrier characteristics can be induced in non-CNS endothelial cells that are cultured with astrocytes (see Alvarez et al., 2013). By filling in the extracellular space around capillaries and releasing secretion factors, these astroglia apparently help maintain the endothelial tight junctions. Also, it is likely that the close interface of astrocytes with both nerve cells and brain capillaries provides the astrocytes with a unique opportunity to coordinate the delivery of oxygen and glucose in the blood with the energy required by activated neurons. There is more discussion of the many functions of astrocytes in Chapter 2.

Before we go on, we should emphasize that the blood–brain barrier is selectively permeable, not impermeable. Although the barrier does reduce diffusion of water-soluble (i.e., ionized) molecules, it does not impede lipid-soluble molecules.

Finally, the blood–brain barrier is not complete. Several brain areas are not isolated from materials in the blood and a limited blood–brain barrier exists in other regions of the brain wherever a functional interaction (e.g., blood monitoring) is required between blood and neural tissue. For example, the area postrema, or chemical trigger zone, is a cluster of cells in the brainstem that responds to toxins in the blood and induces vomiting.

The limited permeability of the blood–brain barrier is important in psychopharmacology because we need to know which drugs remain non-ionized at plasma pH and readily enter the CNS, and which drugs only circulate throughout the rest of the body. Minor differences in drug molecules are responsible for the relative selectivity of drug action. For example, physostigmine readily crosses the blood-brain barrier and is useful for treating the intoxication caused by some agricultural pesticides. It does so by increasing the availability of the neurotransmitter acetylcholine. In contrast, the structurally related but highly ionized drug neostigmine is excluded from the brain and increases acetylcholine only peripherally. Its restriction by the blood–brain barrier means that neostigmine can be used to treat the muscle disease myasthenia gravis without significant CNS side effects, but it would not be effective in treating pesticide-induced intoxication. As mentioned in an earlier section, because many drugs that are ionized do not pass through the

blood–brain barrier, direct delivery of the drug into brain tissue by intracranial injection may be necessary, although at least some drugs can be atomized and delivered intranasally to bypass the blood–brain barrier. A second approach is to develop a prodrug that is lipid soluble and becomes bioactivated by brain enzymes.

PLACENTAL BARRIER A second barrier, unique to women, is found between the blood circulation of a pregnant mother and that of her fetus. The placenta, which connects the fetus with the mother's uterine wall, is the means by which nutrients obtained from the digestion of food, O_2, CO_2, fetal waste products, and drugs are exchanged. As is true for other cell membranes, lipid-soluble substances diffuse easily, and water-soluble substances pass less easily. The potential for transfer of drugs from mother to fetus has very important implications for the health and well-being of the developing child. Potentially damaging effects on the fetus can be divided into two categories: acute toxicity and teratogenic effects.

The fetus may experience acute toxicity in utero after exposure to the disproportionately high drug blood level of its mother. In addition, after birth, any drug remaining in the newborn's circulation is likely to have a dramatic and prolonged action because of slow and incomplete metabolism. It is well known that opiates such as heroin readily reach the fetal circulation and that newborn infants of heroin- or methadone-addicted mothers experience many of the signs of opiate withdrawal. Certain tranquilizers, gaseous anesthetics, alcohol, many barbiturates, and cocaine all readily pass into the fetal circulation to cause acute toxicity. In addition, alcohol, cocaine, and the carbon monoxide in cigarette smoke all deprive the fetus of oxygen. Such drugs pose special problems because they are readily accessible and are widely used.

Teratogens are agents that induce developmental abnormalities in the fetus. The effects of teratogens such as drugs (both therapeutic and illicit), exposure to X-rays, and some maternal infections (e.g., German measles) are dependent on the timing of exposure. The fetus is most susceptible to damaging effects during the first trimester of pregnancy, because it is during this period that many of the fetal organ systems are formed. Each organ system is maximally sensitive to damaging effects during its time of cell differentiation (**TABLE 1.3**). Many drugs can have damaging effects on the fetus despite minimal adverse effects in the mother. For example, the vitamin A–related substance isotretinoin, which is a popular prescription acne medication (Accutane), produces serious birth defects and must be avoided by sexually active young women. Experience has taught us that evaluation of drug safety must consider potential fetal effects, as well as effects on adults. Furthermore, because teratogenic effects are most severe during the time before pregnancy is typically recognized, the use of any drug known to be teratogenic in animals should be avoided by women of childbearing age.

TABLE 1.3 Periods of Maximum Teratogenic Sensitivity for Several Organ Systems in the Human Fetus

Organ system	Days after fertilization
Brain	15–60
Eye	15–40
Genitalia	35–60
Heart	15–40
Limbs	25–35

Depot binding alters the magnitude and duration of drug action

We already know that after a drug has been absorbed into the blood from its site of administration, it circulates throughout the body. Thus high concentrations of drug may be found in all organs that are well supplied with blood until the drug gradually redistributes to all tissues in the body. Drug binding occurs at many inactive sites, where no measurable biological effect is initiated. Such sites, called **drug depots** or **silent receptors**, include several plasma proteins, with albumin being most important. Any drug molecules bound to these depots cannot reach active sites, nor can they be metabolized by the liver. However, the drug binding is reversible, so the drug remains bound only until the blood level drops, causing it to unbind gradually and circulate in the plasma.

The binding of a drug to inactive sites—called **depot binding**—has significant effects on the magnitude and duration of drug action. Some of these effects are summarized in **TABLE 1.4**. First, depot binding reduces the concentration of drug at its sites of action because only freely circulating (unbound) drug can pass across membranes. Onset of action of a drug that binds readily to depot sites may be delayed and its effects reduced because the number of drug molecules reaching the target tissue is dependent on its release from inactive sites. Individual differences in the amount of depot binding explain in part why some people are more sensitive than others to a particular drug.

Second, because binding to albumin, fat, and muscle is rather nonselective, many drugs with similar physiochemical characteristics compete with each other for these sites. Such competition may lead to much-higher-than-expected free drug blood level of the displaced drug, producing a drug overdose. For example, the antiseizure drug phenytoin is highly protein bound, but aspirin can displace some of the phenytoin molecules from the binding sites because aspirin binds more readily. When phenytoin is displaced from plasma protein by aspirin, the elevated drug level may be responsible

TABLE 1.4 Effects of Drug Depot Binding on Therapeutic Outcome

Depot-binding characteristics	Therapeutic outcome
Rapid binding to depots before reaching target tissue	Slower onset and reduced effects
Individual differences in amount of binding	Varying effects:
	High binding means less free drug, so some people seem to need higher doses.
	Low binding means more free drug, so these individuals seem more sensitive.
Competition among drugs for depot-binding sites	Higher-than-expected blood levels of the displaced drug, possibly causing greater side effects, even toxicity
Unmetabolized bound drug	Drug remaining in the body for prolonged action
Redistribution of drug to less vascularized tissues and inactive sites	Termination of drug action

for unexpected side effects. Many psychoactive drugs, including the antidepressant fluoxetine (Prozac) and the tranquilizer diazepam (Valium), show extensive (more than 90% of the drug molecules) plasma protein binding and may contribute to drug interactions in some cases.

Third, bound drug molecules cannot be altered by liver enzymes, because the drug is not free to leave the blood to enter liver cells for metabolism. For this reason, depot binding frequently prolongs the time that the drug remains in the body. This phenomenon explains why some drugs, such as THC, which is stored in fat and is only slowly released, can be detected in urine for many days after a single dose. Such slow release means that an individual could test positive for urinary THC (one active ingredient in marijuana) without experiencing cognitive effects at that time. The prolonged presence of drugs in body fat and inert depots makes pre-employment and student drug testing possible.

Finally, as mentioned previously, redistribution of a drug from highly vascularized organs (e.g., brain) to tissues with less blood flow will reduce drug concentrations in those organs. The redistribution occurs more rapidly for highly lipid-soluble drugs that reach the brain very quickly but also redistribute readily because of the ease of movement through membranes. Those drugs have a rapid onset but short duration of action.

Biotransformation and elimination of drugs contribute to bioavailability

Drugs are eliminated from the body through the combined action of several mechanisms, including biotransformation (metabolism) of the drug and excretion of metabolites that have been formed. Drug clearance reduces blood levels and in large part determines the intensity and duration of drug effects. The easiest way to assess the rate of elimination consists of intravenously administering a drug to establish a peak plasma drug level, then collecting repeated blood samples. The decline in plasma drug concentration provides a direct measure of the clearance rate without complication by absorption kinetics.

DRUG CLEARANCE Drug clearance from the blood usually occurs exponentially and is referred to as **first-order kinetics**. Exponential elimination means that a constant *fraction* (50%) of free drug in the blood is removed during each time interval. The exponential function occurs because very few clearance sites are occupied, so the rate is concentration dependent. Hence when blood levels are high, clearance occurs more rapidly, and as blood levels drop, the rate of clearance is reduced. The amount of time required for removal of 50% of the drug in blood is called the **half-life**, or $t_{½}$. **FIGURE 1.9** provides an example of half-life determination for the stimulant dextroamphetamine (Dexedrine), a drug used to treat attention deficit hyperactivity disorder. Although this drug is essentially eliminated after six half-lives (6 × 10 hours), many psychoactive drugs have half-lives of several days, so clearance may take weeks after even a single dose. A list of the half-lives of some common drugs is provided in **TABLE 1.5**. Keep in mind that clearance

TABLE 1.5 Half-lives of Some Common Drugs

Drug	Trade/street name	Half-life
Cocaine	Coke, big C, snow	0.5–1.5 hours
Morphine	Morphine	1.5–2 hours
Nicotine	Tobacco	2 hours
Methylphenidate	Ritalin	2.5–3.5 hours
THC	Marijuana	20–30 hours
Acetylsalicylic acid	Aspirin	3–4 hours
Ibuprofen	Advil	3–4 hours
Naproxen	Aleve	12 hours
Sertraline	Zoloft	2–3 days
Fluoxetine	Prozac	7–9 days

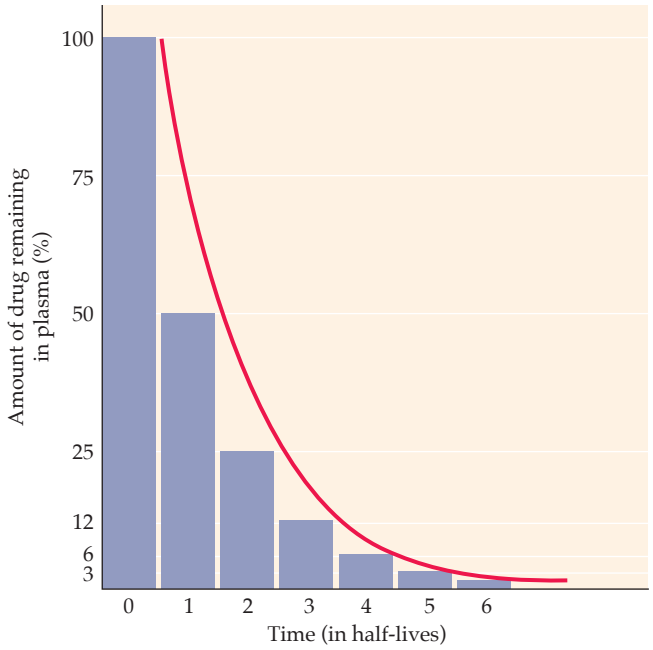

FIGURE 1.9 First-order kinetics of drug clearance Exponential elimination of drug from the blood occurs when clearance during a fixed time interval is always 50% of the drug remaining in blood. For example, the half-life of orally administered dextroamphetamine (Dexedrine) is approximately 10 hours. Therefore, 10 hours (1 half-life) after the peak plasma concentration has been reached, the drug concentration is reduced to about 50% of its initial value. After 20 and 30 hours (i.e., two and three half-lives) have elapsed, the concentration is reduced to 25% and 12.5%, respectively. After six half-lives, the drug is essentially eliminated, with 1.6% remaining. The curve representing the rate of clearance is steeper early on, when the rate is more rapid, and becomes more shallow as the rate of clearance decreases.

from the blood is also dependent on biotransformation rate as well as depot binding and storage in reservoirs such as fat.

Half-life is an important characteristic of a drug because it determines the time interval between doses. For example, because about 88% of the drug is eliminated in three half-lives, a drug given once a day should ideally have a half-life of 8 hours. A shorter half-life would mean that effective blood levels would not be maintained over 24 hours with once-a-day dosing, and this would diminish the effectiveness of the drug. Such a drug would require multiple administrations in one day or use of a sustained-release product. A half-life significantly longer than 8 hours would lead to drug accumulation because there would be drug remaining in the body when the next dose was taken. Drug accumulation increases the potential for side effects and toxicity.

The principal goal of any drug regimen is to maintain the plasma concentration of the drug at a constant desired level for a therapeutic period. The therapeutic window is the range of plasma drug levels that are high enough to be effective, but not so high that they cause toxic effects. However, the target therapeutic concentration is achieved only after multiple administrations. For instance, as **FIGURE 1.10** shows, a predictable fluctuation in blood level occurs over time as a result of the dynamic balance between absorption and clearance. After oral administration at time A, the plasma level of a drug gradually increases to its peak (peak 1) followed by a decrease because of drug biotransformation, elimination, or storage at inactive sites. If first-order kinetics is assumed, after one half-life (time B), the plasma drug level has fallen to one-half its peak value. Half-life determines the time needed to reach the **steady state plasma level**, which is the desired blood concentration of drug achieved when the absorption/distribution phase is equal to the metabolism/excretion phase. For any given daily dose of a drug, the steady state plasma level is approached after a period of time equal to five half-lives (time C). Hence, for a given dosing interval, the shorter the half-life of a drug, the more rapidly the therapeutic level of the drug will be achieved. Drugs with longer half-lives will take longer to reach the desired blood levels. For example, if we needed the blood level of drug X with a half-life of 4 hours to be 1000 mg, we might administer 500 mg at the outset. After 4 hours, the blood level would have dropped to 250 mg, at which time we could administer another 500 mg, raising the blood level to 750 mg. Four hours later, another 500 mg could be added to the current blood level of 375 mg, bringing the new value to 875 mg, and so forth. The

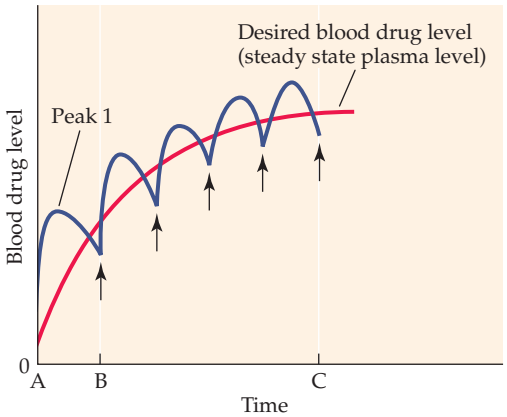

FIGURE 1.10 Achieving steady state plasma levels of drug The scalloped line shows the pattern of accumulation during repeated administration of a drug. The arrows represent the times of administration. The shape of the scallop is dependent on both the rate of absorption and the rate of elimination. The smooth line represents drug accumulation in the blood during continuous intravenous infusion of the same drug.

amount of drug would continue to rise until a maximum of 1000 mg was reached because more drug was given than was metabolized. However, as we reached the steady state level after approximately five half-lives, the amount administered would approximate the amount metabolized (500 mg).

Although most drugs are cleared from the blood by first-order kinetics, under certain conditions some drugs are eliminated according to the zero-order model. **Zero-order kinetics** means that drug molecules are cleared at a constant rate regardless of drug concentration; this is graphically represented as a straight line (**FIGURE 1.11**). It happens when drug levels are high and routes of metabolism or elimination are saturated (i.e., more drug molecules are available than sites). A classic example of a drug that is eliminated by zero-order kinetics is high-dose ethyl alcohol. When two or more drinks of alcohol are consumed in a relatively short time, alcohol molecules saturate the enzyme-binding sites (i.e., more alcohol molecules than enzyme-binding sites are present), and metabolism occurs at its maximum rate of approximately 10 to 15 ml/hour, or 1.0 ounce of 100-proof alcohol per hour regardless of concentration. The rate here is determined by the number of enzyme molecules. Any alcohol consumption that occurs after saturation of the enzyme will raise blood levels dramatically and will produce intoxication. Although zero-order biotransformation occurs at high levels of alcohol, the biotransformation rate shifts to first-order kinetics as blood levels are reduced (see Figure 1.11).

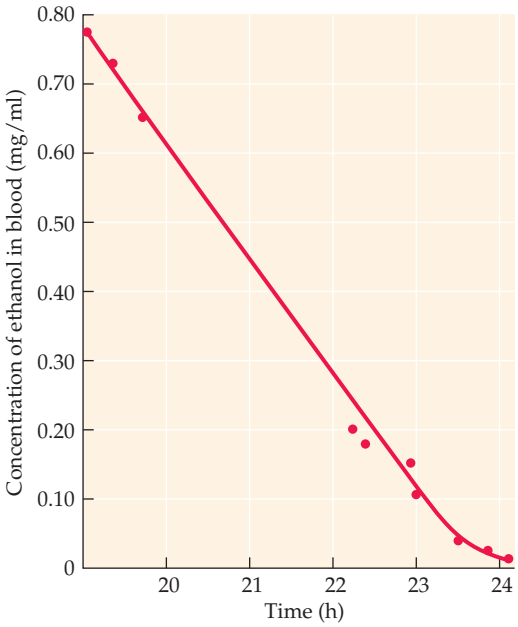

FIGURE 1.11 Zero-order rate of elimination The curve shows the decline of ethanol content in blood after intravenous administration of a large dose to laboratory animals. The *x*-axis represents the time beginning 19 hours after administration. Plotted data show the change from zero-order to first-order kinetics when low concentrations are reached between 23 and 24 hours after administration. (After Marshall, 1953.)

BIOTRANSFORMATION BY LIVER MICROSOMAL ENZYMES Most drugs are chemically altered by the body before they are excreted. These chemical changes are catalyzed by enzymes and can occur in many tissues and organs, including the stomach, intestine, blood plasma, kidney, and brain. However, the greatest number of chemical changes, which we call *drug metabolism* or biotransformation, occur in the liver.

There are two major types of biotransformation. Type I biotransformations are sometimes called phase I because these reactions often occur before a second metabolic step. Phase I changes involve *nonsynthetic* modification of the drug molecule by oxidation, reduction, or hydrolysis. Oxidation is by far the most common reaction; it usually produces a metabolite that is less lipid soluble and less active, but it may produce a metabolite with equal or even greater activity than the parent drug. Type II, or phase II, modifications are *synthetic* reactions that require the combination (called *conjugation*) of the drug with some small molecule such as glucuronide, sulfate, or methyl groups. Glucuronide conjugation is particularly important for inactivating psychoactive drugs. These metabolic products are less lipid soluble because they are highly ionized and are almost always biologically inactive. In summary, the two phases of drug biotransformation ultimately produce one or more inactive metabolites, which are water soluble, so they can be excreted more readily than the parent drug. Metabolites formed in the liver are returned to the circulation and are subsequently filtered out by the kidneys, or they may be excreted into bile and eliminated with the feces. Metabolites that are active return to the circulation and may have additional action on target tissues before they are further metabolized into inactive products. Obviously, drugs that are converted into active metabolites have a prolonged duration of action. **TABLE 1.6** shows several examples of the varied effects of phase I and phase II metabolism. The sedative drug phenobarbital is rapidly inactivated by phase I metabolism. In contrast, aspirin is converted at first to an active metabolite by phase I metabolism, but phase II action produces an inactive compound. Morphine does not undergo phase I metabolism but is inactivated by phase II reactions. Finally, diazepam (Valium), a long-lasting antianxiety drug, has several active metabolites before phase II inactivation. Further, as mentioned previously, some drugs are inactive until they are metabolized. For example, the inactive drug codeine is metabolized in the body to the active drug morphine, making codeine a prodrug.

TABLE **1.6** Varied Effects of Phase I and Phase II Metabolism

Active drug	Active metabolites and inactive metabolites[a]
Phenobarbital	—Phase I→ Hydroxy-phenobarbital
Aspirin	—Phase I→ **Salicylic acid** —Phase II→ Salicylic-glucuronide
Morphine	—Phase II→ Morphine-6-glucuronide
Diazepam	—Phase I→ **Desmethyldiazepam** —Phase I→ **Oxazepam** —Phase II→ Oxazepam-glucuronide

[a]Bold terms indicate active metabolites.

The liver enzymes primarily responsible for metabolizing psychoactive drugs are located on the smooth endoplasmic reticulum, which is a network of tubules within the liver cell cytoplasm. These enzymes are often called **microsomal enzymes** because they exhibit particular characteristics on biochemical analysis. Microsomal enzymes lack strict specificity and can metabolize a wide variety of xenobiotics (i.e., chemicals that are foreign to the living organism), including toxins ingested with food, environmental pollutants, and carcinogens, as well as drugs. Among the most important liver microsomal enzymes is the **cytochrome P450** (**CYP450**) enzyme family. Members of this class of enzyme, which number more than 50, are responsible for oxidizing most psychoactive drugs, including antidepressants, morphine, and amphetamines. Although they are primarily found in the liver, cytochrome enzymes are also located in the intestine, kidney, lungs, and nasal passages, where they alter foreign molecules. Enzymes are classified into families and subfamilies by their amino acid sequences, as well as by the genes encoding them, and they are designated by a number-letter-number sequence such as 2D6. Among the cytochrome enzymes that are particularly important for psychotropic drug metabolism are CYP450 1A2, 3A4, 2D6, and several in the 2C subfamily.

FACTORS INFLUENCING DRUG METABOLISM The enzymes of the liver are of particular interest to psychopharmacologists because several factors significantly influence the rate of biotransformation. These factors alter the magnitude and duration of drug effects and are responsible for significant drug interactions. These drug interactions can either increase bioavailability, causing adverse effects, or reduce blood levels, which may reduce drug effectiveness. Additionally, variations in the rate of metabolism explain many of the individual differences seen in response to drugs. Factors that modify biotransformation capacity include the following: (1) enzyme induction; (2) enzyme inhibition; (3) drug competition; and (4) individual differences in age, gender, and genetics.

Many psychoactive drugs, when used repeatedly, cause an increase in a particular liver enzyme (called **enzyme induction**). Increased numbers of enzyme molecules not only cause the drugs to speed up their own rate of biotransformation two- to threefold, but also can increase the rate of metabolism of all other drugs modified by the same enzyme. For example, repeated use of the antiseizure drug carbamazepine (Tegretol) increases the number of CYP450 3A4 enzyme molecules, leading to more rapid metabolism of carbamazepine and many other drugs, producing a lower blood level and a reduced biological effect. Among the drugs metabolized by the same enzyme are oral contraceptives. For this reason, if carbamazepine is prescribed to a woman who is taking oral contraceptives, she will need an increased hormone dose or an alternative means of birth control (Zajecka, 1993). When drug use is terminated, there is a gradual return to normal levels of metabolism.

Another common example is cigarette smoke, which increases CYP450 1A2 enzymes. People who are heavy smokers may need higher doses of drugs such as antidepressants and caffeine that are metabolized by the same enzyme. Such changes in drug metabolism and elimination explain in part why some drugs lose their effectiveness with repeated use—a phenomenon known as *tolerance* (see the discussion on tolerance later in the chapter); these changes also cause a reduced effect of other drugs that are metabolized by the same enzyme (cross-tolerance). Clearly, drug-taking history can have a major impact on the effectiveness of the drugs that an individual currently takes.

In contrast to drug-induced induction of liver enzymes, some drugs directly inhibit the action of enzymes (**enzyme inhibition**); this reduces the metabolism of other drugs taken at the same time that are metabolized by the same enzyme. In such cases, one would experience a much more intense or prolonged drug effect and increased potential for toxicity. Monoamine oxidase inhibitors (MAOIs), used to treat depression, act in the brain by preventing the destruction of certain neurotransmitters by the enzyme monoamine oxidase (MAO). The same enzyme is found in the liver, where it normally metabolizes amines such as tyramine, which is

found in red wine, beer, some cheeses, and other foods. When individuals who are taking these antidepressants eat foods rich in tyramine, dangerous high blood pressure and cardiac arrhythmias can occur, making normal foods potentially life threatening. Further detail on this side effect of MAOIs is provided in Chapter 18.

In addition, because MAOIs are not specific for MAO, they have the potential to cause adverse effects unrelated to MAO function. They inhibit several microsomal enzymes of the cytochrome P450 family, producing elevated blood levels of many drugs and potentially causing increased side effects or unexpected toxicity.

A second drug–food interaction involves the ingestion of grapefruit juice, which significantly inhibits the biotransformation of many drugs metabolized by CYP450 3A4, including numerous psychiatric medications. A single glass (5 ounces) of grapefruit juice elevates the blood levels of those drugs significantly by inhibiting their first-pass metabolism. The effect is caused by chemicals in grapefruit that are not found in oranges, such as bergamottin. Inhibition persists for 24 hours and dissipates gradually after several days, but it can be a hazard for those taking medications daily, because it causes significant drug accumulation.

A second type of inhibition, based on **drug competition** for the enzyme, occurs for drugs that share a metabolic system. Because the number of enzyme molecules is limited, an elevated concentration of either drug reduces the metabolic rate of the second, causing potentially toxic levels. Cytochrome P450 metabolism of alcohol leads to higher-than-normal brain levels of other sedative–hypnotics, for example, barbiturates or Valium, when administered at the same time, producing a potentially dangerous drug interaction.

Finally, differences in drug metabolism due to genetic and environmental factors can explain why some individuals seem to be extremely sensitive to certain drugs, but others may need much higher doses than normal to achieve an effect. Over 40 years ago, the first **genetic polymorphisms** (genetic variations among individuals that produce multiple forms of a given protein) for drug-metabolizing enzymes were identified. Large variations, for instance, were found in the rate of acetylation of isoniazid, a drug used to treat tuberculosis and subsequently found to relieve depression. Acetylation is a conjugation reaction in which an acetyl group is attached to the drug. These genetic polymorphisms that determine acetylation rate vary across populations. For instance 44% to 54% of American Caucasians and African Americans, 60% of Europeans, 10% of Asians, and only 5% of Eskimos are slow inactivators (Levine, 1973).

The enzymes that have been studied most are in the CYP450 family, and each has multiple polymorphisms. In that family, CYP2D6 (i.e., CYP450 2D6) is of great interest because it is responsible for metabolizing numerous psychotropic drugs, including many antidepressants, antipsychotics, antihistamines, muscle relaxants, opioid analgesics, and others. In a recent study, swabs of epithelial cells from the inside cheek linings of 31,563 individuals were taken and analyzed for the number of copies of the gene for CYP2D6. **FIGURE 1.12A** shows the distribution of samples based on the number (zero, one, two, or three or more) of normal

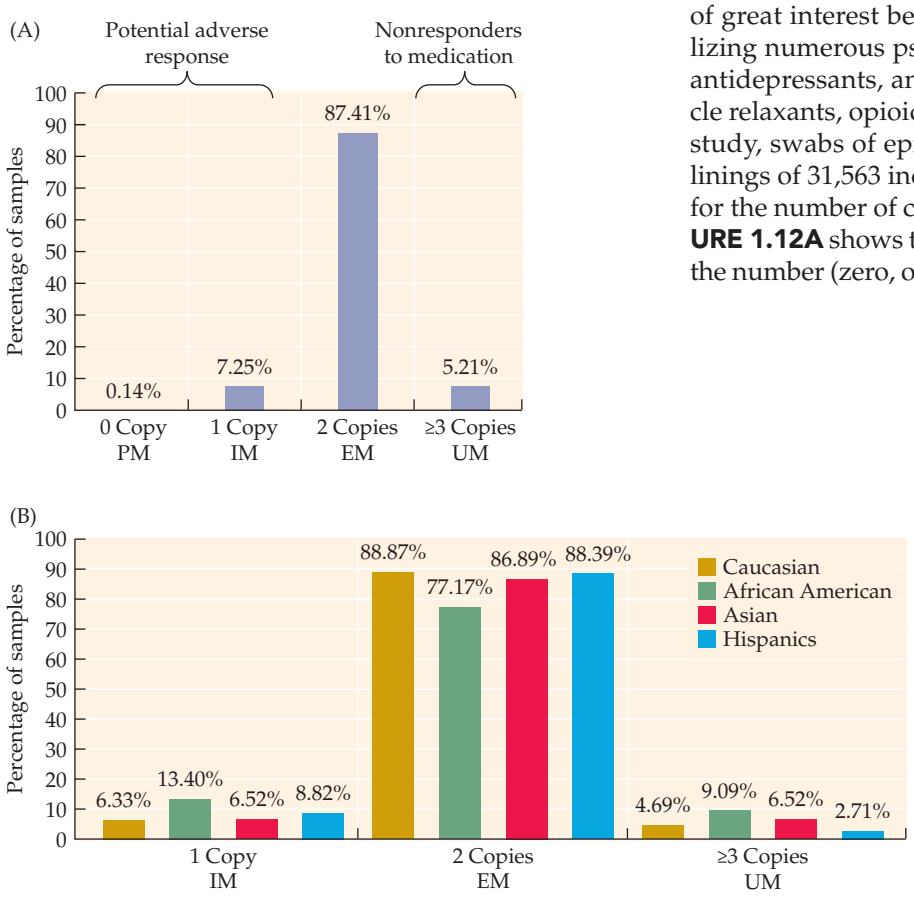

FIGURE 1.12 Four genetic populations based on the number of normal CYP2D6 genes (A) Percentage of samples containing zero, one, two, and three or more copies of the normal CYP2D6 gene from 31,563 individuals. PM, poor metabolizers; IM, intermediary metabolizers; EM, extensive metabolizers; UM, ultrarapid metabolizers. (B) Percentage of samples containing one, two, and three or more copies of the normal CYP2D6 gene in self-reported ethnic groups: Caucasians (yellow), African Americans (green), Asians (red), Hispanics (blue). (After Beoris et al., 2016.)

CYP2D6 genes (Beoris et al., 2016). A small percentage of individuals (0.14%) are very poor metabolizers (PM) and have multiple copies of a polymorphism that is ineffective in metabolizing substrates for the CYP2D6 enzyme. Intermediary metabolizers (IM) are 7.25% of the population tested and have one deficient allele and one normal allele. These two clusters of individuals having poorer metabolism would be expected to have greater bioavailability of those drugs, which may be responsible for adverse drug reactions or toxicity. These individuals would benefit from a reduction in drug dosage. Approximately 87% of the individuals are extensive metabolizers (EM) who have two normal alleles. They are considered extensive metabolizers because the normal enzyme is highly functional and efficient. The fourth group (5.21% of the population tested) are ultrarapid metabolizers (UM) and have multiple (three or more) normal gene copies. The UM group would be expected to have significantly lower blood levels of drug than normal, which may make them nonresponders to the medication. Hence these individuals would benefit from higher drug dosage. Such differences are significant because there may be as much as a 1000-fold difference in rate of metabolism for a particular drug among these individuals. In addition, the data showed there are different distributions of these genotypes in different populations. **FIGURE 1.12B** shows the data for one or more copies of the normal gene (the samples with zero copies are not shown) broken down by self-reported ethnicity (about two-thirds of the individuals provided data on ethnicity). The data show that the frequency of individuals with two copies of CYP2D6 was significantly lower in African Americans than in the other ethnic groups and that the percentage of individuals with one copy was 1.5 to 2.1 times higher in that group. Additionally, the percentage of individuals with three or more copies among African American was 1.4 to 3.4 times higher. These differences indicate greater variation in CYP2D6 metabolism in African Americans, which puts some at greater risk for adverse side effects and others at risk for inadequate response to psychotropic medications. Further discussion of this topic can be found at the end of the chapter in the section on pharmacogenetics and personalized medicine.

Other enzymes also show wide genetic differences. For example, approximately 50% of certain Asian groups (Chinese, Japanese, and Koreans) have reduced capacity to metabolize acetaldehyde, which is an intermediary metabolic step in the breakdown of alcohol. The resulting elevation in acetaldehyde causes facial flushing, tachycardia, a drop in blood pressure, and sometimes nausea and vomiting. The reduced metabolic capacity is caused by a specific mutation in the gene for aldehyde dehydrogenase (Wall and Ehelers, 1995).

Along with variations in genes, other individual differences may influence metabolism. Significant changes in nutrition or in liver function, which accompany various diseases, lead to significantly higher drug blood levels and prolonged and exaggerated effects. Advanced age is often accompanied by a reduced ability to metabolize drugs, while children under age 2 also have insufficient metabolic capacity and are vulnerable to drug overdose. In addition, both the young and the elderly have reduced kidney function, so clearance of drugs for them is much slower. Gender differences in drug metabolism also exist. For example, the stomach enzymes that metabolize alcohol before it reaches the bloodstream are far less effective in women than in men. This means that for an identical dose, a woman will have a much higher concentration of alcohol reaching her blood to produce biological effects. If you would like to read more about some of the clinical concerns related to differences in drug metabolism, see Applegate (1999).

RENAL EXCRETION Although drugs can be excreted from the body in the breath, sweat, saliva, feces, or breast milk, the most important route of elimination is the urine. Therefore, the primary organ of elimination is the kidney. The kidneys are a pair of organs, each about the size of a fist. They are responsible for filtering materials out of the blood and excreting waste products. As filtered materials pass through the kidney tubules, necessary substances such as water, glucose, sodium, potassium, and chloride are reabsorbed into the blood.

Most drugs are readily filtered by the kidney unless they are bound to plasma proteins or are of large molecular size. However, because reabsorption of water from the tubules makes the drug concentration greater in the tubules than in the surrounding blood vessels, many drug molecules are reabsorbed back into the blood. Ionization of drugs reduces reabsorption because it makes the drugs less lipid soluble. Liver biotransformation of drugs into ionized (water-soluble) molecules traps the metabolites in the kidney tubules, so they can be excreted along with waste products in the urine.

Reabsorption from the tubules, similar to diffusion across other membranes (discussed earlier), is pH-dependent. When tubular urine is made more alkaline, weak acids are excreted more rapidly because they become more ionized and are not reabsorbed as well; that is, they are "trapped" in the tubular urine. If the urine is acidic, the weakly acidic drug will be less ionized and more easily reabsorbed; thus excretion will be less. The opposite is true for a weakly basic drug, which will be excreted more readily when tubular urine is acidic rather than basic. This principle of altering urinary pH is frequently used in the treatment of drug toxicity, when it is highly desirable to remove the offending drug from the body as quickly as possible. In the case of

phenobarbital poisoning, for example, kidney excretion of this weakly acidic substance is greatly enhanced by alkalinization of the urine with sodium bicarbonate. This treatment leads to ionization and trapping of the drug within the tubules, from whence it is readily excreted. Acidifying the urine by administering intravenous ammonium chloride increases the percentage of ionization of weakly basic drugs, which enhances their excretion. For example, acidifying the urine increases the rate of excretion of amphetamine and shortens the duration of a toxic overdose episode.

Therapeutic Drug Monitoring

For a drug to be clinically effective while producing minimal side effects, optimum blood levels and hence drug concentration at the target site must be maintained throughout the treatment period. The difficulties in determining the appropriate drug dosage for initial clinical trials with humans and for veterinary medical treatment based on preclinical laboratory animal testing are described in **BOX 1.2**. Optimum blood levels must be determined in clinical trials before Food and Drug Administration (FDA) approval. However, wide variation in rates of absorption, metabolism, and elimination among individuals because of differences in gender, age, genetic profile, disease state, and drug interactions can lead to significant differences in blood levels. Blood levels that are too low prevent desired clinical outcomes, and for individuals with higher-than-normal blood levels, unwanted side effects and toxicity may occur. In the future, pharmacogenetic screening of individuals (see the last section of this chapter) will allow personalized prescription of drug doses, but presently, the appropriate dosage of a drug is determined most often by the clinical response of a given individual. Under some conditions, such as for drugs with serious side effects, multiple blood samples are taken after drug administration to determine plasma levels of drug (**therapeutic drug monitoring**). Short-term blood sampling may be done to establish the optimum dosage for a patient taking a new medication. After each dosage correction by the physician, it may take some time to reach steady state (approximately five half-lives), so monitoring may continue for several days or weeks, until the optimum dosage has been determined. For drugs that must be taken over the life span, periodic monitoring may be

BOX 1.2 Pharmacology in Action

Interspecies Drug Dose Extrapolation

Interspecies drug dose extrapolating is converting or scaling the appropriate dose in one species to another species. It is vital not only in veterinary medicine, but also in drug development when the initial clinical trials with humans are based on preclinical testing in laboratory animals. It is also significant in the laboratory in order to replicate results from one species in another. The goal of dose extrapolation is to find the optimum dose for effectiveness and safety. See Sharma and McNeill (2009) for a full discussion.

Comparing the sizes of the animals is the most obvious example of scaling a drug dose to a different species, but it is not the only factor. Using size alone can have disastrous consequences, as shown by the tragic outcome in Tusko, a 14-year-old Asiatic elephant housed in the Lincoln Park Zoo in Oklahoma City (West et al., 1962). The researchers used only the difference in weight to scale the dose for the elephant from previous experiments with cats. If the elephant's sensitivity to the hallucinogenic lysergic acid diethylamide (LSD) resembled that of humans, the 297 mg given to the elephant would have been an enormous overdose. Almost immediately after the drug was injected into the rump of the 3 1/2-ton elephant with a dart rifle, the elephant stormed around his pen appearing uncoordinated before he collapsed, defecated, and had continuous seizures. His tongue turned blue and he struggled to breath, dying shortly later of

(*Continued*)

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BOX 1.2 Pharmacology in Action (*continued*)

strangulation. There were efforts to save him, but the drugs used may have instead contributed to his death. One must conclude that the elephant's sensitivity to LSD more closely resembled that of humans than of felines.

There are many pharmacokinetic and pharmacodynamic factors that are unrelated to size yet vary significantly among species. For example, protein binding at silent depots varies greatly among species because of differences in affinity to and number of binding sites. Hence for any drug that shows high depot binding in one species, using weight in determining the appropriate dose would not be effective, and correction for the extent of protein binding would increase accuracy. Complex interspecies differences in drug metabolism are also key factors in differences in bioavailability of a given drug. For example, there are major variations among species in the CYP450 enzyme family amino acid sequences. The varied enzyme structures determine which drug substrates are acted upon by the enzyme. Another example of varied metabolism is conjugation with glucuronide, which is important and efficient in humans, while cats lack glucuronidation. Rats are very efficient acetylators, but dogs lack acetylation, while humans are intermediate between the two. It is clear that these differences will lead to variations in the blood levels of drug in different species.

In addition to differences in weight and pharmacokinetic factors, drug targets also contribute to variations in interspecies response to administered drugs. CNS neurotransmitters are differentially distributed in the brains of various species, and differences in the number, affinity, distribution, and regulation of receptors likely explain dramatic differences in drug response. For example, opiate analgesics such as morphine cause CNS depression in primates, dogs, and rats, while they induce excitation in horses and cats. These pharmacodynamic factors are not related to the size of the animal, so to adequately convert the drug dose, one should have some understanding of the receptor characteristics of each species.

performed regularly. Monitoring detects changes in pharmacokinetics due to aging, hormonal changes during pregnancy and menopause, stress, changes in medical condition, or addition of new medications. Many of the monitored psychotropic drugs such as antiepileptic drugs, including carbamazepine, some antidepressants, and drugs used as mood stabilizers such as lithium and valproic acid, are taken on a long-term basis.

Therapeutic drug monitoring is especially important for drugs that have a narrow therapeutic index (i.e., the dose needed for effectiveness is very similar to the dose that causes serious side effects; see the section on pharmacodynamics). Because blood levels rise to a peak and then fall to a trough just before the next administration (see Figure 1.10), drug monitoring can ensure that the peak remains below the blood level associated with toxic effects, while the trough remains in the therapeutic range to maintain adequate symptom relief. Modifying the dosage for a given individual can optimize treatment. In addition, drug monitoring can be used to determine whether the individual is taking the drug according to the prescribed regimen. Failure to comply with the drug treatment protocol often can be corrected by further patient education. The American Association for Clinical Chemistry (2011) provides additional information on therapeutic drug monitoring at www.labtestsonline.org/understanding/analytes/therapeutic_drug/glance.html.

Section Summary

- A placebo is an inert substance that produces effective therapeutic response and side effects. Placebo response depends on the ritual of the therapeutic treatment, which has neurobiological and behavioral effects. Multiple explanations for the effectiveness of placebos include Pavlovian conditioning and expectation of outcome.
- The effects of a drug are determined by (1) how much of the drug reaches its target sites, where it has biological action, and (2) how quickly the drug reaches those sites.
- Interacting pharmacokinetic factors that determine how much free drug is in the blood (bioavailability) include method of administration, rate of absorption and distribution, binding at inactive sites, biotransformation, and excretion.
- The route of administration determines both onset and duration of drug action.
- The method of administration influences absorption of the drug because it determines the area of the absorbing surface, the number of cell layers through which the drug must pass, and the extent of first-pass metabolism.
- Oral and rectal routes of administration are enteral because they involve the gastrointestinal tract; all other methods are called parenteral.

- Each method of administration provides distinct advantages and disadvantages involving rate of onset, blood level of drug achieved, duration of action, convenience, safety, and special uses.
- Absorption is dependent on method of administration, solubility and ionization of the drug, and individual differences in age, sex, and body size.
- Lipid-soluble drugs are not ionized and readily pass through fatty membranes at a rate dependent on the concentration gradient.
- Drugs that are weak acids tend to remain nonionized (lipid soluble) in acidic body fluids such as stomach juices; they are more readily absorbed there than in the more alkaline intestinal fluid. Drugs that are weak bases are more ionized in the acidic stomach fluid, so they are absorbed less readily there than from the more basic intestine, where ionization is reduced.
- Drug distribution is determined by the volume of blood flow to the tissue, but drug concentration in the CNS is limited by the blood–brain barrier that is formed by specialized brain capillaries that have few pores to allow drugs to leave the circulation. Only lipid-soluble drugs readily enter the brain.
- The placental barrier separates maternal circulation from fetal circulation, but it does not impede passage of most drug molecules.
- Drug molecules bound to inactive depots, including plasma proteins, cannot act at target sites, nor can they be metabolized, so the magnitude, onset, and duration of drug action are affected.
- Drug clearance from the blood usually occurs by first-order kinetics such that a constant fraction (50%) of the free drug is removed during each time interval. This interval is called the drug half-life. Some drugs are cleared according to zero-order kinetics, in which clearance occurs at a constant rate regardless of drug concentration.
- The steady state plasma level is the desired blood concentration of drug achieved when the absorption/distribution phase is equal to the metabolism/excretion phase. The steady state plasma level is approached after a period of time equal to five half-lives.
- Enzymatic drug metabolism or biotransformation occurs primarily in the liver and produces chemical changes in the drug molecule that make it inactive and more water soluble. The cytochrome P450 family of enzymes consists of the most important type of liver enzyme for transforming psychotropic drugs.
- Drug metabolism occurs in two steps. Phase I consists of oxidation, reduction, or hydrolysis and produces an ionized metabolite that may be inactive, as active as, or more active than the parent drug. Phase II metabolism involves conjugation of the drug with a simple molecule provided by the body, such as glucuronide or sulfate. Products of phase II metabolism are always inactive and are more water soluble.
- The kidney is most often responsible for filtration of metabolites from the blood before excretion in the urine. Alternatively, metabolites may be excreted into bile and eliminated with the feces.
- Factors that change the biotransformation rate may alter the magnitude and duration of drug effects or cause drug interactions, and they may explain variability in individual response to drugs. Chronic use of some drugs can induce (increase) the quantity of liver enzymes, thereby decreasing bioavailability. Drugs that inhibit liver enzymes increase the blood levels of a drug, enhancing its action. Competition among drugs for metabolism by the same enzyme increases blood levels of one or both drugs. Genetic differences and individual differences in age, sex, nutrition, and organ function may influence the rate of drug metabolism.
- Therapeutic drug monitoring involves taking multiple blood samples to directly measure plasma levels of drug after a drug has been administered. Monitoring is done to identify the optimum dosage for a patient to maximize therapeutic potential and minimize side effects. It is especially important for drugs with serious side effects and when there are changes in an individual's pharmacokinetics due to aging, hormonal changes, stress, the addition of new medications, or other events.

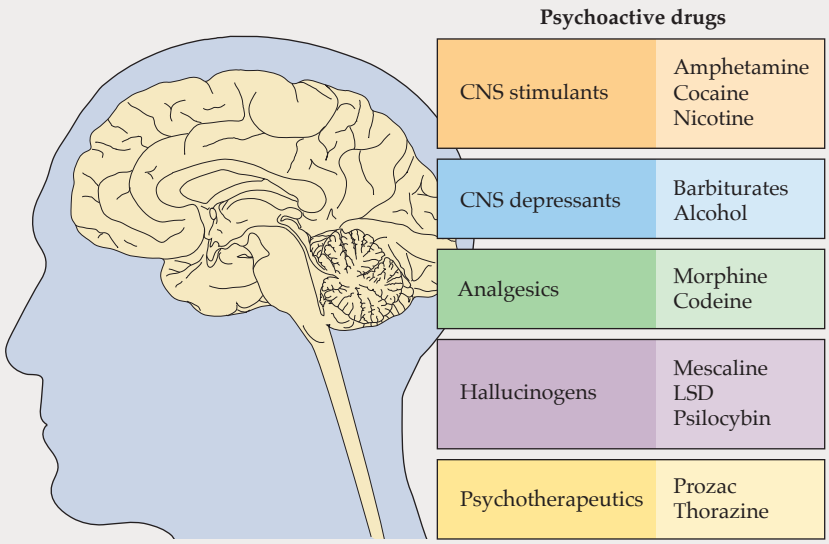
Pharmacodynamics: Drug–Receptor Interactions

Pharmacodynamics is the study of the physiological and biochemical interaction of drug molecules with target tissue that is responsible for the ultimate effects of a drug. Drugs can be classified into a wide variety of categories (**BOX 1.3**), but all the drugs that we are concerned with affect cell function in target tissue by acting on specific molecular targets, including enzymes, transporter proteins, receptors, and others. Knowing which targets a drug acts on and where these targets are located is crucial for understanding what actions and side effects will be produced.

Receptors can be more selectively defined as large protein molecules located on the cell surface or within

BOX 1.3 Pharmacology in Action

Drug Categories



As we learned earlier in this chapter, all drugs have multiple effects, which vary with dose and bioavailability, the nature of the receptors occupied, and the drug-taking history (e.g., tolerance) of the individual. For these reasons, drugs can be categorized in any of several classes, depending on the trait of interest. One might classify drugs according to chemical structure, medical use, legal status, neurochemical effects, abuse potential, behavioral effects, and many other categories. Amphetamine may be described as a CNS stimulant (based on increased brain activity and behavioral arousal), an anorectic used for diet control (medical use), a sympathomimetic (because it neurochemically mimics the effects of the sympathetic nervous system), or a Schedule III drug (a controlled substance based on the federal government's assessment of abuse potential). Because we are particularly interested in brain function and behavior, the classification used in this text emphasizes CNS action and behavioral effects.

CNS stimulants produce increased electrical activity in the brain and behavioral arousal, alertness, and a sense of well-being in the individual. Among drugs in this class are amphetamine, cocaine, and methylphenidate (Ritalin), as well as the methylxanthines, which include caffeine, theophylline, and theobromine. Nicotine may also be included here because of its activating effect on CNS neurons, although behaviorally for some individuals, the drug clearly has a calming effect. Classification is complicated by the fact that drug effects are dose-dependent, and drugs occasionally produce dramatically different effects at different doses. Low and moderate doses of amphetamine, for example, stimulate physical activity, but at high doses, locomotion may be reduced and replaced by meaningless stereotyped, repetitive acts that have clear psychotic characteristics.

CNS depressants include a variety of drugs that depress CNS function and behavior to cause a sense of relaxation and drowsiness. Some of the sedative–hypnotics are useful for these sedating qualities and for their ability to relieve anxiety or induce sleep. At high doses, more profound mental clouding occurs, along with loss of coordination, intoxication, and coma. Significant drugs in this group include the barbiturates (such as Seconal), the benzodiazepines (including Valium), and ethyl alcohol, all of which will be considered in later chapters. Some might include marijuana in this class because of its relaxing and depressant qualities at low doses, although at higher doses, hallucinogenic characteristics may occur prominently.

Analgesics are drugs that frequently have CNS-depressant qualities, although their principal effect is to reduce the perception of pain. The most important drugs in this class are the narcotics. Narcotics, or opiates, such as morphine or codeine are derived from the opium poppy; the synthetic narcotics (sometimes called *opioids*) include heroin, meperidine (Demerol), methadone, and fentanyl. All opiate-like drugs produce relaxation and sleep, as well as analgesia. Under some circumstances, these drugs also produce a powerful sense of euphoria and a desire to continue drug administration. Non-narcotic analgesics, of course, also belong in this class but have little effect on behavior and do not produce relaxation or sleep. They include aspirin, acetaminophen (Tylenol), and ibuprofen (Motrin).

Hallucinogens, or mind-altering drugs, are often called "psychedelics" because their primary effect is to alter one's perceptions, leading to vivid visual illusions or distortions of objects and body image. As a group, these drugs produce a wide variety of effects on brain chemistry and neural activity. They include many naturally occurring substances such as mescaline and psilocybin. Certainly, LSD belongs in this class, as does MDMA (street name: "ecstasy"). The drug PCP (street name: "angel dust") and its analog ketamine

BOX 1.3 Pharmacology in Action (*continued*)

(street name: "special K"), which is used as an animal sedative, might belong in the class of CNS depressants, but their ability to cause profound hallucinogenic experiences and their use as models for psychotic behavior prompt their placement in this category.

Psychotherapeutic drugs as a classification is intended to suggest that some psychoactive drugs are used almost entirely to treat clinical disorders of mood or behavior: antipsychotics, antidepressants, and mood stabilizers. These drugs have distinctly different mechanisms of action and are rarely used outside the therapeutic realm. Antipsychotics reduce symptoms of schizophrenia, including hallucinations and bizarre behavior. Examples include haloperidol (Haldol) and chlorpromazine (Thorazine). Antidepressants also belong in this classification; they are used to treat disorders of mood. Among the most familiar are duloxetine (Cymbalta), sertraline (Zoloft), and fluoxetine (Prozac). Although drugs in this class do reverse the symptoms of clinical depression, they do not produce the effects of CNS stimulants, nor do they produce euphoria. Finally, mood stabilizers reduce the dramatic mood swings between mania and depression that characterize bipolar disorder. Lithium carbonate (Lithonate) is still most often prescribed, but valproate (Depakote) and carbamazepine (Tegretol) are increasingly popular. Each of these types of drugs will be described in subsequent chapters of this text.

Clearly, many of the drugs you may be interested in have not been mentioned: hormones such as the anabolic steroids and contraceptives, inhalants including household products and glues, and others. Many of these would require special categories for classification, but this text addresses some of those topics in Chapter 16.

cells that are the initial sites of action of biologically active agents such as neurotransmitters, hormones, and drugs (all referred to as ligands). A **ligand** is defined as any molecule that binds to a receptor with some selectivity. Because most drugs do not readily pass into neurons, neuropharmacology most often is interested in receptors found on the cell surface that relay information through the membrane to affect intracellular processes (**FIGURE 1.13A**). Which of the many possible intracellular changes occurs depends on whether the receptor is coupled with an ion channel to alter the membrane potential or with a G protein to produce longer-lasting changes such as activation of an enzyme (see Chapter 3). The essential goal of neuropharmacology is to identify drugs that can act at neurotransmitter receptors to enhance or reduce normal functioning of the cell and bring about a clinically useful effect.

The second type of receptor is found within the target cell, either in the cytoplasm (e.g., glucocorticoids) or in the nucleus (e.g., sex steroid receptors). Most of the hormones that act on the brain to influence neural events use this type of receptor. Hormonal binding to intracellular receptors alters cell function by triggering changes in expression of genetic material within

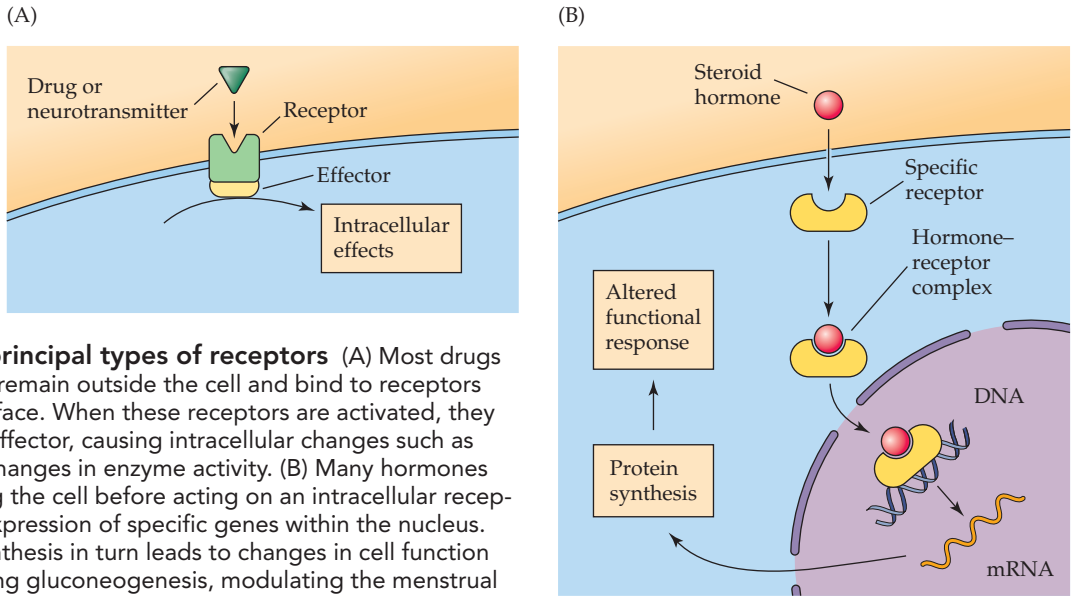


FIGURE 1.13 Two principal types of receptors (A) Most drugs and neurotransmitters remain outside the cell and bind to receptors on the exterior cell surface. When these receptors are activated, they initiate changes in an effector, causing intracellular changes such as movement of ions or changes in enzyme activity. (B) Many hormones are capable of entering the cell before acting on an intracellular receptor that changes the expression of specific genes within the nucleus. The altered protein synthesis in turn leads to changes in cell function that may include altering gluconeogenesis, modulating the menstrual cycle, and others.

the nucleus, producing differences in protein synthesis (**FIGURE 1.13B**). Sex hormones act in this way to facilitate mating behavior and other activities related to reproduction, such as lactation. This mechanism is described more fully in Chapter 3.

Extracellular and intracellular receptors have several common features

Several characteristics are common among receptors in general. The ability to recognize specific molecular shapes is one very important characteristic. The usual analogy of a lock and key suggests that only a limited group of neurochemicals or drugs can bind to a particular receptor protein to initiate a cellular response. These neurochemicals are called **receptor agonists**. Molecules that have the best chemical "fit" (i.e., have the highest **affinity**) attach most readily to the receptor. However, just as one may put a key in a lock but may not be able to turn the key, so too a ligand may be recognized by a receptor, but it may not initiate a biological action. Such ligands are considered to have low **efficacy**. These molecules are called **receptor antagonists** because not only do they produce no cellular effect after binding, but by binding to the receptor, they prevent an "active" ligand from binding; hence they "block" the receptor (**FIGURE 1.14**). **Partial agonists** demonstrate efficacy that is less than that of full agonists but more than that of an antagonist at a given receptor. Finally, when **inverse agonists** bind to a receptor, they initiate a biological action, but it is an action that is opposite to that produced by an agonist. Hence drug action can vary in efficacy along a continuum ranging from full agonist action with maximal efficacy, to partial agonist action to the inactive antagonist, to partial inverse agonist action with some inverse efficacy, to full inverse agonist action that shows maximal inverse efficacy (**FIGURE 1.15**). The importance of these distinctions will become apparent in later chapters as we discuss specific drug classes.

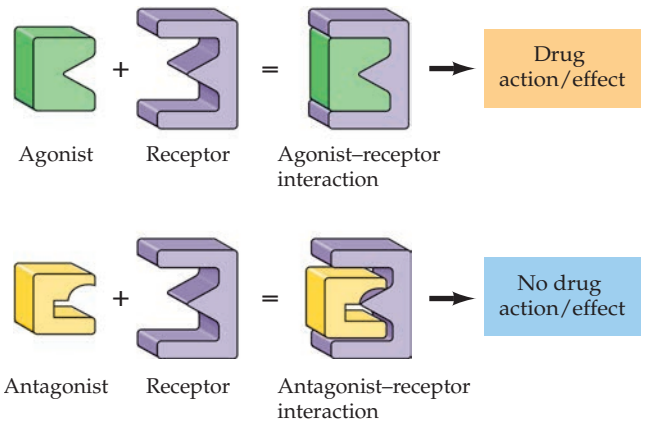


FIGURE 1.14 Agonist and antagonist interactions with receptors The agonist molecule has an excellent fit for the receptor (high affinity) and produces a significant biological response (high efficacy). The antagonist in this case fits less well and has very low efficacy. Note that if both the agonist and the antagonist are present simultaneously, they will compete to fit into the same receptor, producing a partial drug effect. (After Carroll, 1996.)

We should mention here that drugs can show agonist action in ways other than by activating a receptor. In this more general sense, drugs are agonists if they enhance synaptic function by increasing neurotransmitter synthesis or release or by prolonging the action of the neurotransmitter within the synapse. Likewise, drugs that show antagonist action may do so by reducing neurotransmitter synthesis or release or by terminating the action of the neurotransmitter more quickly. These actions will be described more fully in Chapter 3.

A second significant feature of receptors is that binding or attachment of the specific ligand is temporary. When the ligand dissociates (i.e., separates) from the receptor, it has opportunities to attach once again.

Third, ligands binding to the receptor produce a physical change in the three-dimensional shape of the protein, thus initiating a series of intracellular events that ultimately generate a biobehavioral effect. How much intracellular activity occurs depends on the number of interactions with the receptor and the ability of the ligand to alter the shape of the receptor, which reflects its efficacy.

Fourth, although we tend to think about receptors as a permanent characteristic of cells, these proteins in fact have a life cycle, just as other cell proteins do. Not only is there a normal life span for receptors, but receptors are modified both in number (long-term regulation) and in sensitivity (more rapid regulation via second messengers). Long-term regulation, called **up-regulation** when receptor numbers increase and called **down-regulation** when receptors are reduced in number, reflects compensatory changes after prolonged absence of receptor agonists or chronic activation of the receptor, respectively. This phenomenon was initially observed in muscle, where it was found that if the nerve serving a particular muscle was cut (thereby eliminating release of the neurotransmitter from the nerve endings), a compensatory increase in neurotransmitter receptors occurred over the muscle surface. The same phenomenon has been found to occur in the CNS, not only when nerves are severed, but also when nerve activity is chronically reduced by drugs. For instance, chronic use of receptor antagonists leads to subsequent up-regulation of receptors. Likewise, drugs that activate a nerve pathway or that act as agonists at the receptor cause a reduction in receptor proteins if they are administered repeatedly. In each case, the change in receptor number requires

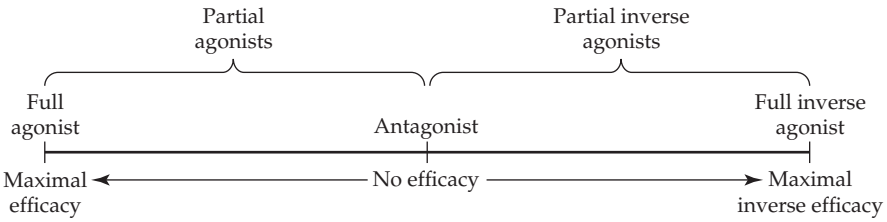


FIGURE 1.15 Continuum of drug efficacy Independent of affinity, drugs can vary in efficacy along a continuum from full agonist action having maximum efficacy to antagonist action with no efficacy to full inverse agonist efficacy. Partial agonists and partial inverse agonists fall in between.

1 to 2 weeks of altered activity. Change in sensitivity due to second messenger–induced function is far more rapid. These changes will be discussed more fully in Chapter 3.

Finally, we have already learned that once drugs are absorbed, they are distributed throughout the body, where multiple sites of action (receptors) mediate different biobehavioral effects. However, the receptor proteins for a given drug or neurotransmitter may have different characteristics in different target tissues. These varied receptors, called **receptor subtypes**, will be discussed more extensively later in the book. The goal of neuropharmacology is to design drugs that bind with greater affinity to one receptor subtype so as to initiate a highly selective therapeutic effect, without acting on related receptor subtypes and producing side effects. For instance, **FIGURE 1.16** shows the three mildly stimulant drugs caffeine, theophylline, and theobromine, all of which belong to the xanthine class and have noticeably similar structures. However, subtle differences in structure are responsible for differences in the magnitude of biological effects, depending on which xanthine receptor subtypes they bind to most effectively. You can see in **TABLE 1.7** that caffeine is more effective at the xanthine receptor subtype in the CNS to produce alertness than are xanthines found in tea (theophylline) or cocoa (theobromine). In contrast, theophylline is the most active of the three in stimulating the heart and causing increased urine output (diuresis).

Dose–response curves describe receptor activity

One important method used to evaluate receptor activity is the **dose–response curve**, which describes the extent of biological or behavioral effect (mean response in a population) produced by a given drug concentration (dose). A typical curve is shown in **FIGURE 1.17**. When plotted on a semilog scale, the curve takes on a classic S-shape. At low doses, the drug-induced effect is slight because very few receptors are occupied. In fact, the threshold dose is the smallest dose that produces a measurable effect. As the dose of the drug is increased, more receptors are activated, and a greater biological response occurs. The ED_{50} (50% effective dose) is the dose that produces half the maximal effect, and maximum response occurs at a dose at which we assume the receptors are fully occupied[1] (we might call it the ED_{100}).

If we were to graph the effects of several pain-relieving drugs, we might find a relationship similar to the one shown in **FIGURE 1.18**. The first three curves show the dose–response characteristics for hydromorphone, morphine, and codeine—all drugs from the opiate analgesic class. For each drug, increasing the concentration produces greater analgesia (elevation in pain

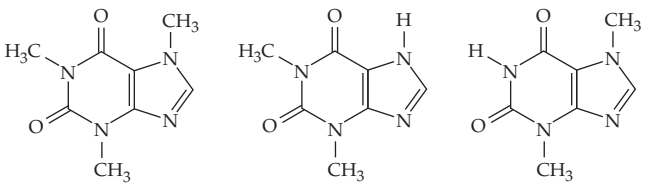


FIGURE 1.16 Chemical structures of the xanthines caffeine, theophylline, and theobromine.

[1]This assumption is not warranted in all cases, however, as can be seen in those models of receptor pharmacology that describe "spare receptors." Readers interested in the complexities of receptor occupancy theory should refer to a standard textbook in pharmacology.

TABLE 1.7 Relative Biological Activity[a] of Xanthines[b]

Biological effects	Caffeine	Theophylline	Theobromine
CNS stimulation	1	2	3
Cardiac stimulation	3	1	2
Respiratory stimulation	1	2	3
Skeletal muscle stimulation	1	2	3
Diuresis	3	1	2

Source: Ritchie, 1975.

[a] Each drug acts more effectively on some xanthine receptor subtypes than others. 1 = most active; 3 = least active.

[b] Derivatives of xanthine are a group of alkaloids used as mild stimulants and bronchodilators.

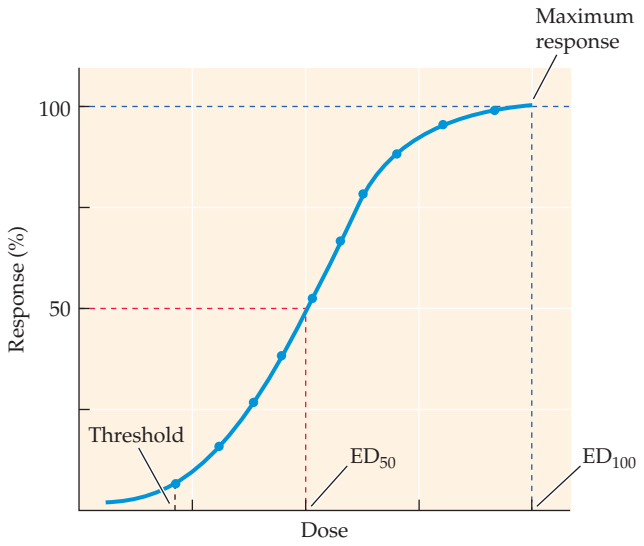


FIGURE 1.17 Dose–response curve The classic S-shape describes the gradual increase in biological response that occurs with increasing doses of a drug (drug–receptor activation). Threshold is the dose that produces the smallest measurable response. The dose at which the maximum response is achieved is the ED_{100} (100% effective dose), and the ED_{50} is the dose that effectively produces 50% of the maximum response.

threshold) until the maximum response is achieved. The absolute amount of drug necessary to produce a specific effect indicates the **potency** of the drug. Differences in potency among these three drugs can be seen by comparing the ED_{50} across all of them. Hydromorphone requires approximately 2 mg, morphine needs 10 mg to achieve the same effect, and codeine needs more than 100 mg. Therefore, morphine is more potent than codeine, and hydromorphone is more potent than either. The relative position of the curves on the *x*-axis indicates potency and reflects the affinity of each drug for the receptor that mediates the measured response. Although the three differ in affinity for the receptor, each reaches the same maximum on the *y*-axis, indicating that they have identical efficacy. The fact that the linear portions of the curves are parallel to one another indicates that they are working by the same mechanism. Although the concept of potency provides some means of comparison, its practical use is limited. As you can see, a lower-potency drug is frequently just as effective and requires only a somewhat higher dose. If the low-potency drug also produces fewer side effects or is less expensive, then it may in fact be the preferred drug. You might consider these issues the next time a drug advertisement makes claims for being the most potent of its kind available.

Figure 1.18 also shows the dose–response curve for aspirin. In contrast to the first three drugs, aspirin is not an opioid, and the distinctive shape of its dose–response curve indicates that although aspirin may relieve pain, it does not act on the same receptors or work by the same mechanism. In addition, regardless of how much aspirin is administered, it never achieves the same efficacy as the opiates.

The therapeutic index calculates drug safety

A second type of dose–response curve does not look at the mean response to a range of drug doses in a group of recipients but instead looks at the cumulative percentage of the population experiencing a particular drug-induced response. This type of curve is used to calculate drug safety. Among the multiple responses to any drug, some are undesirable or even dangerous side effects that need to be evaluated carefully in a therapeutic situation. For example, **FIGURE 1.19** depicts three distinct pharmacological effects produced by drug A, which is prescribed to reduce anxiety. The blue curve shows the percentage of individuals who experience reduced anxiety at various doses of the drug. You can see that even at low doses, some subjects will show reduced anxiety. At somewhat higher doses, the same individuals will respond to the drug and other subjects will begin to respond as well. Ultimately there will be a dose to which 100% of the subjects respond. The purple curve shows the percentage of persons suffering respiratory depression (a toxic effect) from various doses of the same drug. By comparing the ED_{50} for relieving anxiety (i.e., the dose at which 50% of the population

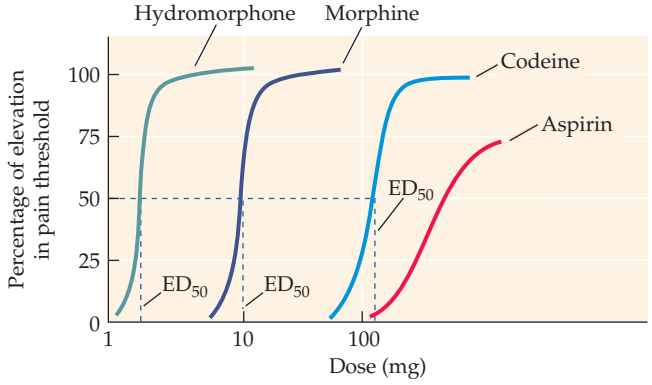


FIGURE 1.18 Dose–response curves for four analgesic agents Each curve represents the increase in pain threshold (the magnitude of painful stimulus required to elicit a withdrawal response) as a function of dose. Knowing the ED_{50} doses for hydromorphone, morphine, and codeine help in comparisons of potency. The linear portions of the curves for the opiate analgesics are parallel, suggesting that they work through the same mechanism. Aspirin is not an opiate and relieves pain by a very different mechanism of action, so the shape of the curve is distinct. In addition, the maximum effectiveness of aspirin never reaches the level of that of the opiates. (After Levine, 1973.)

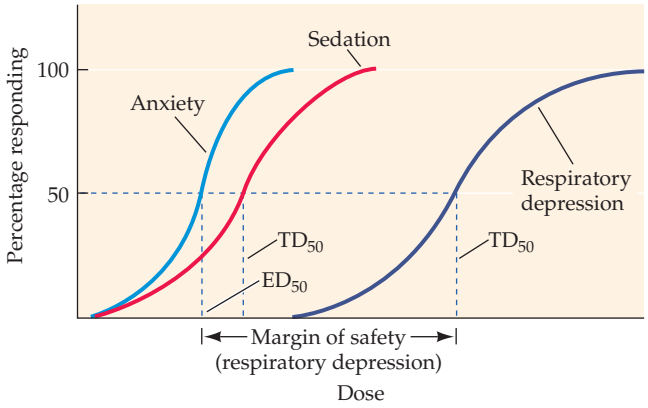


FIGURE 1.19 Comparison of ED_{50} and TD_{50}
The therapeutic index (TI) is calculated by comparing the dose of drug required to produce a toxic effect in 50% of individuals (TD_{50}) with the dose that is effective for 50% (ED_{50}). Each drug may have several therapeutic indices based on its toxic effects or side effects of concern. Because the optimum condition is to have a drug that is effective at a low dose with no toxicity except at very high doses, the TI will be large for a safer drug. The figure shows a large margin of safety for an antianxiety drug that can produce respiratory depression.

shows reduced anxiety) and the TD_{50} (the dose at which 50% of the population experiences a particular toxic effect) for respiratory depression, you can see that for most individuals, the toxic dose is much higher than the dose that produces the desired effect. An alternative interpretation is that at the dose needed to provide significant clinical relief for many patients (50%), none of the patients would be likely to experience respiratory depression. Therefore, pharmacologists would say that the drug has a relatively favorable **therapeutic index** ($TI = TD_{50}/ED_{50}$). In contrast, the dose of drug A that produces sedation and mental clouding (red curve) is not very different from the ED_{50}. This small difference means that the probability is high that a dose that is effective in reducing anxiety is likely to also produce significant mental clouding and sedation, which may represent serious side effects for many people who might use the drug. Another index is the certain safety factor index, and it is defined as the dose of drug that is lethal to 1% of the population compared with the dose that is therapeutically effective in 99% of the population (LD_1/ED_{99}). This index is not used very often, because significant ethical limitations do not allow the lethal doses in humans to be readily determined.

Receptor antagonists compete with agonists for binding sites

We have already introduced the concept of receptor antagonists as those drugs that compete with agonists to bind to receptors but fail to initiate an intracellular effect, thereby reducing the effects of the agonists. These are called **competitive antagonists**. As the name implies, they can be displaced from those sites by an excess of the agonist, because an increased concentration of active drug can compete more effectively for the fixed number of receptors. A simple example will clarify. If we assume that the agonist and the antagonist have similar affinities for the receptor, then if both 100 molecules of drug and 100 molecules of antagonist were present at the receptor, the probability of an agonist acting on the receptor would be 1 to 1. If agonist molecules were increased to 1000, the odds of agonist binding would rise to 10 to 1; at 1,000,000 agonist molecules, the odds would favor agonist binding by 10,000 to 1. Certainly at this point, the presence of the antagonist is of no consequence for the biobehavioral effect as measured.

FIGURE 1.20A illustrates the effect of a competitive antagonist, naloxone, on the analgesic effect of morphine. The blue line shows a typical dose–response curve for the analgesic effect of morphine. When participants were pretreated with naloxone, the dose–response curve shifted to the right (red line), demonstrating that for any given dose of morphine, naloxone-pretreated subjects showed less analgesia. The addition of naloxone diminished the potency of morphine. The figure also shows that the inhibitory action of naloxone was overcome by increasing the amount of morphine administered; that is, the same maximum effect (analgesia) was achieved, but more morphine was required. If you look at the chemical structures of the two drugs in Figure 11.3, you will see the striking similarity and will understand how the two drugs compete to be recognized by the same receptor protein.

We have emphasized receptor antagonism because, in combination with the concept of dose dependency, it is a vital tool in pharmacology. If we want to know whether a specific ligand–receptor interaction is responsible for a particular biological effect, the biological effect must be shown to occur in proportion to the amount of ligand present (dose) and, furthermore, the effect must be reduced in the presence of a competitive antagonist.

Of course, other types of antagonism can occur. **Noncompetitive antagonists** are drugs that reduce the effects of agonists in ways other than competing for the receptor. For example, a noncompetitive antagonist may impair agonist action by binding to a portion of the receptor other than the agonist-binding site, by disturbing the cell membrane supporting the receptor, or by interfering with the intracellular processes that were initiated by the agonist–receptor association. **FIGURE 1.20B** illustrates the effect of a noncompetitive antagonist on the analgesic effect of morphine. In general, the shape of the dose–response curve will be distorted, and the same maximum effect is not likely to be reached.

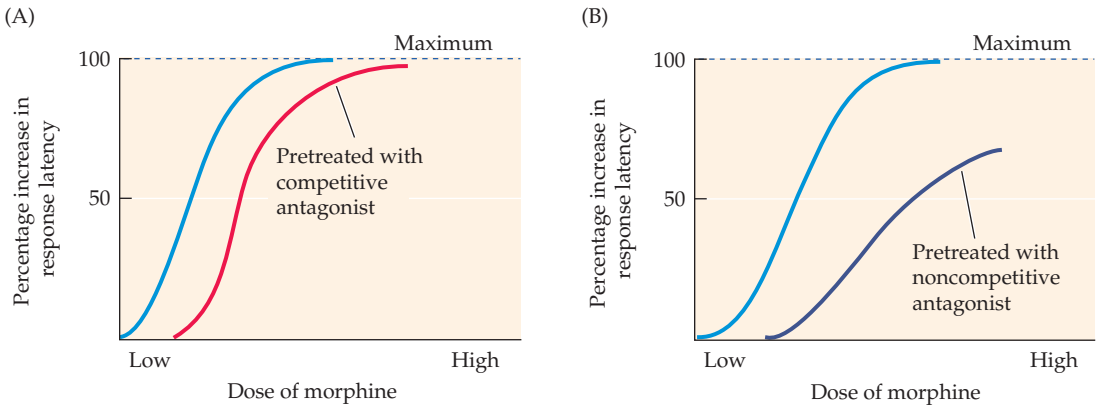
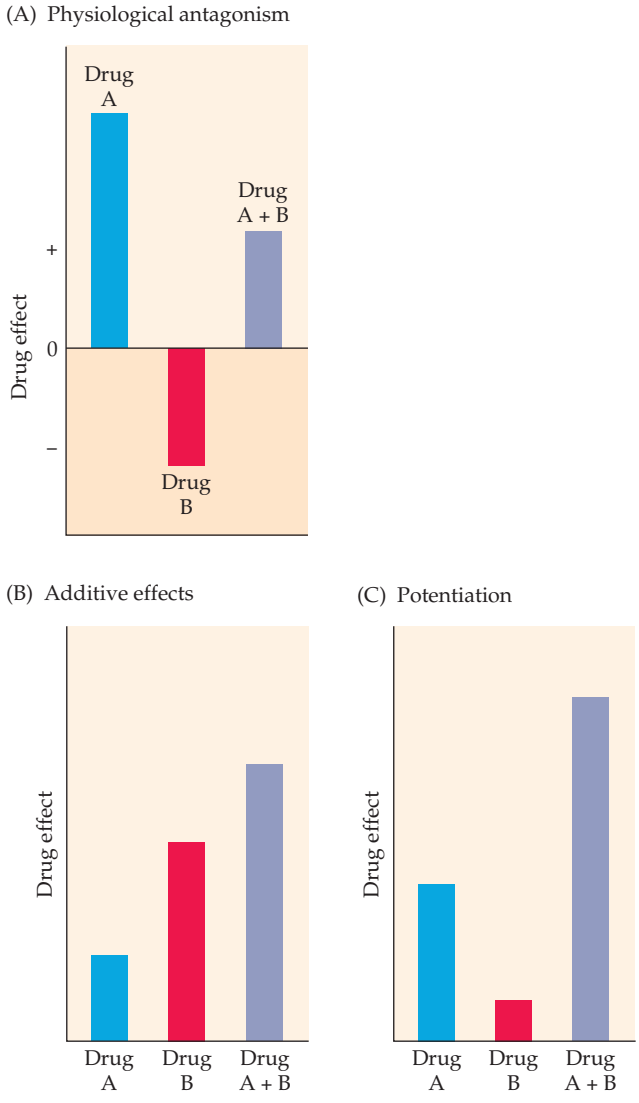


FIGURE 1.20 Drug antagonism (A) The effect of a competitive antagonist (naloxone) on the analgesic effect of morphine. The addition of a competitive antagonist essentially reduces the potency of the agonist, as is shown by the parallel shift of the dose–response curve to the right. (B) In contrast, adding a noncompetitive antagonist usually produces a distinct change in the shape of the dose–response curve, indicating that it does not act at the same receptor site. Also, regardless of the increase in morphine, the maximum efficacy is never reached.



Furthermore, although pharmacologists are concerned with molecular actions at the receptor, biobehavioral interactions can result in several different possible outcomes. **Physiological antagonism** (**FIGURE 1.21A**) involves two drugs that act in two distinct ways but interact in such a way that they reduce each other's effectiveness in the body. For example, one drug may act on receptors in the heart to increase heart rate, and the second may act on distinct receptors in the brainstem to slow heart rate. Clearly, two agents may have **additive effects** if the outcome exceeds the effect of either drug alone (**FIGURE 1.21B**). Finally, **potentiation** refers to the situation in which the combination of two drugs produces effects that are greater than the sum of their individual effects (**FIGURE 1.21C**). Potentiation often involves issues of pharmacokinetics such as altered metabolic rate or competition for depot binding, which may elevate free drug blood levels in unexpected ways.

Biobehavioral Effects of Chronic Drug Use

Many prescription and over-the-counter drugs are taken on a regular basis for chronic medical or psychiatric conditions. These drugs are taken for periods of weeks, months, or even years. Recreational drugs are most often used repeatedly rather than on only a single occasion. When a drug is used on several occasions (i.e.,

FIGURE 1.21 Possible results of the interaction of two drugs (A) Physiological antagonism results when two drugs produce opposite effects and reduce each other's effectiveness. (B) Additive effects are seen when the combined drug effect equals the sum of each drug alone. (C) Potentiation is said to occur when combined drug effects are greater than the sum of individual drug effects.

chronically administered), changes in the magnitude of response to the drug frequently occur. Most often, the response diminishes with chronic use (tolerance), but occasionally, the effects are increased (sensitization). In some cases, selected effects of a particular drug decrease while others increase in magnitude, as is true for the stimulant drug amphetamine. It should be clear that an individual's drug-taking history has a significant influence on drug action.

Repeated drug exposure can cause tolerance

Drug **tolerance** is defined as a diminished response to drug administration after repeated exposure to that drug. In other words, tolerance has developed when increasingly larger doses of a given drug must be administered to obtain the same magnitude of biological effect that occurred with the original dose. Development of tolerance to one drug can diminish the effectiveness of a second drug. This phenomenon, called **cross-tolerance**, serves as the basis for a number of drug interactions. For example, the effective anticonvulsant dose of phenobarbital is significantly larger in a patient who has a history of chronic alcohol use than in a patient who has not developed tolerance to alcohol.

CHARACTERISTICS OF TOLERANCE Although the appearance of tolerance varies, several general features are worth mentioning. These characteristics are summarized in **TABLE 1.8**. First, as is true for biological processes in general, tolerance is reversible; that is, it gradually diminishes if use of the drug is stopped. Additionally, the extent of tolerance that develops is dependent on the pattern of drug administration, that is, the dose and frequency of drug use, as well as the environment in which it occurs. Chronic heroin users may take as much as 1800 mg without ill effects, despite the fact that the lethal range for a novice heroin user is 200 to 400 mg.

However, regardless of dose and frequency, some drugs induce tolerance relatively rapidly (e.g., LSD), while others require weeks of chronic use (barbiturates) or never cause significant tolerance (antipsychotics).

TABLE 1.8 Significant Characteristics of Tolerance

It is reversible when drug use stops.
It is dependent on dose and frequency of drug use and the drug-taking environment.
It may occur rapidly, after long periods of chronic use, or never.
Not all effects of a drug show the same degree of tolerance.
Several different mechanisms explain multiple forms of tolerance.

In some cases, tolerance develops during just a single administration, as when an individual experiences significantly greater effects of alcohol as his blood level rises than he experiences several hours later, when his blood level has fallen to the same point. This form of tolerance is called **acute tolerance**.

It is important to be aware that not all biobehavioral effects of a particular drug demonstrate tolerance equally. For example, morphine-induced nausea and vomiting show rapid development of tolerance, but the constipating effects of the drug rarely diminish even after long-term use. Sometimes the uneven development of tolerance is beneficial, as when tolerance develops for the side effects of a drug but not for its therapeutic effects. At other times, the uneven development of tolerance poses a hazard, as when the desired effects of a drug diminish, requiring increased doses, but the lethal or toxic effects do not show tolerance. Chronic barbiturate use is one such example. As more drug is taken to achieve the desired effect, the dose gets increasingly close to the lethal dose that causes respiratory depression.

Finally, several types of tolerance may occur and have distinct mechanisms. Although some drugs never induce tolerance at all, others may cause several types of tolerance, as shown in **TABLE 1.9**. The three principal forms are metabolic tolerance, pharmacodynamic tolerance, and behavioral tolerance. A fourth type, called acute tolerance, was described above with respect to alcohol.

METABOLIC TOLERANCE (DRUG DISPOSITION TOLERANCE) **Drug disposition tolerance**, or **metabolic tolerance**, occurs when repeated use of a drug reduces the amount of that drug that is available at the target tissue. The most common form of drug disposition tolerance occurs when drugs increase their own rate of metabolism. It is clear that many drugs are capable of liver microsomal enzyme induction (see the discussion on biotransformation earlier in this chapter), which results in increased metabolic capacity. A more efficient metabolism reduces the amount of drug available to target tissue and diminishes drug effects. All drugs metabolized by the induced enzyme family will likewise show a reduced effect (cross-tolerance). Drug disposition tolerance requires repeated administration over time for protein synthesis to build new enzyme proteins. Well-documented examples of inducers of liver enzymes such as cytochrome P450 include drugs from many classes, including the sedative phenobarbital, the antibiotic rifampicin, the antiseizure medication phenytoin, cigarette smoke, and anabolic steroids.

PHARMACODYNAMIC TOLERANCE The most dramatic form of tolerance that develops to the central actions of certain drugs cannot be explained on the basis of

TABLE **1.9** Types of Tolerance Exhibited by Selected Drugs

Drug or drug class	Metabolic tolerance	Pharmacodynamic tolerance	Behavioral tolerance
Barbiturates	+	+	+
Alcohol	+	+	+
Morphine	+	+	+
Amphetamine	–	+	+
Cocaine	–	+	+
Caffeine	–	+	?
Nicotine	–	+	?
LSD	–	+	–

Note: + indicates that the drug produces the type of tolerance indicated; – indicates that the drug does not produce the indicated type of tolerance; ? indicates that evidence is mixed regarding whether the drug produces the indicated type of tolerance.

altered metabolism or altered concentration of drug reaching the brain. **Pharmacodynamic tolerance** occurs when changes in nerve cell function compensate for continued presence of the drug. In an earlier section, we described the normal response to chronic receptor activation as receptor down-regulation. Once receptors have down-regulated, a given amount of drug will have fewer receptors to act on and therefore will produce less of a biological effect. Compensatory up-regulation (increased receptor number) occurs in cases in which receptor activation is chronically reduced. Other compensatory cellular adjustments to chronic drug use will be described in later chapters that focus on individual agents such as ethanol, amphetamine, caffeine, and others. These drug-induced compensatory changes also help to explain the **withdrawal syndrome** that occurs when chronic drug users abruptly stop using the drug. Because the adaptive mechanism produces effects opposite to the initial drug effects, when the drug is no longer present, the adaptive mechanism remains functioning and so causes a rebound withdrawal syndrome, overshooting basal levels. For example, alcohol has sedating effects and depresses CNS function, while withdrawal is characterized by a hyperexcitable state. The occurrence of the withdrawal or abstinence syndrome is the hallmark of **physical dependence**, which is defined as a physiological state in which the body adapts to the chronic presence of a drug and elicits a drug-specific withdrawal syndrome if the drug is abruptly stopped or dosage is reduced. Chapter 11 on opioids and Chapter 9 on drug abuse provide a more complete discussion along with examples.

BEHAVIORAL TOLERANCE Although many instances of tolerance can be attributed to cell physiology and chemistry, a behavioral component involving learning and adaptation has been demonstrated by numerous investigators. **Behavioral tolerance** (sometimes called *context-specific tolerance*) is seen when tolerance occurs in the same environment in which the drug was administered, but tolerance is not apparent or is much reduced in a novel environment. Several types of learning (classical conditioning and operant conditioning) may play a part in the development of behavioral tolerance, as well as in the withdrawal syndrome characteristic of physical dependence (see Chapter 9).

Pavlovian conditioning, or **classical conditioning**, plays an important role in drug use and in tolerance. In the original experiments with Pavlov's dogs, the meaningless bell (neutral stimulus) was presented immediately before the meat (unconditioned stimulus) was presented, which elicited reflexive salivation (unconditioned response). After repeated pairings, the bell took on the characteristic of a conditioned stimulus, because when presented alone, it could elicit salivation—now a conditioned response. Hence the bell was a cue that predicted what was about to happen. Since many psychoactive drugs elicit reflexive effects such as cortical arousal, elevated blood pressure, or euphoria, they can act as unconditioned stimuli, and the drug-taking procedure or stimuli in the environment may become conditioned stimuli that elicit a conditioned response even before the drug is administered (**FIGURE 1.22**). These associations with the drug may explain why the various rituals and procedures of drug procurement and use may elicit effects similar to those produced by the drug itself and remind the individual of how the drug feels, causing intense craving. Anticipation of drug effects may also explain behavioral tolerance.

Siegel (1985) suggests that tolerance is due at least in part to the learning of an association between the effects of a given drug and the environmental cues that reliably precede the drug effects. For development of tolerance, the "anticipatory" response (conditioned

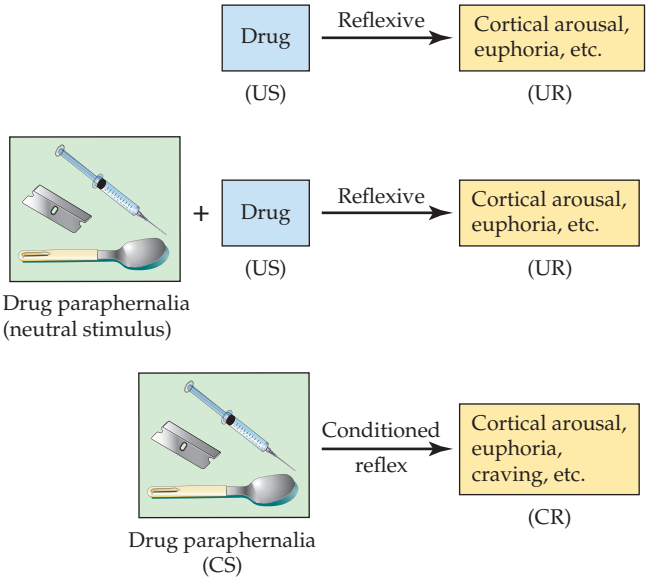


FIGURE 1.22 Classical conditioning of drug-related cues Although drug-taking equipment and the immediate environment initially serve as meaningless stimuli for the individual, their repeated pairing with the drug (unconditioned stimulus; US), which naturally elicits euphoria, arousal, or other desirable effects (unconditioned response; UR), gives the drug-taking equipment new meaning. Ultimately, the equipment and the environment alone (now a conditioned stimulus; CS) could elicit drug effects (conditioned response; CR) in the absence of the drug.

response) must be compensatory in nature; that is, the environment associated with administration of the drug elicits physiological responses *opposite* to the effect of the drug. For instance, in animals repeatedly experiencing the hyperthermic effects of injected morphine, the injection procedure alone *in the same environment* leads to a compensatory drop in body temperature. **FIGURE 1.23** shows the results of a study of two groups of rats given an identical course of morphine injections (5 mg/kg SC for 10 days), which would normally produce evidence of tolerance to the hyperthermic effect. On test day, rats given morphine in the identical environment (the "same" animals) showed the expected decrease in hyperthermia—an indication of tolerance. Animals given morphine in a novel environment (the "different" animals) did not show the same extent of tolerance. The conclusion drawn is that classically conditioned environmental responses contribute to the development of tolerance to morphine. Some researchers believe that environmentally induced tolerance may explain why addicts who use their drug in a new environment or who alter their drug-taking routine may suddenly show much greater response to the same dose of the drug they had used the day before. This phenomenon may explain at least some of the fatal drug overdoses that occur each year. Although environment is clearly significant in drug tolerance, keep in mind that neural changes that underlie learning or behavioral tolerance are subtle alterations in physiology that may be similar to pharmacodynamic tolerance.

The appearance of tolerance to a psychoactive drug is often manifested in a task in which **operant conditioning** plays some part. For example, Leblanc et al. (1976) showed that administration of alcohol (2.5 g/kg IP) to rats initially disrupted the performance of traversing a moving belt, but repeated administrations had less and less effect. The improved performance could be identified as a type of tolerance, but the apparent tolerance could be due to learning of a new skill (the ability to run a treadmill while under the influence of the drug), which would be expected to improve with practice. How can we know that the improvement was not due to changes in metabolic rate or to pharmacodynamic tolerance? The answer can be found by adding a second group of animals that have the same number of alcohol treatments and the same number of practice sessions on the treadmill but receive the drug *after* each practice session. If tolerance in the first group was due to metabolic changes, the extent of tolerance in the two groups should be identical, but in fact, the second group in such trials has shown significantly less tolerance. The same type

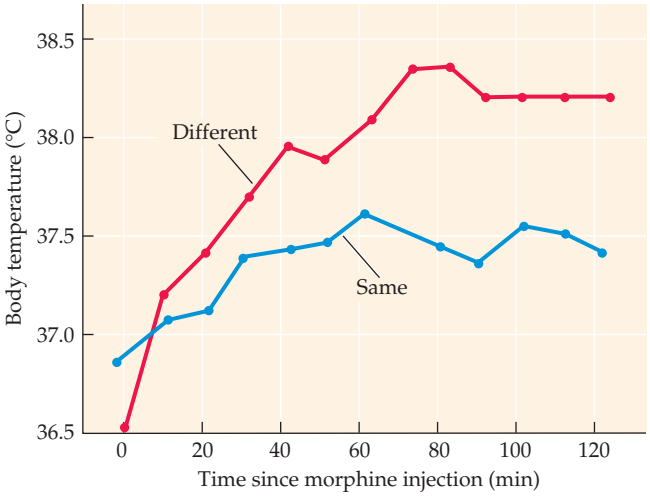


FIGURE 1.23 Tolerance to morphine-induced hyperthermia After an identical series of prior morphine injections (5 mg/kg SC for 10 days), rats were tested with a morphine injection, and changes in body temperature were measured for the next 2 hours. One group of rats was given morphine in the same environment in which they were previously treated ("same"), and the second group was tested in a novel environment ("different"). Animals treated in the same environment show much less hyperthermia; this indicates tolerance. (After Siegel, 1978.)

of tolerance is demonstrated by the individual with alcohol use disorder who learns to maneuver fairly efficiently while highly intoxicated, to avoid detection, whereas a less experienced alcohol abuser with the same blood alcohol level may appear behaviorally to be highly intoxicated.

Vogel-Sprott developed these ideas further by suggesting that if tolerance were due to learning, then reinforcement of a compensatory response to the drug effect should be necessary for tolerance to develop. In a series of experiments, participants given ethyl alcohol were monetarily rewarded when their motor skill task performance while intoxicated matched their scores while sober. Under these conditions, researchers measured rapid development of tolerance through repeated drinking sessions for the participants who were given feedback about their performance (i.e., how their score compared with their nonalcohol score) and a monetary reward; they found minimal or no increase in tolerance to drinking sessions for participants who were given no feedback about performance and for whom reward was unrelated to sober performance (Vogel-Sprott, 1997). Hence, reinforcing the ability to resist the effects of alcohol on performance led to behavioral tolerance. Young and Goudie (1995) is an excellent source for additional details on the role of classical and operant conditioning in the development of tolerance to behavioral effects of drugs.

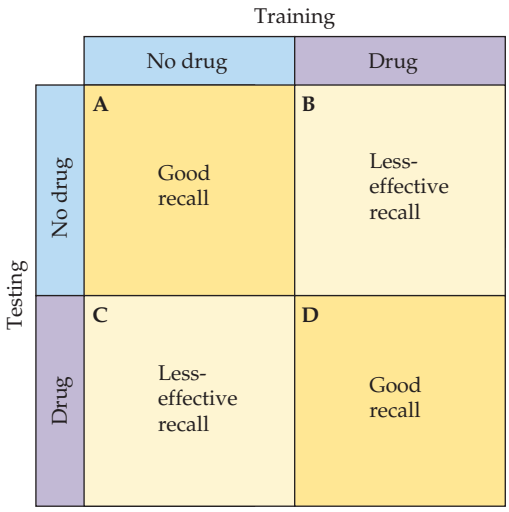


FIGURE 1.24 Experimental design to test state-dependent learning Four different conditions show the difficulties involved in transferring learning from one drug state to another. Individuals trained without drug and tested without drug (A) show maximum performance that is not much different from the performance of those trained and tested under the influence of the drug (D). However, subjects asked to perform in a state different from the training condition (B and C) showed recall that was less efficient.

STATE-DEPENDENT LEARNING **State-dependent learning** is a concept that is closely related to behavioral tolerance. Tasks learned under the influence of a psychoactive drug may subsequently be performed better in the drugged state than in the nondrugged state. Conversely, learning acquired in the nondrugged state may be more available in the nondrugged state. This phenomenon illustrates the difficulty in transferring learned performance from a drugged to a nondrugged condition (**FIGURE 1.24**). An example of this is the alcohol abuser who during a binge hides his supply of liquor for later consumption but is unable to find it while he is sober (in the nondrugged state). Once he has returned to the intoxicated state, he can readily locate his cache.

One explanation for state dependency is that the drug effect may become part of the environmental "set," that is, it may assume the properties of a stimulus itself. A drugged subject learns to perform a particular task in relation to all internal and external cues in the environment, including, it is argued, drug-induced cues. Thus in the absence of drug-induced cues, performance deteriorates in much the same way as it would if the test apparatus were altered. It has been shown in animal studies that the decrease in performance is very much related to the change in environmental cues and that a particular drug state does provide readily discriminable stimuli. A comparison of state-dependent learning with the cuing properties of drugs as discriminative stimuli (see Chapter 4) is provided by Koek (2011).

Chronic drug use can cause sensitization

Despite the fact that repeated drug administration produces tolerance for many effects of a given drug, sensitization can occur for other effects of the same drug. **Sensitization**, sometimes called *reverse tolerance*, is the enhancement of particular drug effects after repeated administration of the same dose of drug. For instance, prior administration of cocaine to animals significantly increases motor activity and stereotypy (continual repetition of a simple action such as head bobbing) produced by subsequent stimulant administration. Chronic administration of higher doses of cocaine has been shown to produce an increased susceptibility to cocaine-induced catalepsy, in which the animal remains in abnormal or distorted postures for prolonged intervals, as well as hyperthermia and convulsions (Post and Weiss, 1988). Cocaine and amphetamine are examples of drugs that induce tolerance for some effects (euphoria) and sensitization for others (see Chapter 12).

As is true for tolerance, the development of sensitization is dose-dependent, and the interval between

treatments is important. Further, cross sensitization with other psychomotor stimulants has been documented. Conditioning also plays a significant role in the appearance of sensitization. Pretreatment with the stimulant followed by testing in dissimilar environments yields significantly less sensitization than occurs in the identical environment. However, in contrast to drug tolerance, the augmentation of response to drug challenge tends to persist for long periods of abstinence, indicating that long-term physiological changes may occur as a result of stimulant administration. Further discussion of sensitization will be provided in Chapter 3.

Pharmacogenetics and Personalized Medicine in Psychiatry

Pharmacogenetics (sometimes called *pharmacogenomics*) is the study of the genetic basis for variability in drug response among individuals. Its goals are to identify genetic factors that confer susceptibility to specific side effects and to predict good or poor therapeutic response. By referring to genetic information on each patient, it should be possible for the clinician to select the most appropriate drug for that individual—the ultimate in personalized medicine.

Variability in response to a given drug among individuals may be explained by heritability of pharmacokinetic or pharmacodynamic factors. Genetic variation in pharmacokinetic factors such as absorption, distribution, and elimination leads to differences in bioavailability after a fixed dose of drug and to subsequent differences in biobehavioral effects. Of the pharmacokinetic factors, pharmacogenetic evaluation of the drug-metabolizing enzymes has been the most studied because variations in metabolic rate produce large differences in drug blood level among individuals and in subsequent clinical effects, as well as side effects. The many genetic polymorphisms (i.e., variations of a gene) of the cytochrome P450 enzyme family are of particular significance in neuropsychopharmacology because those enzymes are responsible for metabolizing most psychoactive drugs. In an earlier section you learned that in the general population there are four CYP2D6 genotypes, based on the number of copies of the functional CYP2D6 gene, that correspond to poor, intermediary, extensive, and ultrarapid metabolizers (see Figure 1.12). Genetic knowledge of an individual's metabolic function can be used to adjust the dose of drug to achieve optimum blood level and subsequent optimum therapeutic response without adverse side effects (Kirchheiner and Seeringer, 2007). An example of this principle can be seen in **FIGURE 1.25**. The top of the figure shows the standard dose of a drug metabolized by CYP450 enzymes and the estimated dosages appropriate for the four genotypes, shown in the middle. The bottom of the figure shows four sets of plasma concentration time courses, one set for each genotype. The blue curves show the plasma levels without dosage correction. The red curves represent the target concentrations with dosage adjusted for genotype. Plasma levels above the dashed line indicate a potential for adverse side effects. Plasma levels below the red curve indicate inadequate drug concentration for effective therapeutics. Hence for individuals who have an identified polymorphism for poor metabolism, a significantly lower dose should be administered to achieve the desired blood level. In those individuals, the standard dose produces blood levels significantly higher than optimum. The

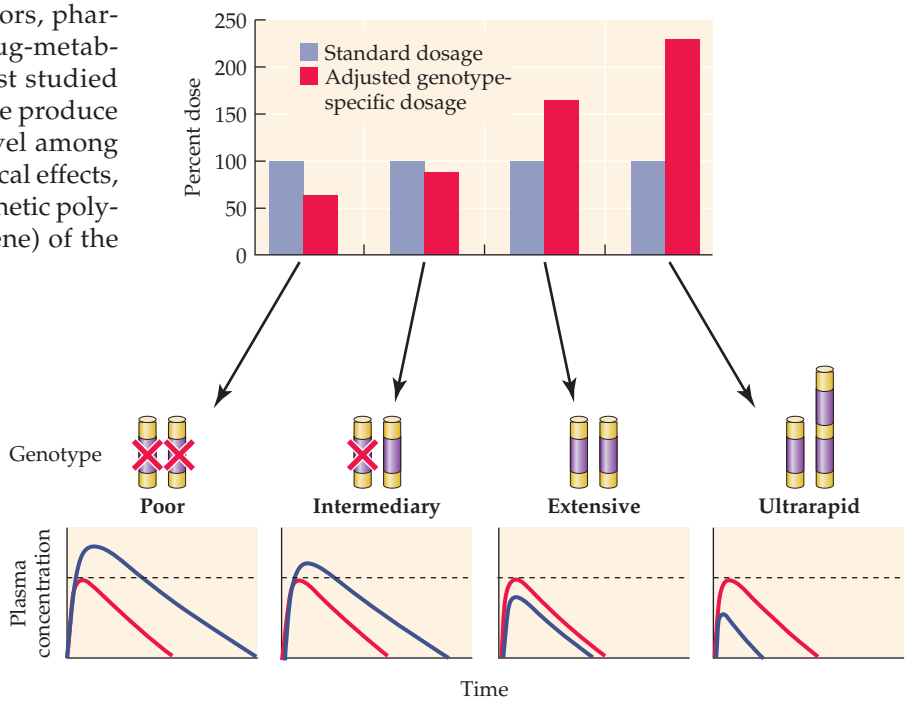


FIGURE 1.25 Drug dosage adjustments based on genotype Compared with standard dosages (purple) theoretical dosages (red) were estimated based on drug-metabolizing genotype in order to achieve equal drug plasma concentration time courses to maximize therapeutic efficacy and avoid unwanted adverse effects. See text for details. (After Kirchheiner and Seeringer, 2007.)

intermediary metabolizers with one normal allele and one inactive polymorphism would also be administered a slightly reduced dosage to optimize plasma concentration. In contrast, individuals with two highly functional genes (extensive metabolizers) should be administered a somewhat higher than normal dosage to ensure therapeutic effectiveness. Finally, the ultrarapid metabolizers having additional high-functioning polymorphisms would need a still higher dosage to reach adequate plasma concentrations for effective treatment. Hence knowing the genetic variation in metabolizing enzymes in individuals helps to predict the bioavailability of a given drug so that dosage modifications can produce optimum therapeutic concentrations while avoiding adverse drug events.

Knowing the inherited level of function of a drug-metabolizing enzyme is clearly useful in determining the correct dose of a drug for a given individual, but it is important to recall that multiple genetic factors, demographic factors (e.g., gender, ethnicity, and age), environmental factors including diet, cigarette smoking, and concomitant use of multiple medications also contribute to the rate of drug metabolism (Arranz and Kapur, 2008). Hence genetic profile explains only a portion of the variance in drug blood levels and in clinical outcomes.

Genetic polymorphisms may also influence pharmacodynamic drug targets, that is, sites of action of the agent. These sites include receptors, transporters, and other membrane proteins, as well as downstream intracellular signaling cascades. Pharmacogenomic studies of drug response typically examine the association between drug effects and polymorphisms in genes suspected to have a role in the mechanism of action of that drug or in the pathophysiology of the disorder. Efforts are made to correlate the gene variant with the therapeutic or side effects of a given drug. One example involves antidepressants that target the reuptake transporter to increase the function of the neurotransmitter serotonin. Several studies have shown that individuals with the long variant of the gene for the serotonin transporter have a better response to treatment with particular drugs and are more likely to show remission (i.e., complete elimination of symptoms of depression) than individuals with the short allele, at least in Caucasian populations (Schosser and Kasper, 2009). Likewise, the short allele is associated with reduced efficacy of those antidepressants.

It is anticipated that routine genetic screening of patients will provide several advantages. Predicting how patients with distinct genetic profiles will respond to a particular medication before treatment begins would avoid the current costly and time-consuming method of trial and error to determine the best drug and the appropriate dose. Additionally, it is anticipated that genotyping will identify those individuals who are most susceptible to dangerous or debilitating side effects. An early example of this is the pioneering study by Chung and coworkers (2004). They found that there is a strong association in Han Chinese individuals between the HLA-B*1502 allele and life-threatening skin reactions caused by carbamazepine, a drug frequently prescribed to reduce seizures. The toxic side effects include high fever, fatigue, and rapidly developing red, blistering, target-like lesions that may be the cause of death in approximately 5% of patients. They found that HLA-B*1502 was present in 100% of individuals (44 out of 44) experiencing the toxic side effect, while only 3 out of 101 (3%) of carbamazepine-tolerant patients had the allele. This significant genetic marker has subsequently been used to prevent carbamazepine-induced toxicity by genotyping for HLA-B*1502 and avoiding carbamazepine therapy in individuals who carry it (Chen et al., 2011). Differences in allele frequencies among world populations may explain apparent differences in drug response among ethnic groups. The fact that the genetic allele is found in 8% of Han Chinese but only 1% to 2% of Caucasians may be responsible for the lower rate of carbamazepine-induced toxicity in Caucasians. Minimizing side effects and serious toxic reactions not only would protect patients, but also should increase compliance with the drug regimen, and this would lead to fewer instances of relapse.

Despite encouraging basic biomedical research showing the potential of genomic screening, multiple challenges remain for the routine application of pharmacogenomics in clinical practice. One major challenge is that patients are not routinely genotyped, so a database of genetic information that can predict clinically meaningful treatment response outcomes remains to be built. Performing such studies for the database is difficult, expensive, and labor intensive because they require a large sample size for statistical validity and prolonged periods of monitoring therapeutic outcomes and side effects. However, Urban and Goldstein (2014) suggest including genetic screening in several phases of clinical trials leading to FDA approval of new drugs (see Chapter 4 for a discussion of the testing phases). Such screening would enhance the database and potentially detect markers that predict drug efficacy or adverse drug reactions, which might help pharmaceutical companies identify individuals for the final phase of testing. Although genotyping can be relatively expensive at this point in time, it is anticipated that with increased use, the costs will continue to diminish and genetic screening may become a routine procedure. However, at present, only a few drugs are labeled with genomic information to guide clinical decisions and encourage routine genetic screening of patients.

Another challenge for pharmacogenetics involves the procedural difficulties involved in making the statistical cost–benefit analyses needed to show that genetic screening adds sufficient value in patient outcome to justify expenditures of the limited health care budget. Thus far, clinical use of genetic screening has been limited to selecting specialized drugs for unusual patients and regulating the dosage of drugs when the therapeutic index is narrow. However, screening may also be beneficial when it is difficult to predict which individuals will experience particularly dangerous adverse effects. It must be concluded that pharmacogenetics holds great promise for better psychiatric treatment, but further development will be needed if it is to reach its clinical potential. For more information you might want to check out an NIH website such as www.genome.gov/27530645.

Section Summary

- Drugs have biological effects because they interact with receptors on target tissues.
- Drugs or ligands that bind and are capable of changing the shape of the receptor protein and subsequently altering cell function are called agonists.
- Ligands that attach most readily are said to have high affinity for the receptor.
- Antagonists are capable of binding and may have high affinity while producing no physiological change; hence they have little or no efficacy. Antagonists "block" agonist activity by preventing agonists from binding to the receptor at the same moment.
- Inverse agonists bind to a receptor and initiate a biological action that is opposite that produced by an agonist.
- In general, binding of the specific ligand to a receptor is reversible.
- Receptor number changes to compensate for either prolonged stimulation (causing down-regulation) or absence of receptor stimulation (causing up-regulation).
- Dose–response curves show that with increasing doses, the effect increases steadily until the maximum effect is reached.
- The ED_{50} is the dose that produces a half-maximal (50%) effect. The more potent drug is one that has the lower ED_{50}.
- Comparison of the TD_{50} (50% toxic dose) with the ED_{50} (50% effective dose) for a single drug provides the therapeutic index, which is a measurement of drug safety.
- Competitive receptor antagonists reduce the potency of an agonist; this is shown by a parallel shift of the dose–response curve to the right with no change in the maximum effect.
- Biobehavioral interactions of drugs can produce physiological antagonism, additive effects, or potentiation.
- Chronic drug use may lead to a reduction in biobehavioral effects called tolerance, but in some circumstances, drug effects increase with repeated use—a phenomenon called sensitization.
- Cross-tolerance may occur if repeated use of one drug reduces the effectiveness of a second drug.
- Tolerance is generally a reversible condition that depends on dose, frequency of use, and the drug-taking environment. Not all drugs induce tolerance, and not all effects of a given drug undergo tolerance to the same extent or at the same rate.
- Metabolic tolerance occurs when drugs increase the quantity of the liver's metabolizing enzymes; this more rapidly reduces the effective blood level of the drug.
- Pharmacodynamic tolerance depends on adaptation of the nervous system to continued presence of the drug by increasing or decreasing receptor number or by producing other compensatory intracellular processes.
- Behavioral tolerance occurs when learning processes and environmental cues contribute to the reduction in drug effectiveness. Pavlovian conditioning and operant conditioning can contribute to the change in drug response.
- Sensitization is dependent on dose, intervals between treatments, and the drug-taking environment. Cross sensitization with other drugs in the same class can occur. Unlike tolerance, sensitization is not readily reversible, and it persists for long periods of abstinence caused by long-term physiological changes to cells.
- Pharmacogenetics strives to identify genetic polymorphisms in individuals that predict good or poor therapeutic response to a specific drug or susceptibility to specific side effects. Genetic variation in pharmacokinetic factors such as rate of drug metabolism is responsible for determining drug blood level and subsequent biobehavioral effects. Genetic polymorphisms may influence the targets for drugs such as receptors and transporters.

STUDY QUESTIONS

1. What is the placebo effect? Discuss several possible explanations for the placebo effect. How might it be used clinically and in research?
2. What are the principal pharmacokinetic factors? Explain how they determine bioavailability.
3. Discuss the advantages and disadvantages of several methods of administering drugs.
4. Describe the factors that influence the absorption of lipid-soluble and ionized drugs.
5. What is the blood–brain barrier, and why is it important to psychopharmacology?
6. What is depot binding? Discuss three ways that it can alter the magnitude and duration of drug action.
7. Compare and contrast synthetic and nonsynthetic biotransformations. Why are these metabolic changes to the drug molecule necessary for excretion?
8. What are the factors that modify biotransformation?
9. What is a drug receptor? Provide several characteristics that are common among receptors in general.
10. Define *agonist*, *antagonist*, *partial agonist*, and *inverse agonist* as they relate to drug–receptor interactions.
11. What is a dose–response curve? How does the curve show threshold response, ED_{50}, and maximum response? Draw a curve to show your thinking.
12. What is the difference between potency and efficacy? How is that shown graphically?
13. What is the therapeutic index? Why is it important?
14. What effect does a competitive antagonist have on drug effects? Compare its effect on potency and efficacy.
15. Define drug tolerance and describe three types of tolerance and their mechanisms. Compare tolerance with sensitization.
16. Describe pharmacogenetics and its role in personalized medicine. Give one example of its potential use in clinical treatment.

Go to the *Psychopharmacology* Companion Website at oup-arc.com/access/meyer-3e for animations, web boxes, flashcards, and other study aids.

CHAPTER 2

The myelin sheath of a neuron damaged by MS is being repaired by an oligodendrocyte. (Carol Werner/Medical Images.)

Structure and Function of the Nervous System

MICHELLE WAS A FIRST-SEMESTER SENIOR ENGLISH MAJOR when she first noticed that she was unusually fatigued and often felt dizzy and weak, barely able to lift her legs to climb stairs. She said it felt as though she were walking through oatmeal. As the semester wore on, Michelle found she had increasing trouble reading her assignments because of blurred vision and a sense that the words were moving on the page. Although these symptoms disappeared temporarily, they recurred with greater intensity just a few weeks later. When she realized she was almost too weak to walk and began slurring her speech, she made an appointment to see her hometown doctor. After a series of tests, Michelle's doctor told her she had multiple sclerosis (MS)—a progressive, degenerative autoimmune disease in which the immune system attacks the nerve cells in the brain and spinal cord, producing sensory and motor deficits. The doctor tried to reassure her. He said that MS is a disease that is characterized by remissions (absence of symptoms) and relapses and that some people go years before experiencing another episode. During remission, many people lead perfectly normal lives. However, it is a disease that can be treated but not cured. Fortunately, there are disease-modifying drugs that reduce the relapse rate and subsequently slow the progression of MS. This chapter describes nervous system structure and function. By the end of the chapter, you should have a good idea of what causes MS and of why Michelle experienced the particular symptoms she did.

As we already know, psychopharmacology is the study of how drugs affect emotion, memory, thinking, and behavior. Drugs can produce these widespread effects because they modify the function of the human brain, most often by altering the chemical nature of the nervous system. To gain an understanding of drug action, we first need to know a bit about individual nerve cell structure and electrochemical function. Second, we need to have an essential understanding of how these individual cells form the complex circuits that represent the anatomical basis for behavior. We hope that for most readers, Chapter 2 will be a review of (1) the structure of nerve cells, (2) electrochemical properties of neurons, and (3) anatomy

of the nervous system, as we put the individual neurons together into functional units. Chapter 3 follows up with greater detail on the chemical nature of nerve cell function. ■

Cells of the Nervous System

All tissues in the body are composed of cells, and the special characteristics of different types of cells determine the structures and functions of the tissues and the organs. Understanding how those cells became specialized (differentiated) is of tremendous importance to basic science as well as clinical research. **BOX 2.1** describes embryonic stem cells and their potential in research and therapeutics. Embryonic stem cells destined to form the nervous system become two primary types of cells: nerve cells called **neurons** and supporting cells called **glial cells** that provide metabolic support, protection, and insulation for neurons (see the section on glial cells later in the chapter). The principal function of neurons is to transmit information in the form of electrical signaling over long distances. **Sensory neurons**, which are sensitive to environmental stimuli, convert physical stimuli in the world around us and in our internal environment into an electrical signal and transmit that information to circuits of **interneurons**, which are nerve cells within the brain and spinal cord. Interneurons form complex interacting neural circuits and are responsible for conscious sensations, recognition, memory, decision making, and cognition. In turn, **motor neurons** direct a biobehavioral response appropriate for the situation. Although these neurons have common features, their structural arrangements and sizes vary according to their specific functions. **FIGURE 2.1** provides some examples of the many possible shapes of neurons that were first described by the nineteenth-century histological studies of the Spanish neuroanatomist Ramón y Cajal. For much of the twentieth century, neuroscientists relied on the same set of techniques developed by early neuroanatomists to describe and categorize the diversity of cell types in the nervous system.

Histological methods that prepare tissue for microscopic study involve preparing very thin slices of the brain after it has been perfused with a salt solution to remove the blood, and treating the tissue with fixative that kills potentially damaging microorganisms, stops enzymatic damage, and hardens normally soft tissue. After slicing, one of several types of stain is applied to make fine cellular details visible. The Golgi technique, developed in 1873 by the Italian scientist Camillo Golgi, stains only a few cells in their entirety for detailed visualization of individual neurons (see Figure 2.1); others selectively stain myelin to view bundles of axons. Still others selectively stain cell bodies or degenerating axons that identify damaged cells. After the tissue is stained, slices are examined with light or electron microscopy. Although variations on this basic technique are still frequently used, from the late 1970s onward, remarkable new technologies (see Chapter 4) in cell biology and molecular biology provided investigators with many additional tools with which to identify minute differences in the structural features of neurons, trace their multiple connections, and evaluate physiological responses.

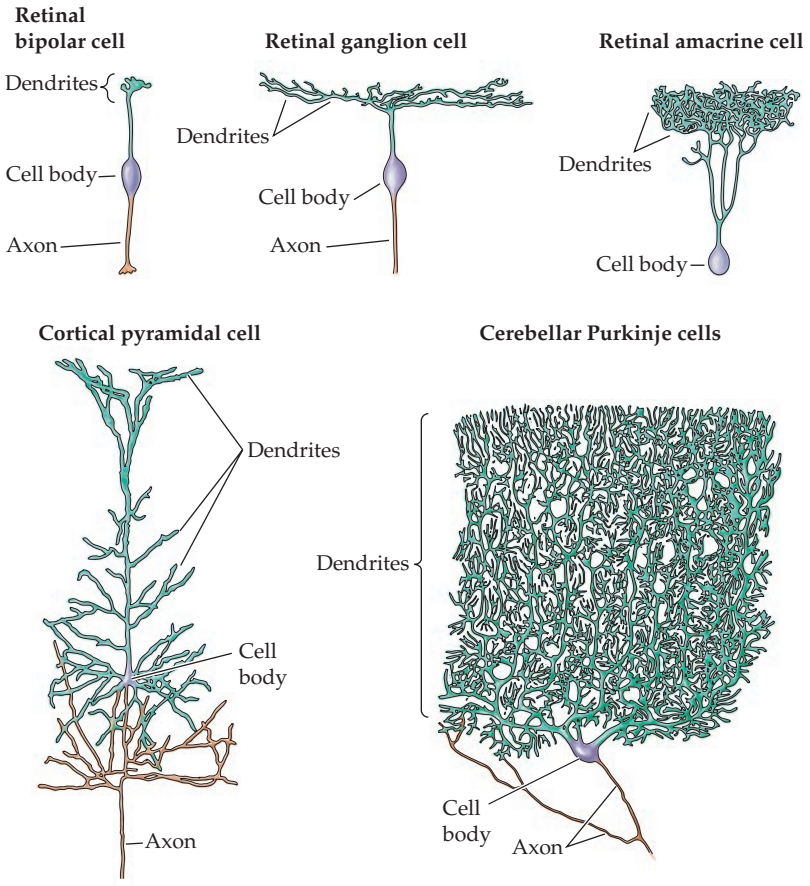


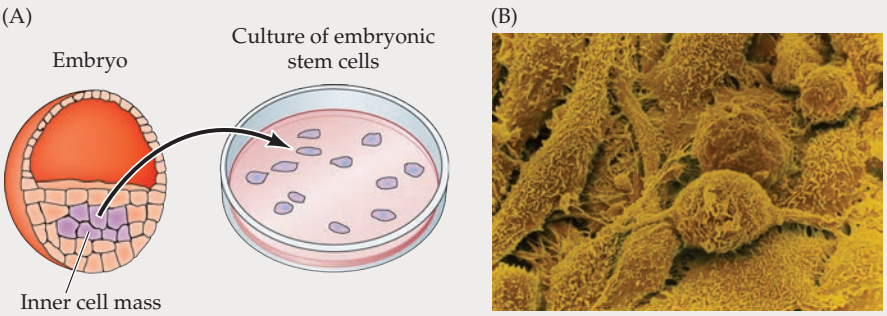
FIGURE 2.1 Varied shapes of neurons These drawings are from actual nerve cells stained by the Golgi technique. Neurons are drawn to different scales to show their varied structures.

Neurons have three major external features

Although neurons come in a variety of shapes and sizes and utilize various neurochemicals, they have several

BOX 2.1 The Cutting Edge

Embryonic Stem Cells



Culture of embryonic stem cells (A) Embryonic stem cells are cultured from the inner cell mass of an early-stage embryo. (B) Scanning electron micrograph of cultured embryonic stem cells. (© Dr. Yorgos Nikas/Science Source.)

Stem cells are undifferentiated (i.e., unspecialized) cells that have the ability to proliferate; that is, they replicate themselves over long periods of time by cell division. A second distinguishing feature is that although they are unspecialized, stem cells have the capacity to become any specific tissue or organ cell type, such as red blood cell, muscle, or neuron, each with its unique structure and functions. This is possible because all cells of the body have identical genetic material, but some genes are activated and others are silenced to produce a cell type with all the appropriate proteins to perform its specialized functions. Hence in a nerve cell, particular genes are silenced, and in a heart cell, other genes are silenced. Embryonic stem cells are derived primarily from a portion of very early-stage embryos that would normally become the three germ layers that ultimately develop into all the different tissues of the body. The cells are maintained in a laboratory cell culture dish and multiply, potentially yielding millions of embryonic stem cells (see **Figure**). If after 6 months, the cells have not differentiated into specific tissue cells, they are considered pluripotent, having more than one potential outcome. Scientists attempt to control differentiation to a specific cell type by changing the chemicals in the culture dish or by inserting specific genes into the cells to provide direction.

There are several potential benefits from stem cell research. First, in the laboratory, the differentiated cells can be used to develop model systems to improve our understanding of how an organism develops from a single cell. Knowing something about what genes and molecular controls direct normal differentiation may provide important clues about the nature of disease-causing aberrations and the causes of cancer and birth defects, both of which are due to abnormal cell differentiation and cell division. This understanding can lead to new strategies for treatment. Second, drug development can be more efficient if multiple cell lines are used to screen new drugs for potential toxic effects on various cell types from multiple organs. Such preliminary testing would also reduce harm to animals or humans. Third, the most publicized potential application of stem cell research is the use of stem cells for cell transplantation therapies for degenerative diseases or diseased organs. Promising results from initial research with stem cells involved animal models of Parkinson's disease, which showed that administered stem cells migrate to the damaged area of the brain and replace lost dopamine neurons, producing significant improvement in motor function. Others used the cells to replace lost oligodendroglial cells that provide myelin in a rat model of human demyelinating disease (Brüstle et al., 1999). Stem cells can also be directed to form specific classes of CNS neurons. For example, by providing appropriate inductive signals and transcription factors, stem cells can be directed to become motor neurons. These cells replicate in the spinal cord, extend axons, and form synapses with target muscle (Wichterle et al., 2002).

Successes with rat models have encouraged early trials in human patients. Efforts have been made to replace inactive pancreatic β-cells in individuals with type 1 diabetes to restore normal levels of insulin. Additional trials have been initiated to evaluate stem cell use in Parkinson's disease, amyotrophic lateral sclerosis (ALS), macular degeneration, and severe burns. The first trials to treat patients with paraplegia after injury to the thoracic region of the spinal cord are under way. The list of neurodegenerative disorders that might someday be tackled by stem cell transplantation is long and includes Alzheimer's disease, ALS, stroke, brain trauma and tumors, MS, Tay-Sachs disease, Duchenne muscular dystrophy, and many others. Evidence also suggests that there is reduced proliferation of brain stem cells in patients with schizophrenia, depression, and bipolar disorder, which may someday be corrected by stem cell transplantation.

(*Continued*)

BOX 2.1 The Cutting Edge (*continued*)

The potential for this type of treatment is enormous, but whether results in humans will resemble those of the animal research must still be determined. Among the hurdles remaining is the need to increase basic research into the cellular events that lead to differentiation of pluripotent stem cells into the specific types of cells needed. In addition, steps may be needed to modify the stem cells to avoid immune rejection of the tissue. Finally, methods of delivery of the cells into the appropriate part of the body will need to be developed for each type of cell therapy. Clearly, treatment of brain disorders is particularly challenging.

A second approach to cell therapy is to use adult stem cells, which are found in many tissues, including brain, blood, skin, and heart. These cells normally function to repair the damaged tissue of the organ in which they reside and so are not pluripotent but are limited in differentiation to cell types within that organ. For instance, cardiac progenitor cells normally repair heart muscle, although at too slow a rate to help someone with significant damage, such as after a heart attack. There is some limited evidence for transdifferentiation of adult cells, which means that certain stem cell types can differentiate into cell types characteristic of tissues other than that of their origin. However, transdifferentiation is somewhat controversial among researchers, and it is not clear whether it occurs in humans. Hence in general, adult stem cells are considered much more limited in their therapeutic potential than those derived from embryos. A second limitation with adult stem cells is that each tissue has only a small number of stem cells, so isolating them is difficult. Furthermore, once they are removed from the body, their ability to divide is limited, so it is more difficult to make the large quantities needed for transplantation. The number of adult cells further decreases with age, and the older cells may have more DNA damage, which may explain their shorter life span compared with pluripotent stem cells. One potential advantage of using adult stem cells is that there might be less risk of immune rejection, because the patient's cells would be isolated, multiplied in cell culture, and then readministered to that same patient.

Psychopharmacology is particularly interested in neural stem cells that are found in only two brain areas: the subventricular zone that lines the lateral ventricles and the hippocampal dentate gyrus. These cells support neurogenesis—the birth of new nerve cells throughout the life span—but also differentiate into oligodendroglia and astrocytes. The importance of neurogenesis in the hippocampus has become an important focus of research into the mechanism of action of antidepressants—a topic that will be discussed further in Chapter 18.

In an effort to overcome the ethical and political hurdles imposed on embryonic stem cell research that have restricted government funding, scientists have developed *induced* pluripotent stem cells (IPS cells). IPS cells are created by genetically reprogramming mature cells to develop the characteristics of the embryonic stem cell, essentially reversing the cell's differentiated fate. These cells have the cell markers of embryonic cells, reproduce in cell culture, and can develop into a wide variety of cells. Further, they avoid the controversial use of human embryos. IPS cells have been useful in drug development and testing and basic research, and a few small studies have been performed in human patients who were undergoing heart surgery. Stem cells injected into the blood or directly into the injured heart seemed to help heart function. However, these studies, although encouraging, are very preliminary. Another advantage of IPS cells is that they would be a match to the cell donor, so they should not be rejected by the immune system. However, a significant downside is that genetic reprogramming requires the use of viral vectors to get the genetic material into the adult cells. Since the use of the retrovirus may produce cancers, alternative nonviral techniques must be developed. Furthermore, more careful evaluation and comparison of IPS cells with embryonic stem cells and adult stem cells are needed to promote understanding of how they differentiate and to evaluate the potential for causing genetic errors or cancer. Unfortunately, some research suggests that IPS cells are less efficient at proliferation than embryonic stem cells, have higher rates of cell death, and prematurely lose their ability to divide (Feng et al., 2010). It is clear that much more research is needed before these cells will be useful therapeutically. A good source of further information is the NIH website Stem Cell Information (NIH, n.d.).

principal external features in common (**FIGURE 2.2**). These features include (1) the **soma**, or cell body, which contains the nucleus and other organelles that maintain cell metabolic function; (2) the **dendrites**, which are treelike projections from the soma that receive information from other cells; and (3) the **axon**, the single tubular extension that conducts the electrical signal from the cell body to the terminal buttons on the axon terminals. Like all other cells, neurons are enclosed by a semipermeable membrane and are filled with a

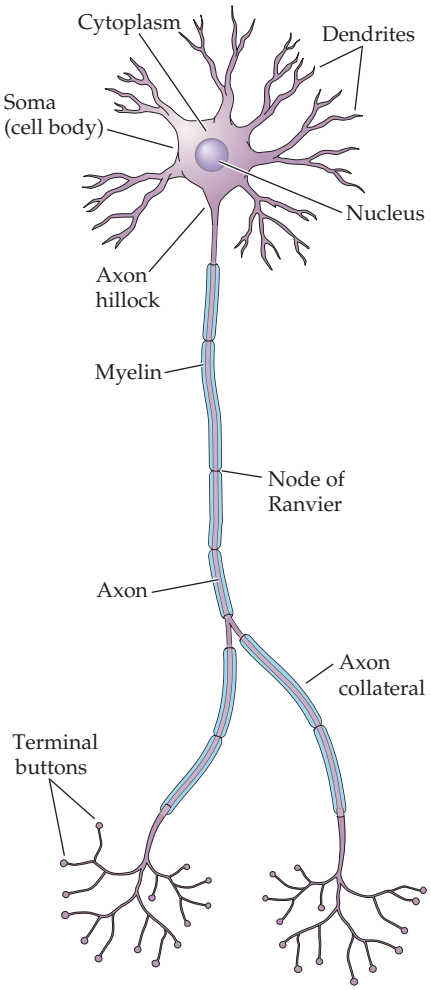
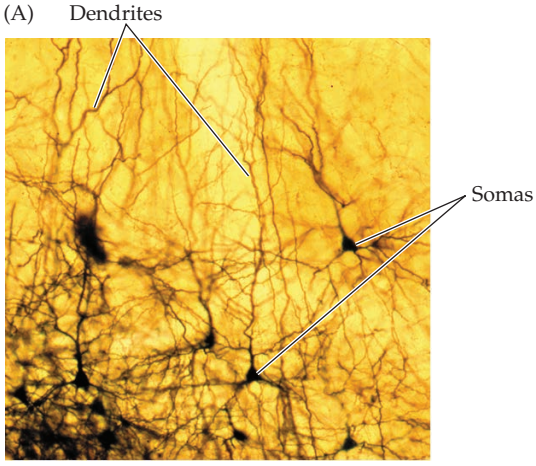


FIGURE 2.2 Principal parts of neurons Despite differences in size and shape, most neurons have numerous features in common.

salty, gelatinous fluid—the **cytoplasm**. Neurons are also surrounded by salty fluid (**extracellular fluid**), from which they take oxygen, nutrients, and drugs, and into which they secrete metabolic waste products that ultimately reach the blood and then are filtered out by the kidneys (see Chapter 1). Like other cells, neurons have **mitochondria**, which are responsible for generating energy from glucose in the form of adenosine triphosphate (ATP). Mitochondria are found throughout the cell, but particularly where energy needs are great. Since neurons use large quantities of ATP, mitochondrial function is critical for survival, and ATP is synthesized continually to support neuron function. The assumption that the rate of synthesis of ATP reflects neuron activity is an underlying premise of several neurobiological techniques that give us the opportunity to visualize the functioning of brain cells (see Chapter 4 for a discussion of positron emission tomography [PET] and functional magnetic resonance imaging [fMRI]).

DENDRITES The general pattern of neuron function involves the dendrites and soma receiving information from other cells across the gap between them, called the **synapse**. On the dendrites of a single neuron, as well as on the soma, there may be thousands of receptors that respond to neurochemicals released by other neurons. Depending on the changes produced in the receiving cell, the overall effect may be either excitatory or inhibitory. Hence each neuron receives and integrates a vast amount of information from many cells; this function is called **convergence**. The integrated information in turn can be transmitted to a few neurons or to thousands of other neurons; this process is known as **divergence**. If we look a bit more carefully using higher magnification, we see that dendrites are usually covered with short **dendritic spines** (**FIGURE 2.3**)



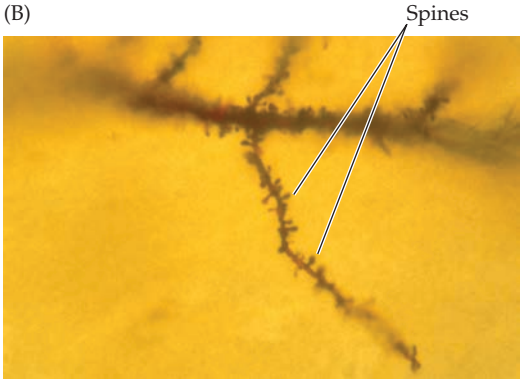


FIGURE 2.3 Dendritic trees with spines (A) Light micrograph of neurons in the human cerebral cortex. Branching dendrites are clearly visible. (B) Higher magnification shows multiple spines all along the dendrite. (A, courtesy of A.-S. LaMantia and D. Purves; B, Mathilde Renard/Medical Images.)

that dramatically increase the receiving surface area. The complex architecture of the dendritic tree reflects the complexity of synaptic connections with other neurons and determines brain function. Dendrites and their spines exhibit the special feature of being constantly modified and can change shape rapidly in response to changes in synaptic transmission (Fischer et al., 1998). Long-lasting changes in synaptic activity change the size and number as well as the shape of dendritic spines from thin to mushroom shaped; this apparently serves as the basis for more efficient signaling. These changes occur throughout life and permit us to continue to learn new associations as we interact with our environment.

Evidence suggesting the importance of dendritic spines to learning comes from studies of human patients and animal models of mental impairment. Individuals with intellectual disabilities have dendritic spines that are unusually small and immature looking; this may result from either a failure of maturation of small spines into larger spines or an inability to maintain spine structure. It is impossible to retain knowledge acquired during development without the large spines, and that failure manifests as intellectual deficiencies. In contrast, individuals with schizophrenia who experience a profound thought disorder have dendritic spines of a normal *size* but reduced spine *density*, particularly in the prefrontal cortex. Although their intelligence is in the normal range, cognitive and negative symptoms of the disorder include poor working memory, lack of attention, poor episodic memory, and low motivation—all of which may be explained by poor connectivity between neurons. Further investigation into the cellular mechanisms of dendritic spine size and density may provide the means to visualize and modify spine dynamics and perhaps may lead to new ways to diagnose and treat brain disorders. For further details on dendritic spine dynamics, the reader is directed to a review by Kasai and colleagues (2010).

AXONS AND TERMINAL BUTTONS The single long extension from the soma is the axon. Axons are tubular in structure and are filled with axoplasm (i.e., cytoplasm within the axon). Axons vary significantly in both length and diameter. Their function is to transmit the electrical signal (action potential) that is generated at the **axon hillock** down the length of the axon to the terminals. The axon hillock is that portion of the axon that is adjacent to the cell body.

Although there is usually only one axon for a given neuron, axons split or bifurcate into numerous branches called **axon collaterals**, providing the capacity to influence many more cells. At the ends of the axons are small enlargements called **terminal buttons**, which are located near the dendrites or somas of other cells.

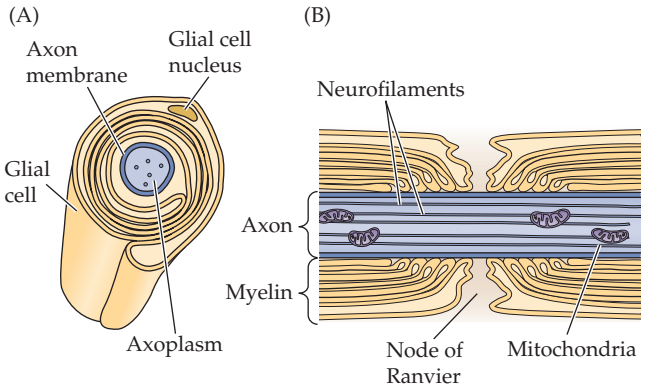


FIGURE 2.4 Myelin sheath (A) Cross section of an axon with multiple layers of glial cell wraps forming the myelin sheath. (B) Longitudinal drawing of a myelinated axon at a node of Ranvier.

Terminal buttons are also called *boutons* or *axon terminals*. Terminal buttons contain small packets (**synaptic vesicles**) of neurochemicals (called **neurotransmitters**) that provide the capacity for chemical transmission of information across the synapse to adjacent cells or to the target organ. Neurons are frequently named according to the neurotransmitter that they synthesize and release. Hence cells that release dopamine are dopaminergic neurons, those that release serotonin are serotonergic, and so forth.

Most axons are wrapped with a fatty insulating coating, called **myelin**, created by concentric layers of glial cells (**FIGURE 2.4A**). There are two types of glial cells that form the myelin sheath: Schwann cells, which myelinate peripheral nerves that serve muscles, organs, and glands; and oligodendroglia, which myelinate nerves within the brain and spinal cord. The myelin sheath provided by both types of glial cells is not continuous along the axon but has breaks in it where the axon is bare to the extracellular fluid. These bare spots, called **nodes of Ranvier** (**FIGURE 2.4B**), are the sites at which the action potential is regenerated during conduction of the electrical signal along the length of the axon. The myelin sheath increases the speed of conduction along the axon; in fact, the thicker the myelin, the quicker the conduction. While a small number of neurons are unmyelinated and conduct slowly, others are thinly wrapped, and some rapidly conducting neurons may have a hundred or more wraps. Myelination also saves energy by reducing the effort required to restore the neuron to its resting state after transmission of the electrical signal.

The best example of the importance of myelin to neuron function comes from multiple sclerosis (MS), the disease that was introduced in the opening vignette. MS is an autoimmune disease in which the immune system attacks a protein in the myelin produced by oligodendrocytes (but not Schwann cells), so the myelin loss is confined to the brain, spinal cord, and optic

nerves. The particular symptoms experienced depend on which neurons have lost their myelin sheath, and they vary greatly from person to person and even within the same person over time. Among the most common symptoms are fatigue; numbness; poor coordination and balance; vision problems; bladder, bowel, and sexual dysfunction; cognitive problems; and depression. There are many more less-common symptoms, as well as what are called secondary symptoms because they result from the primary conditions. For example, the motor symptoms may lead to inactivity that in turn may cause muscle weakness and bone loss. Although myelin can be repaired by oliogodendroglia the repair is slow and not very effective in MS. However researchers are hopeful that new drugs will be developed that reverse nerve damage by facilitating remyelination to improve symptoms (for example, see Dombrowski et al., 2017). Currently available pharmacotherapies and greater detail on MS is provided in Chapter 20. See Recommended Readings on the Companion Website to access *What Is Multiple Sclerosis*, a brief animation of the causes of MS. The impact of having MS is described in one of many available YouTube video clips as well.

SOMA The cell body is responsible for the metabolic care of the neuron. Among its important functions is the synthesis of proteins that are needed throughout the cell for growth and maintenance. These proteins include such things as enzymes, receptors, and components of the cell membrane. Within the nucleus are pairs of chromosomes that we inherited from our parents. **Chromosomes** are long strands of deoxyribonucleic acid (DNA), and **genes** are small portions of chromosomes that code for the manufacture of a specific protein molecule. Hence the **coding region** of a gene provides the "recipe" for a specific protein such as a receptor or an enzyme. Although every cell in the body contains the full genetic library of information, each cell type manufactures only those proteins needed for its specific function. Hence liver cells manufacture enzymes to metabolize toxins, and neurons manufacture enzymes needed to synthesize neurotransmitters and carry out functions necessary for neural transmission. In addition, which specific genes are activated is determined in part by our day-to-day experience. Neurobiologists are finding that experiences such as prolonged stress and chronic drug use may turn on or turn off the production of particular proteins by modifying transcription factors. **Transcription factors** are nuclear proteins that direct protein production. Transcription factors such as CREB bind to the **promoter region** of the gene adjacent to the coding region, modifying its rate of transcription.

Transcription occurs in the nucleus, where messenger RNA (mRNA) makes a complementary copy of the active gene. After moving from the nucleus to the cytoplasm, mRNA attaches to organelles called **ribosomes**, which decode the recipe and link the appropriate amino acids together to form the protein. This process is called **translation**. Some of the basic steps in protein synthesis are shown in **FIGURE 2.5**.

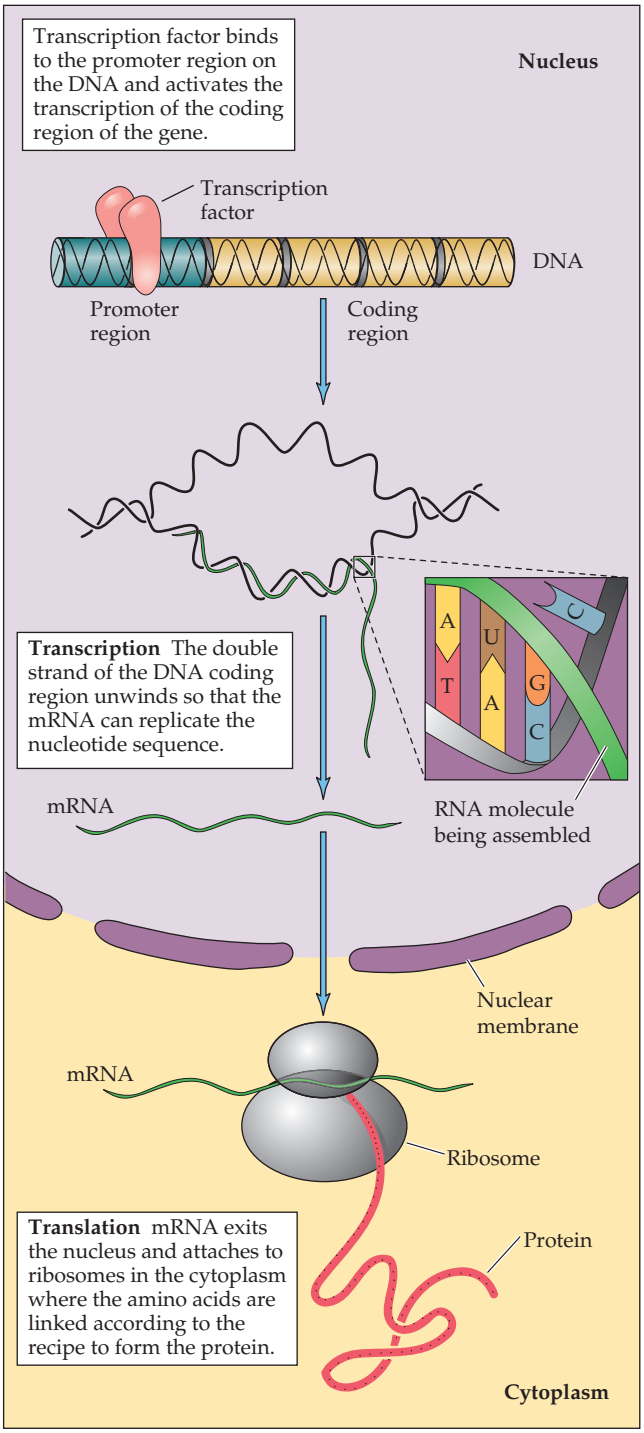


FIGURE 2.5 Stages of protein synthesis Activation of a gene by a transcription factor initiates the formation of mRNA within the nucleus, followed by translation into a protein on the ribosomes in the cytoplasm.

EPIGENETICS We already said that environmental events can alter the rate of gene expression through induction of transcription factors. In addition, there are longer-lasting environmentally induced epigenetic modifications that determine which genes will be turned on or off and how much gene expression occurs. These changes may persist through the lifetime of the organism and may even be passed on to future generations if modifications are present in the germ cell line (i.e., eggs and sperm). These events occur despite the fact that the basic structure of the DNA is not altered. Instead, the simple covalent attachment of methyl groups (**DNA methylation**) to particular locations on a gene usually decreases expression of that gene. A second type of modification is **chromatin remodeling**. Chromatin is a complex of small spherical histone proteins around which the DNA wraps (**FIGURE 2.6A**). Environmentally induced acetylation, methylation, or phosphorylation of the lysine residues of histone tails can loosen the chromatin structure, allowing transcription factors to bind to the DNA and activate expression of the gene (**FIGURE 2.6B**). In other cases, chemical modification of the histone tails makes the chromatin more tightly packed, which represses gene expression by physically limiting the access of transcription factors (**FIGURE 2.6C**).

Epigenetic modification of gene expression has been understood since the 1970s because the phenomenon is central to cell differentiation in the developing fetus. Because all cells in the organism have identical DNA, they differentiate into organ-specific cells only when epigenetic processes turn on some of the genes and turn off others in utero. However, pre- or postnatal epigenetic modification can also occur in response to environmental demands such as starvation or overabundance of food, stress, poor prenatal nutrition, childhood abuse and neglect, exposure to environmental toxins, and so forth. For example, significant overeating leads to weight gain but also produces epigenetic changes that cause the genes for obesity to be overexpressed and the genes for longevity to be underexpressed. These epigenetic modifications not only contribute to the weight gain and shortened life span of the individual but also can be passed on to offspring when the epigenetic modification is in the egg or sperm. This in turn increases the probability of obesity and early mortality in the offspring (Kaati et al., 2007). For more detail see Transgenerational Epigenetic Transmission below. A brief video, *The Epigenome at a Glance*, from the University of Utah is available online (Genetic Science Learning Center, 2013, July 15). For additional online materials and interactive activities, go to the parent site: Learn.genetics.utah.edu/content/epigenetics/.

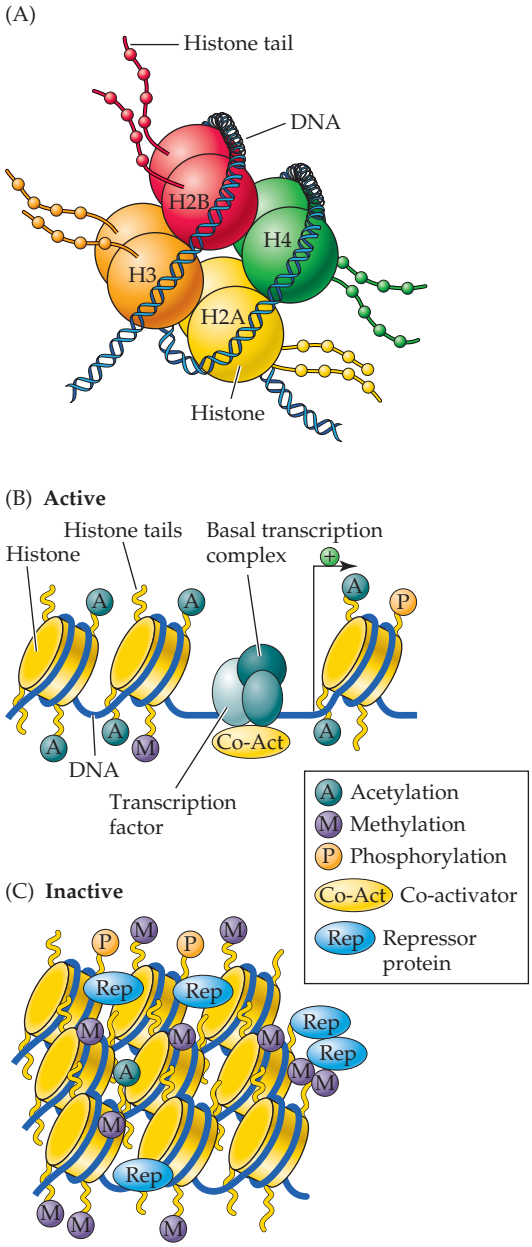


FIGURE 2.6 Epigenetic regulation of gene transcription (A) Chromatin is a complex of DNA, histone proteins, and nonhistone proteins (not shown). (B) When histone tails are acetylated, charges open up the chromatin, creating an active state that allows transcription factors to bind to the promoter region of a gene to enhance transcription. (C) The inactive state of chromatin is caused by methylation of histone tails, which pulls the chromatin tighter and prevents the binding of transcription factors, reducing transcription of the gene. (A, after Levenson and Sweatt, 2005 and Tsankova et al., 2007; B,C after Tsankova et al., 2007.)

TRANSGENERATIONAL EPIGENETIC TRANSMISSION The concept that parents' environmental exposure can influence their offspring's behavior, metabolism, or disease status is an intriguing concept, although the mechanism of transfer is not well understood. It is clear that maternal experiences during pregnancy are capable of affecting fetal development, since the

uterus is the fetus's initial environment. However, recent evidence suggests that environmental events occurring *before* conception can also impact the health and behavior of the offspring. Many studies have shown that the offspring of extremely stressed parents have greater risk for mental and physical difficulties even when parental trauma predated conception. This would suggest that the trauma can cause epigenetic modifications to the genome and be passed on via gametes (eggs and sperm). More recently it has been shown that the mother's experiences are not the only influence on the phenotype of the offspring. The father's environment can also pass on epigenetic markers on sperm or in seminal fluid (see Bowers and Yehuda, 2016). Since experiments on humans would not be ethical, it is easier to study the mechanisms of transgenerational epigenetic transmission in male rodents because they do not participate in raising the young. In contrast, interpretation of data in female rats must consider multiple factors, including maternal influence on intrauterine fetal development, nurturing behaviors with the young, and lactation (Rando, 2012). In numerous rodent studies young male animals were provided with a normal diet or one that lacked protein or contained excess fat. When they reached adulthood, they were mated with females that had always had a normal diet. When the offspring of the pair were evaluated, they found that the abnormal diets of the fathers altered metabolic factors such as glucose control (in humans, associated with diabetes) and cholesterol metabolism (in humans, associated with heart disease) as well as blood pressure and other cardiovascular irregularities (Rando, 2015).

Transgenerational inheritance goes well beyond dietary modification. Dias and Ressler (2014) used male mice and conditioned them with foot shock, which produced a distinct startle response, when one odor (acetophenone) or another (propanol) was presented. These odors were chosen because they activate different receptors in the olfactory system. After the association was established, the males were mated with healthy female mice. The male offspring resulting from the mating were tested for their behavioral sensitivity to the odors. Dias and Ressler found that the young unconditioned animals showed greater sensitivity to the odor their parents had been conditioned to, compared with control mice. Further, the offspring whose fathers associated acetophenone with foot shock were not more sensitive to propanol and vice versa. Hence mice who had never experienced the odor before testing responded differentially to the odors, based on the earlier paternal painful experience associated with a particular odor. This may represent an evolutionarily adaptive way to pass on information about potentially harmful environmental stimuli to future generations. Expanding on their results, the researchers found that the neuroanatomical pathway associated with the acetophenone olfactory receptor (Olfr151) was enhanced in the acetophenone-sensitive offspring compared with the other mice. Further, those same offspring and their fathers showed DNA hypomethylation (an epigenetic marker described above).

Epigenetic mechanisms became a major focus of research when it became clear that epigenetic differences caused by environmental factors could potentially explain a lot of previously unanswered questions. For instance, it may explain why monozygotic twins who have identical genes do not necessarily develop the same disorders, such as schizophrenia or bipolar disorder, cancer, or diabetes. Apparently, despite the identical genes, environmental events have caused those genes to be expressed in different patterns in the two individuals. Epigenetic events may also help to explain the persistence of the drug-taking behavior characteristic of addiction (see Chapter 9). Drugs of abuse cause neuroadaptive changes that are very long lasting and that persist, despite years of abstinence. These changes in structural and behavioral plasticity are associated with increased activation of specific transcription factors. However, these transcription factors return to baseline levels after several months of abstinence, so they are not likely to maintain drug dependence over prolonged periods. One explanation may be that drugs of abuse enhance histone acetylation of multiple genes, which would produce much more persistent changes in gene expression. The potential for clinical use of epigenetic manipulations in drug treatment programs is suggested by a study showing that inhibition of an enzyme that removes acetyl groups from histone, which increased acetylation in nucleus accumbens, reduced drug-seeking behavior in laboratory animals and also reduced the probability of relapse when the mice were re-exposed to cocaine (Malvaez et al., 2010).

An additional potentially significant role for epigenetic mechanisms may help to explain the etiology of many complex disorders, such as anxiety and depression, that have not shown a strong genetic link despite the newest sophisticated molecular genetic techniques. For these and other psychiatric disorders, the genetic polymorphisms identified so far contribute only about 1% of the risk for developing the disease. Hence the environment along with epigenetic mechanisms may have a greater part in initiating the disorder. Epigenetic changes may also help to explain why some disorders, such as autism and many neurological disorders, appear to run in families and yet have no classic genetic transmission (Sweatt, 2013). Finally, epigenetic mechanisms may also help us understand the link between early life events such as abuse or neglect and the increased occurrence of clinical depression (see Chapter 18) and anxiety disorders (see Chapter 17) later in life.

The ultimate goal for neuropharmacology is to develop drugs that can be used to manipulate epigenetic

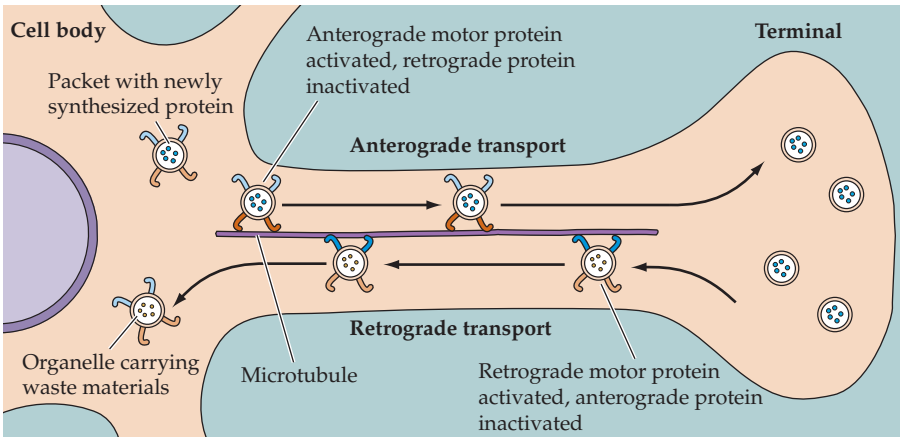


FIGURE 2.7 Axoplasmic transport The movement of newly synthesized proteins from the soma to the axon terminals (anterograde) is powered along the microtubules by a motor protein called kinesin. Old proteins are carried from the terminals to the soma (retrograde) by the motor protein dynein.

factors, for example, to deacetylate histone or increase methylation of DNA to treat psychiatric disorders that have genetic components, such as autism, schizophrenia, depression, Alzheimer's disease, and others. In essence, the drug could be used to enhance gene expression that may have been suppressed by the disorder or turn off expression of genes that are associated with the development of symptoms.

AXOPLASMIC TRANSPORT Having said that proteins are synthesized within the soma and knowing that proteins are needed throughout the neuron, we must consider how these proteins are moved to the required destination. The process is called **axoplasmic transport**, and it depends on structures of the cytoskeleton. The **cytoskeleton**, as the name suggests, is a matrix composed of tubular structures, which include microtubules and neurofilaments that form a mesh-like mass that provides shape for the cell. In addition, the microtubules, which run longitudinally down the axon, provide a stationary track along which small packets of newly synthesized protein are carried by specialized motor proteins (**FIGURE 2.7**). Movement of materials occurs in both directions. Newly synthesized proteins are packaged in the soma and transported in an anterograde direction toward the axon terminals. At the terminals, the contents are released, and retrograde axonal transport carries waste materials from the axon terminals back to the soma for recycling.

Abnormalities of the cytoskeleton constitute one of several pathological features of the brain in people with Alzheimer's disease—the neurofibrillary tangles. These tangles are made up of long, paired, spiral neurofilaments braided together. The neurofilament proteins, called tau, are normally important in keeping the microtubules running parallel and longitudinally directed down the axon. Tau normally has phosphate molecules attached to it, but in Alzheimer's disease a large number of additional phosphate groups attach. This hyperphosphorylation causes tau to separate from the microtubules, and tau becomes twisted together to form tangles and accumulates as a mass in the soma. The microtubules disintegrate destroying the material transport system, and the axons shrivel up so neurons can no longer communicate with each other. The number of such tangles is directly related to the extent of cognitive impairment, which demonstrates clearly the importance of the cytoskeleton to brain function. Alzheimer's disease is discussed in detail in Chapter 20.

Characteristics of the cell membrane are critical for neuron function

One of the more important characteristics of neurons is the cell membrane. In Chapter 1 we learned that the neuronal membrane is essentially a phospholipid bilayer that prevents most materials from freely passing (see Figure 1.5), unless they are lipid soluble. In addition to phospholipids, membranes have proteins inserted into the bilayer. Many of these proteins are **receptors**—large molecules that are the initial sites of action of neurotransmitters, hormones, and drugs. Details of these receptors and their functions are described in Chapter 3. Other important proteins associated with the membrane are enzymes that catalyze biochemical reactions in the cell. A third important group of proteins consists of ion channels and transporters. Because the membrane is not readily permeable to charged molecules, special devices are needed to move molecules such as amino acids, glucose, and metabolic products across the membrane. Movement of these materials is achieved by transporter proteins, which are described further in Chapter 3. In addition, charged particles (ions), such as potassium (K^+), sodium (Na^+), chloride (Cl^-), and calcium (Ca^{2+}), that are needed for neuron function can be moved through the membrane only via ion channels. These channels are protein molecules that penetrate through the cell membrane and have a water-filled pore through which ions can pass.

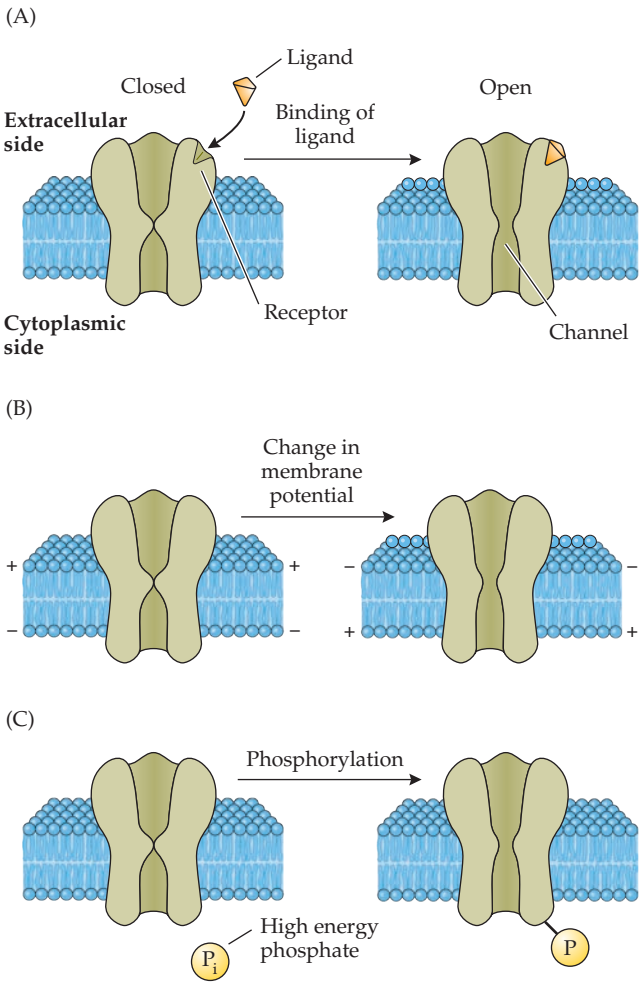


FIGURE 2.8 Ion channels (A) When a ligand (neurotransmitter, hormone, or drug) binds to a receptor on the channel, the ligand-gated channel protein changes shape and opens the gate, allowing passage of a specific ion. (B) A voltage-gated channel is opened when the electrical potential across the membrane near the channel is altered. (C) Modification of a channel by a second messenger, which produces intracellular phosphorylation (addition of a phosphate group) and regulates the state of the channel. (After Siegelbaum and Koester, 2000.)

Ion channels have several important characteristics. First, they are relatively specific for particular ions, although some allow more than one type of ion to pass through. Second, most channels are not normally open to allow free passage of the ions, but are in a closed configuration that can be opened momentarily by specific stimuli. These channels are referred to as **gated channels**. Two types of channels of immediate interest to us are **ligand-gated channels** and **voltage-gated channels**. Looking at the ligand-gated channel in **FIGURE 2.8A**, you can see that when a drug, hormone, or neurotransmitter binds to a receptor that recognizes the ligand, the channel protein changes shape and opens the gate, allowing flow of a specific ion either into or out of the cell. The direction in which an ion moves is determined by its relative concentration; it always travels from high to low concentration. Hence, given an open gate, Na^+, Cl^-, and Ca^{2+} will move into the cell, while K^+ moves out (see details on local potentials in the next section). A second type of channel, which will be of importance later in this chapter, is the type that is opened by voltage differences across the membrane. These voltage-gated channels are opened not by ligands, but by the application of a small electrical charge to the membrane surrounding the channel (**FIGURE 2.8B**). Other channels are modified by second messengers (**FIGURE 2.8C**), but discussion of these will have to wait until Chapter 3. Regardless of the stimulus opening the channel, it opens only briefly and then closes again, limiting the total amount of ion flux.

Glial cells provide vital support for neurons

Glial cells have a significant role in neuron function because they provide physical support to neurons, maintain the chemical environment of neurons, and provide immunological function. The four principal types include oligodendroglia, Schwann cells, astrocytes, and microglia. **Schwann cells** and **oligodendroglia**, described earlier, produce the myelin sheath on neuronal axons of the peripheral nervous system (PNS) and the central nervous system (CNS), respectively. Schwann cells and oligodendroglia differ in several ways in addition to their location in the nervous system. Schwann cells (**FIGURE 2.9A**) are dedicated

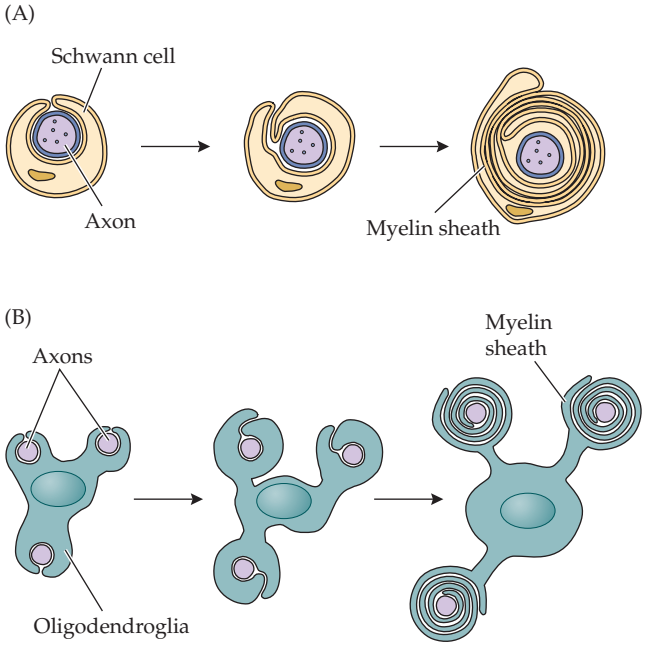
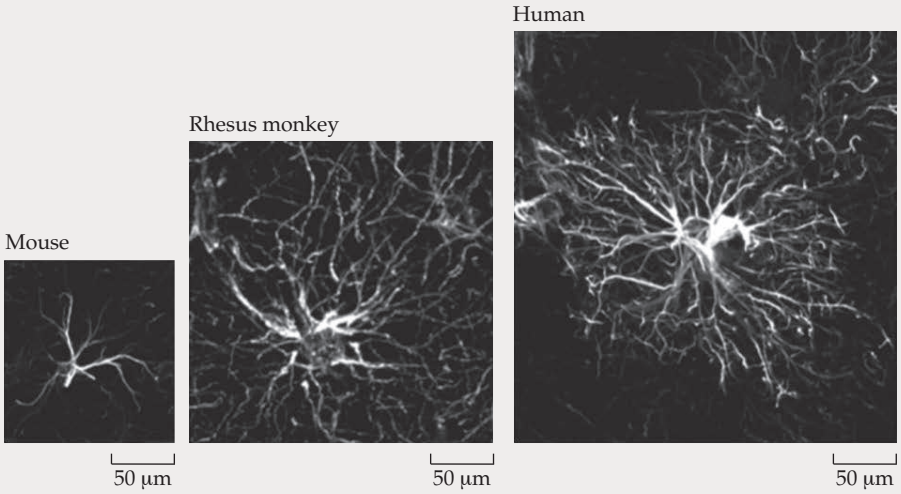


FIGURE 2.9 Glial cells forming myelin (A) Schwann cells in the PNS dedicate themselves to a single axon and wrap many times to form the myelin for one segment. (B) Each oligodendroglia in the CNS sends out multiple sheetlike arms that wrap around segments of multiple nearby axons to form the myelin sheath.

BOX 2.2 Of Special Interest

Astrocytes

Although astrocytes were previously believed to act merely as a "brain glue" by providing support for the all-important neurons, in the last 30 years many new vital functions have been identified for these cells. Their importance to nervous system function is suggested by the fact that the ratio of astroglia to neurons increases with the complexity of brain function. In worms, there is one astrocyte for every six neurons (1:6), the ratio in rodent cortex is 1:3, and in human cortex there are slightly more astrocytes than neurons (1.4:1) (Sofroniew and Vinters, 2010). Not only does the ratio of astrocytes to neurons change, but the astrocyte domain (soma plus processes) increases with increasing complexity of brain function (see **Figure**).



Astrocytes in cortex of an adult mouse, a rhesus monkey, and a human Although all show a bushy morphology, the size of the astrocyte increases with increasing complexity of brain function. (From Kimelberg and Nedergaard, 2010.)

Much of the research has been focused on how the glial cells respond to tissue damage, because they are potentially novel therapeutic targets for many diseases of the nervous system that involve gliosis (activation and accumulation of glial cells in response to CNS injury). These diseases include Alzheimer's disease, neuropathic pain, multiple sclerosis, Parkinson's disease, seizure disorders, migraine, and many others. The response to CNS injury begins with the arrival of microglia, which provide a rapid response to inflammatory signals caused by brain damage. The arrival of microglia initiates the action of astrocytes, called astrogliosis. Gliosis, a reactive change in glial cells following CNS damage, is characterized by an increase in filaments of the cytoskeleton, leading to cell enlargement and buildup of scar tissue. Depending on the nature and extent of damage, astroglia can have either beneficial or detrimental effects. For instance, in some cases they release anti-inflammatory molecules, but in other circumstances they may release pro-inflammatory neurotoxic substances. These neurotoxic substances may contribute further to inflammatory disorders such as multiple sclerosis. Additionally, while astroglia protect against programmed cell death, in other cases the astroglial scar tissue prevents the regeneration of axons. Although the scar tissue may impair recovery, in other cases it may prevent the spreading of an infectious agent to healthy brain tissue by creating a physical barrier (see Sofroniew and Vinters, 2010).

Astroglia do not have electrical excitability, as neurons do, but are capable of communicating with nearby cells (both neurons and other glia) by altering their intracellular calcium concentration. Neurons can signal to the astrocytes by releasing neurotransmitters, including glutamate, GABA (γ-aminobutyric acid), acetylcholine, and norepinephrine. The astrocytes can reciprocally influence the function of neighboring neurons, so there is a coordinated network of glial–neuron activity.

Furthermore, astroglia also have proteins in their membranes, called transporters, that remove certain neurotransmitters from the synapse, hence terminating their synaptic action. This function is especially important for the amino acid neurotransmitter glutamate. Uptake of glutamate by astrocytes has several functions. After glutamate is taken up, the astrocyte converts it to glutamine. Glutamine can be transported back into neurons, where it can be converted back into glutamate for neurotransmission (Boison et al., 2010). The uptake of glutamate by astrocytes is also important when synaptic glutamate levels are high for prolonged periods, because glutamate can have damaging effects (excitotoxicity) on neurons (see Chapter 8). Impaired uptake of glutamate by astrocytes has been implicated in some neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), and also disorders associated with severe energy failure, such as severe head trauma or status epilepticus, which is characterized by prolonged, uncontrolled seizures. Multiple studies have shown

BOX 2.2 Of Special Interest (*continued*)

structural and physiological abnormalities in astrocytes in epileptic tissue such as in the hippocampus. Others have found that animals that have been genetically modified to prevent glutamate uptake have been highly susceptible to excitotoxicity and many have developed epileptic seizures (see Boison et al., 2010). Failure of astroglia function not only prevents glutamate uptake but also impairs the ability to remove excess synaptic K^+ and maintain the appropriate salt–water balance and extracellular pH.

In addition to the significant role of astrocytes in responding to tissue damage, modulating synaptic function, and maintaining the extracellular fluid, astrocytes also surround all the blood vessels in the brain (see Figure 1.8) and so in that way play a large role in regulating CNS blood flow. They respond to the neurotransmitters released by adjacent neurons by increasing their intracellular Ca^{2+}. This in turn causes the release of vasoactive chemicals, such as prostaglandins, that produce vasodilation. They regulate the amount of blood flow in a given brain region based on the level of synaptic activity of hundreds of neurons. In this way astroglia can coordinate the availability of oxygen and glucose in the blood with the amount of neural activity. As you will see in Chapter 4, that principle is the basis for fMRI, which scans the brain for increased blood flow and oxygenation to identify those areas that are most metabolically active. Although it is unclear what the precise chemical mediators between the astrocytes and blood vessels are, much more research is ongoing (Howarth, 2014).

Additionally, because astrocytes bridge the gap between blood vessels and neurons, they are able to take up glucose from the blood and store it in the form of glycogen. During times when blood glucose is low and/or neurons show enhanced electrical activity, the astrocytes convert glycogen to lactate and transfer it to neurons to provide energy to the cells. Several excellent reviews on astrocyte function and their role in CNS disorders and brain pathology are available (Kimelberg and Nedergaard, 2010; Sofroniew and Vinters, 2010).

to a single neuron, and these PNS axons, when damaged, are prompted to regenerate axons because of the Schwann cell response. First, the Schwann cells release growth factors, and second, they provide a pathway for regrowth of the axon toward the target tissue. Oligodendroglia, (**FIGURE 2.9B**) in contrast, send out multiple paddle-shaped "arms," which wrap many different axons to produce segments of the myelin sheath. In addition, they do not provide nerve growth factors when an axon is damaged, nor do they provide a path for growth.

Two other significant types of glial cells are the astrocytes and microglia. **Astrocytes** are large, star-shaped cells that have numerous extensions. They intertwine with neurons and provide structural support; in addition, they help to maintain the ionic environment around neurons and modulate the chemical environment as well by taking up excess neurochemicals that might otherwise damage cells. Because astrocytes have a close relationship with both blood vessels and neurons, it is likely that they may aid the movement of necessary materials from the blood to nerve cells. Greater detail on astrocyte function is provided in **BOX 2.2**. **Microglia** are far smaller than astrocytes and act as scavengers that collect at sites of neuron damage to remove dying cells. In addition to their phagocytotic function, microglia are the primary source of immune response in the CNS and are responsible for the inflammation reaction that occurs after brain damage. Chapter 19 describes their potential role in the etiology of schizophrenia. **TABLE 2.1** summarizes the functions of glial cells.

TABLE 2.1 Functions of Glial Cells

Cell	Function
Astrocytes	Provide structural support
	Maintain ionic and chemical environment
	Store nutrients to provide energy for neurons
	Perform gliosis
	Regulate CNS blood flow
	Coordinate reciprocal glia–neuron activity
Microglia	Perform phagocytosis
	Provide immune system function
Schwann cells	Form myelin sheath on a single axon in the PNS
	Release growth factors following neuron damage
	Provide a channel to guide axons to targets
Oligodendroglia	Form myelin sheath on multiple axons in the CNS
	Inhibit regrowth of axons following neuron damage

Section Summary

- Neurons are surrounded by a cell membrane and are filled with cytoplasm and the organelles needed for optimal functioning.
- Among the most important organelles are the mitochondria, which provide energy for the metabolic work of the cell.
- The principal external features of a neuron are the soma, treelike dendrites, and a single axon extending from the soma that carries the electrical signal all the way to the axon terminals.
- Axon terminals contain synaptic vesicles filled with neurotransmitter molecules that are released into the synapse between cells when the action potential arrives.
- The dendrites of a neuron are covered with minute spines that increase the receiving surface area of the cell. These spines are reduced in size in individuals with intellectual impairment and reduced in number in those with schizophrenia.
- Thousands of receptors that respond to neurotransmitters released by other neurons are found on the dendrites, dendritic spines, and soma of the cell.
- The axon hillock is located at the juncture of soma and axon and is responsible for summation (or integration) of the multiple signals required to generate an action potential.
- Conduction of the action potential along the axon is enhanced by the insulating property of the myelin created by nearby glial cells.
- The nucleus of the cell is located within the soma, and protein synthesis occurs there. Transcription of the genetic code for a specific protein by mRNA occurs within the nucleus, and translation of the "recipe," carried by the mRNA, occurs on the ribosomes in the cytoplasm. Ribosomes link together appropriate amino acids to create the protein.
- Changes in synaptic activity increase or decrease the production of particular proteins by activating transcription factors in the nucleus.
- Epigenetics is the study of how environmental demands such as diet, environmental toxins, stress, prenatal nutrition, and many others turn on or turn off the expression of specific genes. Although epigenetic markers do not modify DNA, they can last a lifetime and may be transmitted to future generations. Two common markers are DNA methylation and chromatin remodeling.
- Future drug development will target epigenetic factors to treat psychiatric disorders that have genetic components by turning on protective genes or turning off genes associated with symptom development.
- Newly manufactured proteins are packaged into vesicles in the soma and are moved by motor proteins that slide along the neuron's microtubules (part of the cytoskeleton) to the terminals (anterograde transport). Protein waste and cell debris are transported from the terminals back to the soma (retrograde transport) for recycling.
- The cell membrane is a phospholipid bilayer that prevents most materials from passing through, unless the material is lipid soluble. Special transporters carry other essential materials, such as glucose, amino acids, and neurotransmitters into the cell. Ion channels allow ions such as Na^+, K^+, Cl^-, and Ca^{2+} to move across the membrane. Other proteins associated with the membrane include receptors and enzymes.
- Four types of glial cells are found in the nervous system. Schwann cells and oligodendroglia produce the myelin sheath on peripheral and central nervous system neurons, respectively. Astrocytes regulate the extracellular environment of the neurons, regulate CNS blood flow, and provide physical support and nutritional assistance. Microglia act as phagocytes to remove cellular debris and provide immune function.

Electrical Transmission within a Neuron

The transmission of information within a single neuron is an electrical process and depends on the semipermeable nature of the cell membrane. When the normal resting electrical charge of a neuron is disturbed sufficiently by incoming signals from other cells, a threshold is reached that initiates the electrical signal (action potential) that conveys the message along the entire length of the axon to the axon terminals. This section of the chapter looks at each of the stages: resting membrane potential, local potentials, threshold, and action potential.

Ion distribution is responsible for the cell's resting potential

All neurons have a difference in electrical charge inside the cell compared with outside the cell, called the **resting membrane potential**. This can be measured by placing one electrode on the exterior of the cell in the extracellular fluid and a second, much finer microelectrode into the intracellular fluid inside the cell (**FIGURE 2.10**). The inside of the neuron is more negative than the outside, and a voltmeter would tell us that the difference is approximately –70 millivolts (mV), making the neuron **polarized** in its resting state.

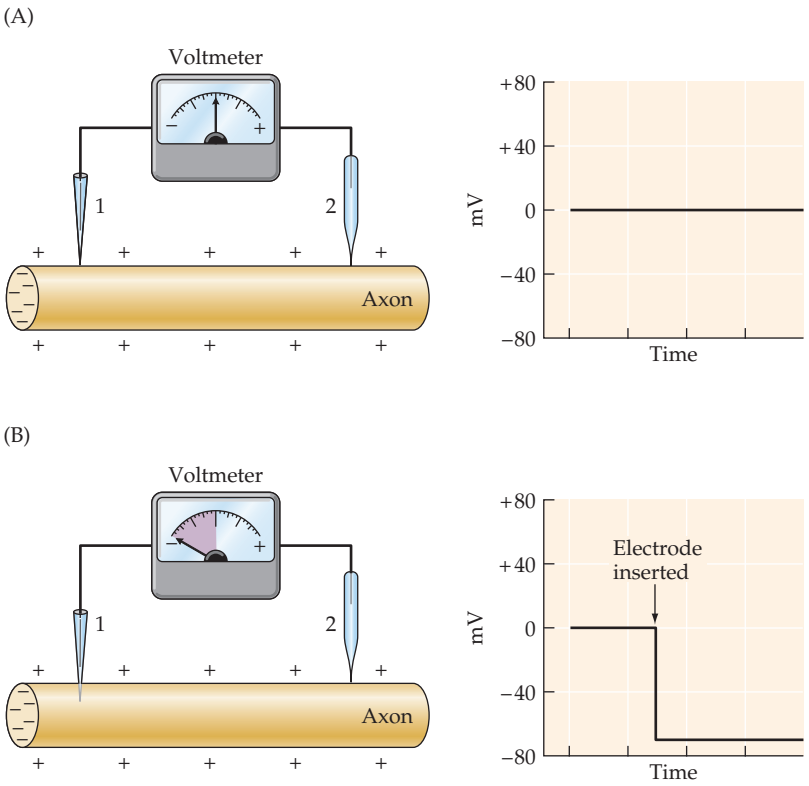


FIGURE 2.10 Membrane potential recording from a squid axon
(A) When both electrodes are applied to the outside of the membrane, no difference in potential is recorded. (B) When the microelectrode is inserted into the axoplasm, a voltage difference between inside and outside is recorded. The graph shows the voltage change when one electrode penetrates the cell.

Selective permeability of the membrane and uneven distribution of ions inside and outside the cell are responsible for the membrane potential. This means that when the cell is at rest, there are more negatively charged particles (ions) inside the cell and more positively charged ions outside the cell. **FIGURE 2.11** shows the relative concentration of different ions on either side of the membrane. Inside we find many large, negatively charged molecules, such as proteins and amino acids, which cannot leave the cell. Potassium is also in much higher concentration (perhaps 20 times higher) inside the cell than outside. In contrast, Na^+ and Cl^- are present in greater concentration outside the cell than inside.

Several forces are responsible for this ion distribution and membrane potential. The concentration gradient and electrostatic pressure for the K^+ ion are particularly important; K^+ moves more freely through the membrane than other ions because some of its channels are not gated at the resting potential. Recall that ions move through relatively specific channels and that most are gated, meaning that they are normally held closed until opened by a stimulus. Since the inside of the cell normally has numerous large, negatively charged materials that do not move through the membrane, the positively charged K^+ ion is pulled into the cell because it is attracted to the internal negative charge, that is, by **electrostatic pressure** (see Figure 2.11). However, as the concentration of K^+ inside rises, K^+ responds to the concentration gradient

	Na^+	K^+	Cl^-	Ca^{2+}	$^-$ Protein
Concentration outside cell	440	20	560	10	Few
Concentration inside cell	50	400	40–150	0.0001	Many

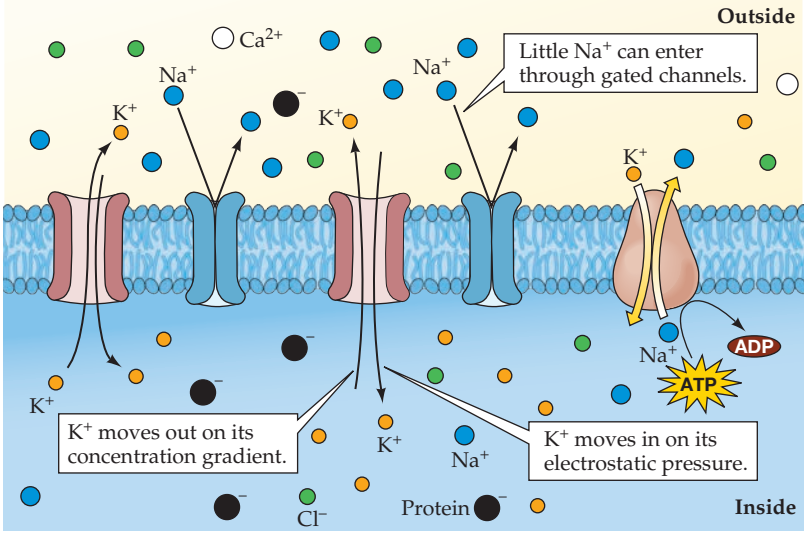


FIGURE 2.11 Distribution of ions inside and outside a neuron at resting potential Na^+ and Cl^- are more concentrated outside the cell and cannot move in freely through their gated channels. Some K^+ channels are not gated, allowing the concentration of the ion to force K^+ outward while electrostatically it is pulled in. At –70 mV, equilibrium between the two forces is reached. The Na^+–K^+ pump helps to maintain the ion distribution. It requires significant energy (ATP) to move ions against their concentration gradients.

by moving out of the cell. The concentration gradient is a force that equalizes the amount or concentration of material across a biological barrier. When the two forces on K^+ (inward electrostatic pressure and outward concentration gradient) are balanced at what is called the **equilibrium potential for potassium**, the membrane potential is still more negative inside (–70 mV). In addition, because small amounts of Na^+ leak into the cell, an energy-dependent pump contributes to the resting potential by exchanging Na^+ for K^+ across the membrane. For every three ions of Na^+ pumped out by this **Na^+–K^+ pump**, two K^+ ions are pumped in, keeping the inside of the cell negative.

In summary, all cells are polarized at rest, having a difference in charge across their membranes. The potential is due to the uneven distribution of ions across the membrane, which occurs because ions move through relatively specific channels that normally are not open. K^+ has greater ability to move freely through ungated channels. Although all cells are polarized, what makes neurons different is that rapid changes in the membrane potential provide the means for neurons to conduct information; this, in turn, influences hundreds of other cells in the nervous system. The rapid change in membrane potential that is propagated down the length of the axon is called the **action potential**. For a cell to generate an action potential, the membrane potential must be changed from resting (–70 mV) to the **threshold** for firing (–50 mV). At –50 mV, voltage-gated Na^+ channels open, generating a rapid change in membrane potential. Before we look closely at the action potential, let's see what happens to a neuron to cause the membrane potential to change from resting to threshold.

Local potentials are small, transient changes in membrane potential

Although the membrane potential at rest is –70 mV, various types of stimuli that disturb the membrane can open ion channels momentarily, causing small, local changes in ion distribution and hence electrical potential differences called **local potentials**. To visualize the small changes in membrane potential, we attach our electrodes to an amplifier and to a computer that measures and records the changing voltage over time (**FIGURE 2.12**). For instance, applying a small, positive electrical current or momentarily opening gated Na^+

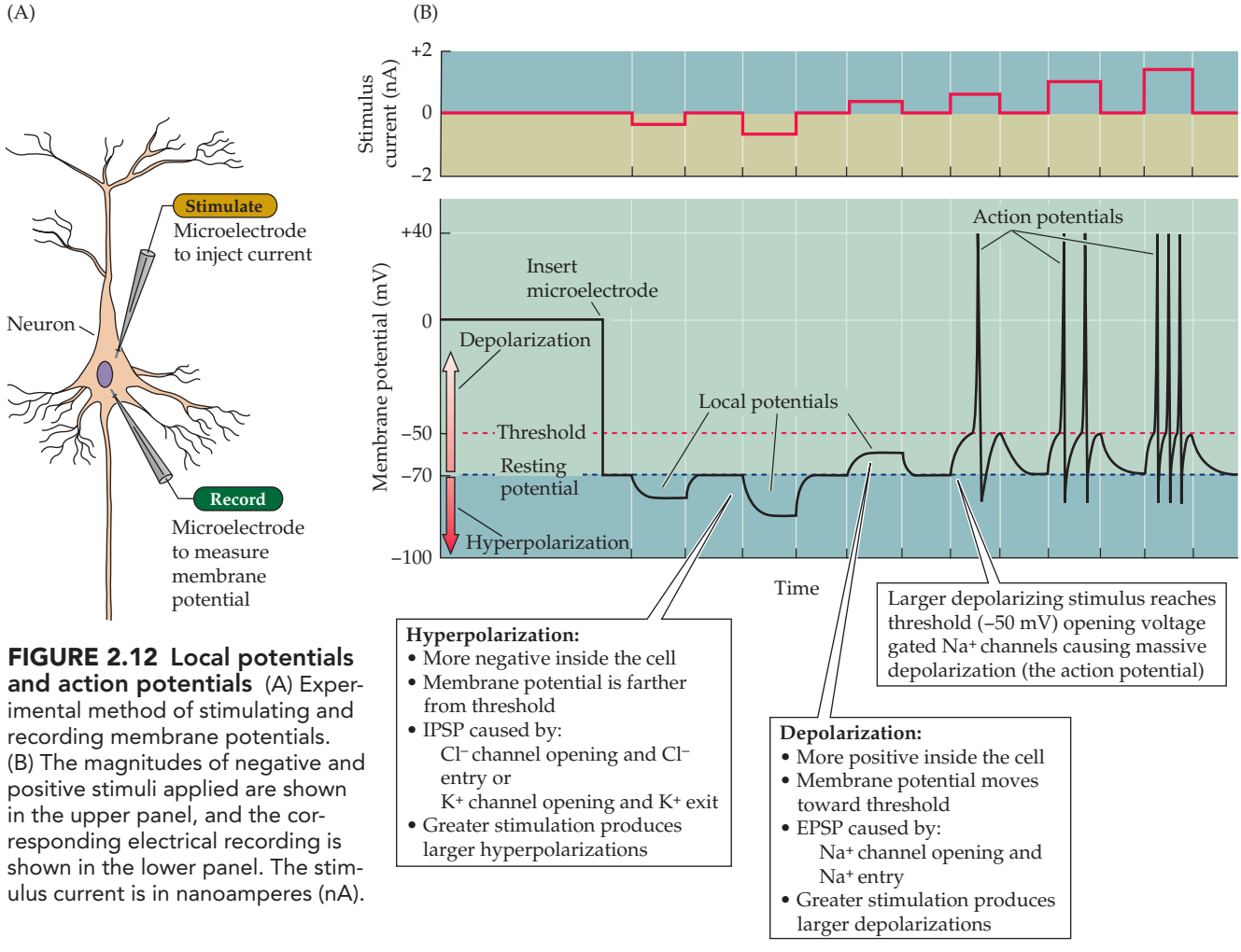


FIGURE 2.12 Local potentials and action potentials (A) Experimental method of stimulating and recording membrane potentials. (B) The magnitudes of negative and positive stimuli applied are shown in the upper panel, and the corresponding electrical recording is shown in the lower panel. The stimulus current is in nanoamperes (nA).

channels allows a relatively small number of Na^+ ions to enter the cell. These ions enter because Na^+ is more concentrated outside than inside, so the concentration gradient drives the ions in. The oscilloscope shows that positively charged ions make the inside of the cell slightly more positive in a small, localized area of the membrane, bringing the membrane potential a tiny bit closer to the threshold for firing. This change is called a local **depolarization** and is excitatory. Other stimuli may open Cl^- channels, which allow Cl^- into the cell because the ion's concentration is greater on the outside of the cell. The local increase in the negatively charged ion makes the cell slightly more negative inside and brings the resting potential farther away from threshold. This **hyperpolarization** of the membrane is inhibitory. Finally, if gated K^+ channels are opened by a stimulus, K^+ is driven outward locally on the basis of its concentration gradient. Because positively charged ions leave the cell, it becomes just slightly more negative inside, making the membrane potential farther from threshold and causing local hyperpolarization. These local potentials are of significance to psychopharmacology because when drugs or neurotransmitters bind to particular receptors in the nervous system, they may momentarily open specific ion channels (see Figure 2.8), causing an excitatory or inhibitory effect. Because neurotransmitters act on the postsynaptic membrane, the effects are called **excitatory postsynaptic potentials** (**EPSPs**) or **inhibitory postsynaptic potentials** (**IPSPs**).

These local potentials (hyperpolarizations and depolarizations), generated on the dendrites and cell body, have several significant characteristics. First, they are graded, meaning that the larger the stimulus, the greater is the magnitude of hyperpolarization or depolarization. Also, as soon as the stimulus stops, the ion channels close and the membrane potential returns to resting levels. These local potentials decay rapidly as they passively travel along the cell membrane. Finally, local potentials show summation, sometimes called **integration**, meaning that several small depolarizations can add up to larger changes in membrane potential, as several hyperpolarizations can produce larger inhibitory changes. When hyperpolarizations and depolarizations occur at the same time, they cancel each other out. The receptor areas of a neuron involved in local potential generation receive information from thousands of synaptic connections from other neurons that at any given instant produce IPSPs or EPSPs (as well as other biochemical changes to be described in Chapter 3). Integration of EPSPs and IPSPs occurs in the axon hillock (**FIGURE 2.13**) and is responsible for generation of the action potential if the threshold for activation is reached.

Sufficient depolarization at the axon hillock opens voltage-gated Na^+ channels, producing an action potential

The summation of local potentials at the axon hillock is responsible for generation of the action potential. The

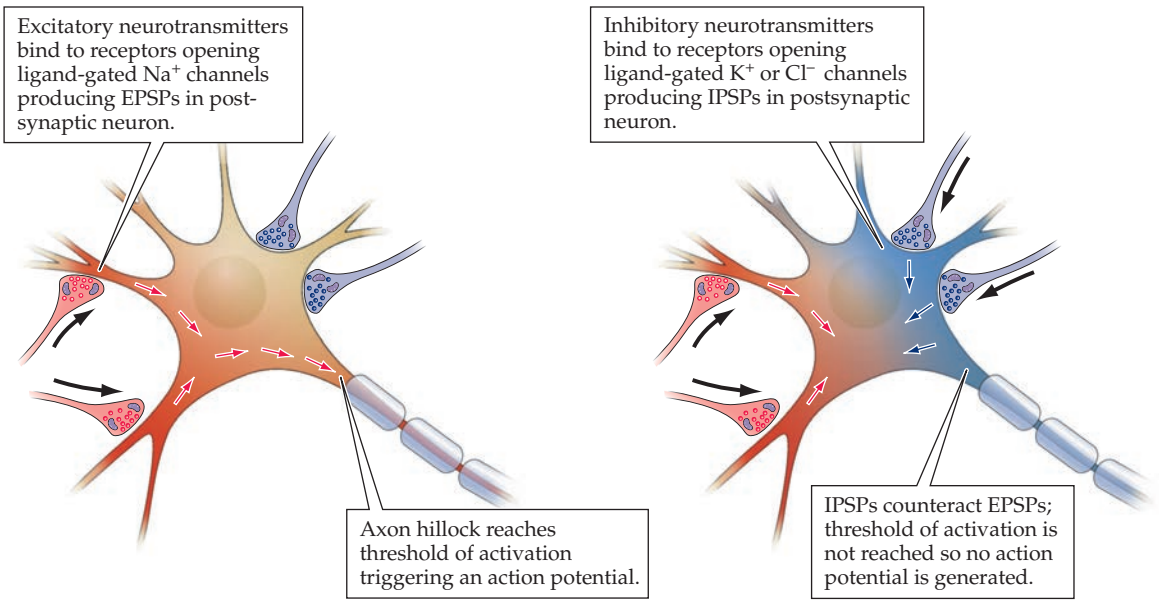


FIGURE 2.13 Summation of local potentials Many inhibitory and excitatory synapses influence each neuron, causing local electrical potentials (IPSPs and EPSPs) as well as biochemical changes. At each instant in time, the electrical potentials summate and may reach the threshold for firing. Integration of electrical events occurs at the axon hillock, where the action potential is first generated. The action potential is then conducted along the axon to the axon terminals.

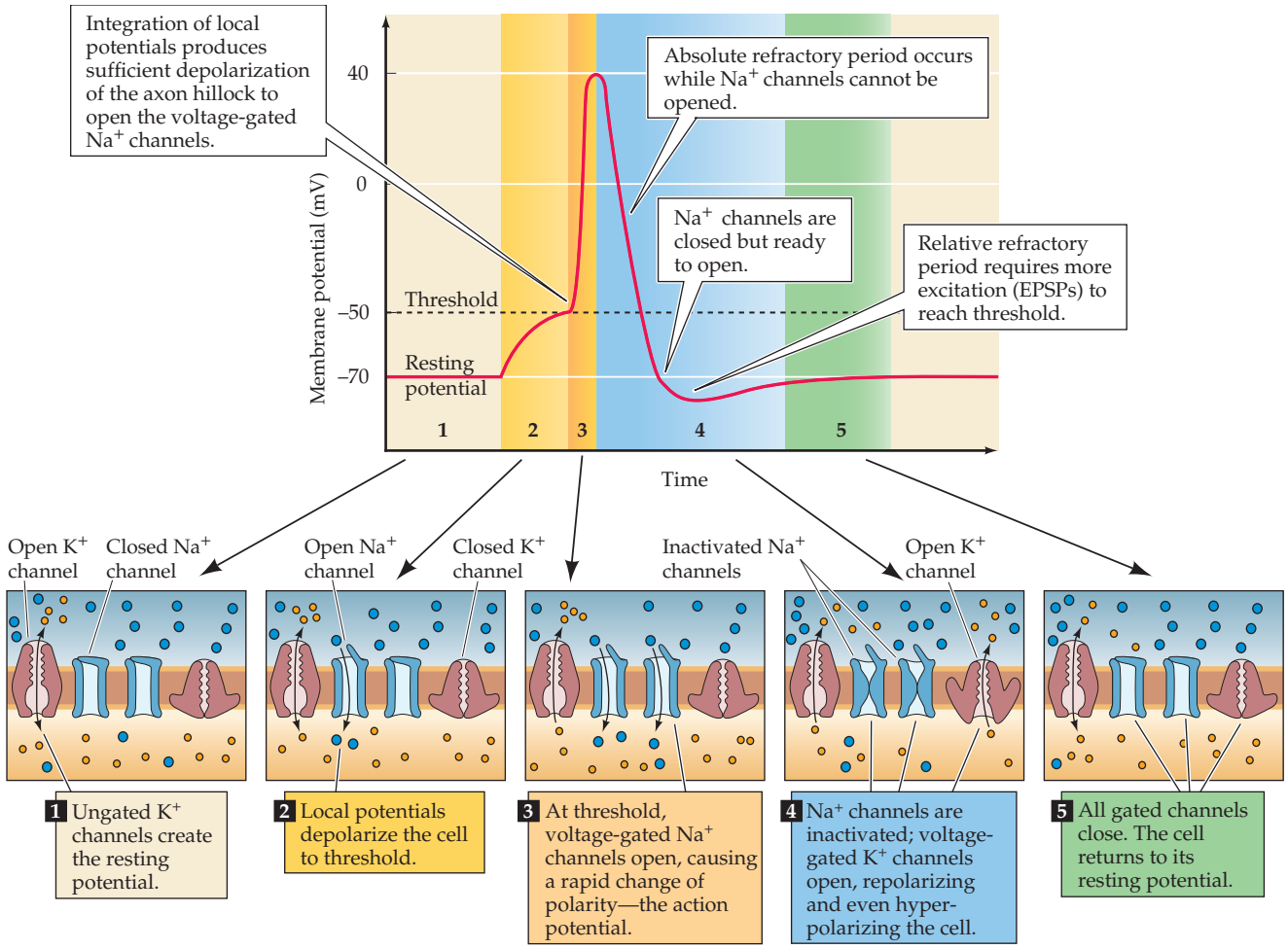


FIGURE 2.14 Stages of the action potential
Opening and closing of Na^+ and K^+ channels are responsible for the characteristic shape of the action potential.

–50 mV membrane potential (threshold) is responsible for opening large numbers of Na^+ channels that are voltage gated, that is, the change in voltage across the membrane near these channels is responsible for opening them (**FIGURE 2.14**). Because Na^+ is much more concentrated outside the cell, its concentration gradient moves it inward; in addition, since the cell at threshold is still negative inside, Na^+ is driven in by the electrostatic pressure. These two forces move large numbers of Na^+ ions into the cell very quickly, causing the rapid change in membrane potential from –50 mV to +40 mV (called *the rising phase of the action potential*) before the Na^+ channels close and remain closed for a fixed period of time while they reset. The time during which the Na^+ channels are closed and cannot be opened, regardless of the amount of excitation, prevents the occurrence of another action potential and is called the **absolute refractory period**. The closing of Na^+ channels explains why the maximum number of action potentials that can occur is about 1200 impulses per second. The action potential is a rapid change in membrane potential that lasts only about 1 millisecond. When the membrane potential approaches resting levels, the Na^+ channels are reset and ready to open.

Meanwhile, during the rising phase, the changing membrane potential due to Na^+ entry causes voltage-gated K^+ channels to open, and K^+ moves out of the cell. K^+ channels remain open after Na^+ channels have closed, causing the membrane potential to return to resting levels. The membrane potential actually overshoots the resting potential, so the membrane remains hyperpolarized for a short time until the excess K^+ diffuses away or is exchanged for Na^+ by the Na^+–K^+ pump. Because the membrane is more polarized than normal, it is more difficult to generate an action potential. The brief hyperpolarizing phase is called the **relative refractory period** because it takes more excitation to first reach resting potential and further depolarization to reach threshold. The relative refractory period explains why the intensity of stimulation determines rate of firing. Low levels

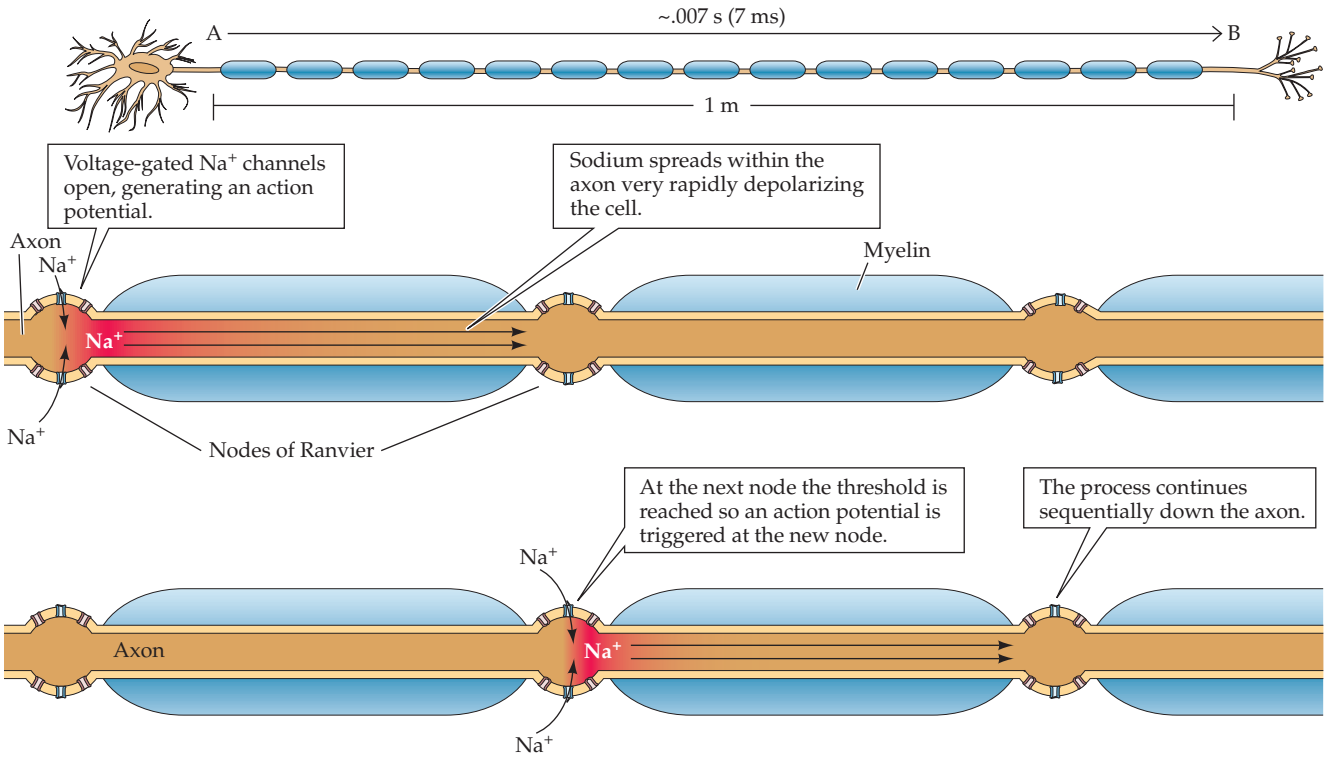


FIGURE 2.15 Conduction along myelinated axons
The generation of the action potential at one node spreads depolarization along the axon, which in turn changes the membrane potential to threshold and opens voltage-gated Na^+ channels at the next node of Ranvier.

of excitation cannot overcome the relative refractory period, but with increasing excitation, the neuron will fire again as soon as the absolute refractory period has ended.

If the threshold is reached, an action potential occurs (first at the hillock). Its size is unrelated to the amount of stimulation; hence it is considered *all-or-none*. Reaching the threshold will generate the action potential, but more excitatory events (EPSPs) will not make it larger; fewer excitatory events will not generate an action potential at all. The action potential moves along the axon because positively charged Na^+ ions spread passively to nearby regions of the axon, which changes the membrane potential to threshold and causes the opening of other voltage-gated Na^+ channels (**FIGURE 2.15**). The regeneration process of the axon potential continues sequentially along the entire axon and does not decrease in size; hence it is called *nondecremental* (i.e., it does not decay). In myelinated axons, the speed of conduction is as much as 15 times quicker than in nonmyelinated axons because regeneration of the action potential occurs only at the nodes of Ranvier. This characteristic makes the conduction seem to jump along the axon, so it is called **saltatory conduction**. In addition, myelinated axons use less energy because the Na^+–K^+ pump, which uses large amounts of ATP, has to work only at the nodes rather than all along the axon. Now that we understand normal neuron firing, it is worth a look at **Web Box 2.1**, which describes abnormal firing during epileptic seizures. The reader may also find the animations of electrophysiological processes on the Companion Website to be helpful. **TABLE 2.2** lists characteristics specific to local and action potentials.

Drugs and poisons alter axon conduction

As we will learn, most drugs act at synapses to modify chemical transmission. However, a few alter action potential conductance along the axon. Drugs that act as local anesthetics, such as procaine (Novocaine), lidocaine

TABLE 2.2 Characteristics of Local Potentials and Action Potentials

Local potentials	Action potentials
Graded	All-or-none
Decremental	Nondecremental
Spatial and temporal summation	Intensity of stimulus coded by rate of firing
Produced by opening of ligand-gated channels	Produced by opening of voltage-gated channels
Depolarization or hyperpolarization	Depolarization

(Xylocaine), and benzocaine (found in Anbesol), impair axonal conduction by blocking voltage-gated Na^+ channels. It should be apparent that if voltage-gated Na^+ channels cannot open, an action potential cannot occur, and transmission of the pain signal cannot reach the brain. Hence the individual is not aware of the damaging stimulus. Several antiepileptic drugs also block voltage-gated Na^+ channels but in a more subtle manner. One such drug is phenytoin (Dilantin), which apparently reduces Na^+ conduction during rapid, repeated, and sustained neuronal firing, a condition that characterizes seizure activity. This selectivity can occur because rather than blocking the channel, phenytoin selectively binds to closed Na^+ channels. Since it takes time for the drug to unbind, its presence prolongs the refractory state of the channel, slowing down the firing rate.

Neurotoxins that bind to Na^+ channels have much more striking effects. One of these is saxitoxin, which is a poison that blocks voltage-gated Na^+ channels throughout the nervous system when it is ingested. Saxitoxin is found in shellfish exposed to "red tide," a common event along the nation's coastlines that is caused by large concentrations of microscopic red dinoflagellates of the species *Gonyaulax*, a marine plankton that produces the neurotoxin. Oral ingestion circulates the toxin throughout the body and causes conduction failure and subsequent death due to suffocation. It is a hazard not only for humans, but also for fish, birds, and animals such as manatees. A second toxin, tetrodotoxin, is also found in several types of fish, including the Japanese puffer fish, which is considered one of the most poisonous vertebrates in the world. However, it is considered a delicacy in Japan, where chefs are specially trained in the art of preparing the fish without the toxic liver, gonads, and skin. Nevertheless, there are numerous poisonings each year caused by the binding of tetrodotoxin to the voltage-gated Na^+ channels, which prevents action potentials. Symptoms include facial numbness, nausea, vomiting, and abdominal pain followed by increasing paralysis, first of the limbs and then of the respiratory muscles. Cardiac dysfunction and coma can occur if the individual survives long enough. Death, which occurs in 50% of cases, can occur as soon as 4 to 6 hours after ingestion (Benzer, 2015).

Section Summary

- At rest, neurons have an electrical charge across the membrane of –70 mV (resting potential), with the inside being more negative than the outside.
- The resting potential results from the balance between two competing forces on K^+ ions. Electrostatic pressure moves K^+ inward because it is attracted by negatively charged molecules trapped inside the cell. The concentration gradient for K^+ pushes ions out of the cell in an effort to distribute them evenly.
- The Na^+–K^+ pump also helps to maintain the negative membrane potential by exchanging three Na^+ ions (moved out of the cell) for two K^+ ions (taken in).
- Local potentials are small, short-lived changes in membrane potential found largely on the soma and dendrites after the opening of ligand-gated channels.
- Excitatory postsynaptic potentials (EPSPs), or depolarizations, occur when ligand-gated Na^+ channels open and allow Na^+ to enter the cell on its concentration gradient, making the cell slightly more positive and bringing the membrane potential closer to the threshold for firing. Opening Cl^- channels allows Cl^- to enter on its concentration gradient, making the cell more negative and farther from the threshold, causing hyperpolarizations called inhibitory postsynaptic potentials (IPSPs). When ligand-gated K^+ channels open, K^+ exits on its concentration gradient, leaving the cell more negative inside and farther from the threshold producing an IPSP.
- The summation of all EPSPs and IPSPs occurring at any single moment in time occurs at the axon hillock. If the threshold (–50 mV) is reached, voltage-gated Na^+ channels open, allowing large amounts of Na^+ to enter the cell to produce the massive depolarization known as the action potential.
- At the peak of the action potential (+40 mV), voltage-gated Na^+ channels close and cannot be opened until they reset at the resting potential, so no action potential can occur during this time (the absolute refractory period).
- As the cell becomes more positive inside, voltage-gated K^+ channels open and K^+ exits from the cell, bringing the membrane potential back toward resting levels. The overshoot by K^+ causes the cell to be more polarized than normal, so it is more difficult to reach the threshold to generate another action potential (relative refractory period).
- The action potential moves down the length of the axon by sequential opening of voltage-gated Na^+ channels.
- In myelinated axons, regeneration of the action potential occurs only at the nodes of Ranvier, producing a rapid, saltatory conduction that is more energy efficient because the Na^+–K^+ pump needs to exchange ions only at the nodes.
- The characteristics of local and action potentials are summarized in Table 2.2.

Organization of the Nervous System

Thus far we have described the structure of individual neurons and their ability to conduct electrical signals. Clearly, neurons never function individually but form interacting circuits referred to as *neural networks*. Such complexity allows us to make coordinated responses to changes in the environment. For example, as we perceive a potential danger, we suddenly become vigilant and more acutely aware of our surroundings. Meanwhile, internal organs prepare us for action by elevating heart rate, blood pressure, available energy sources, and so forth. Most of us will also calculate the probable outcome of fighting or running before taking a defensive or aggressive stance. Even simple responses require complex coordination of multiple nuclei in the brain and spinal cord. The following section describes the organization of neurons into brain regions that serve specific functions. This section provides only the highlights of functional neuroanatomy and emphasizes those brain structures that receive more attention in subsequent chapters. **BOX 2.3** provides a quick review of the terms used to describe the location of structures in the nervous system.

BOX 2.3 The Cutting Edge

Finding Your Way in the Nervous System

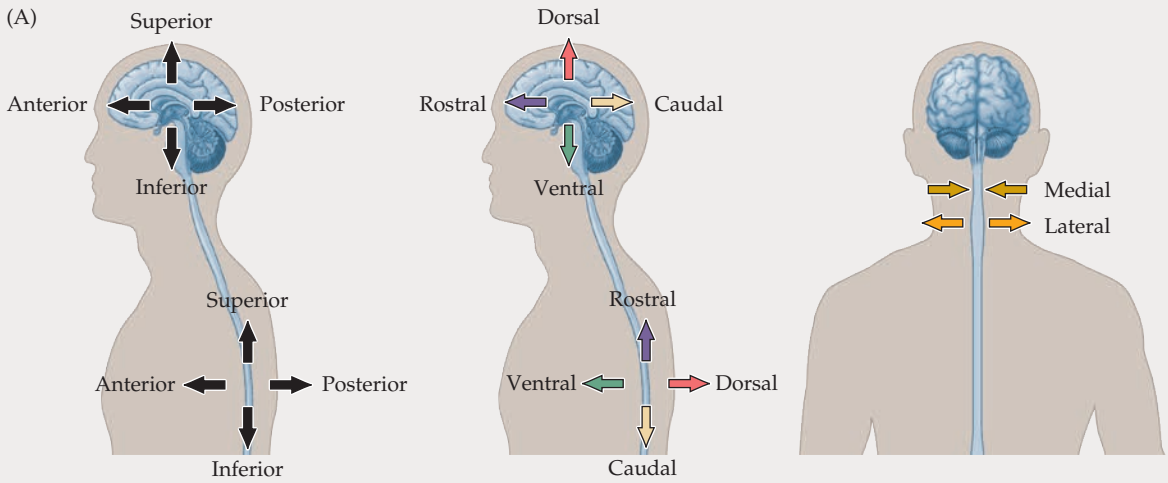
To discuss anatomical relationships, a systematic method to describe location in three dimensions is needed. The directions are based on the **neuraxis**, an imaginary line beginning at the base of the spinal cord and ending at the front of the brain. For most animals, the neuraxis is a straight line; however, because humans walk upright, the neuraxis bends, changing the relationship of the brain to the spinal cord (**Figure A**). For this reason, both the top of the head and the back of the body are called **dorsal**; **ventral** refers to the underside of the brain and the front surface of the body. To avoid confusion, sometimes the top of the human brain is described as **superior** and the bottom as **inferior**. In addition, the head end of the nervous system is **anterior** or **rostral**, and the tail end is **posterior** or **caudal**. Finally, **medial** means "toward the center or midline of the body," and **lateral** means "toward the side." We can describe the location of any brain area using three pairs of dimensional descriptors: dorsal/ventral, anterior/posterior and medial/lateral.

Much of our knowledge about the structure of the nervous system comes from examining two-dimensional slices (**Figure B**). The orientation of the slice (or **section**) is typically in any one of three different planes:

- **Horizontal** sections are slices parallel to the horizon.
- **Sagittal** sections are cut on the plane that bisects the nervous system into right and left halves. The **midsagittal** section is the slice that divides the brain into left and right symmetrical pieces.
- **Coronal** (or **frontal**) sections are cut parallel to the face.

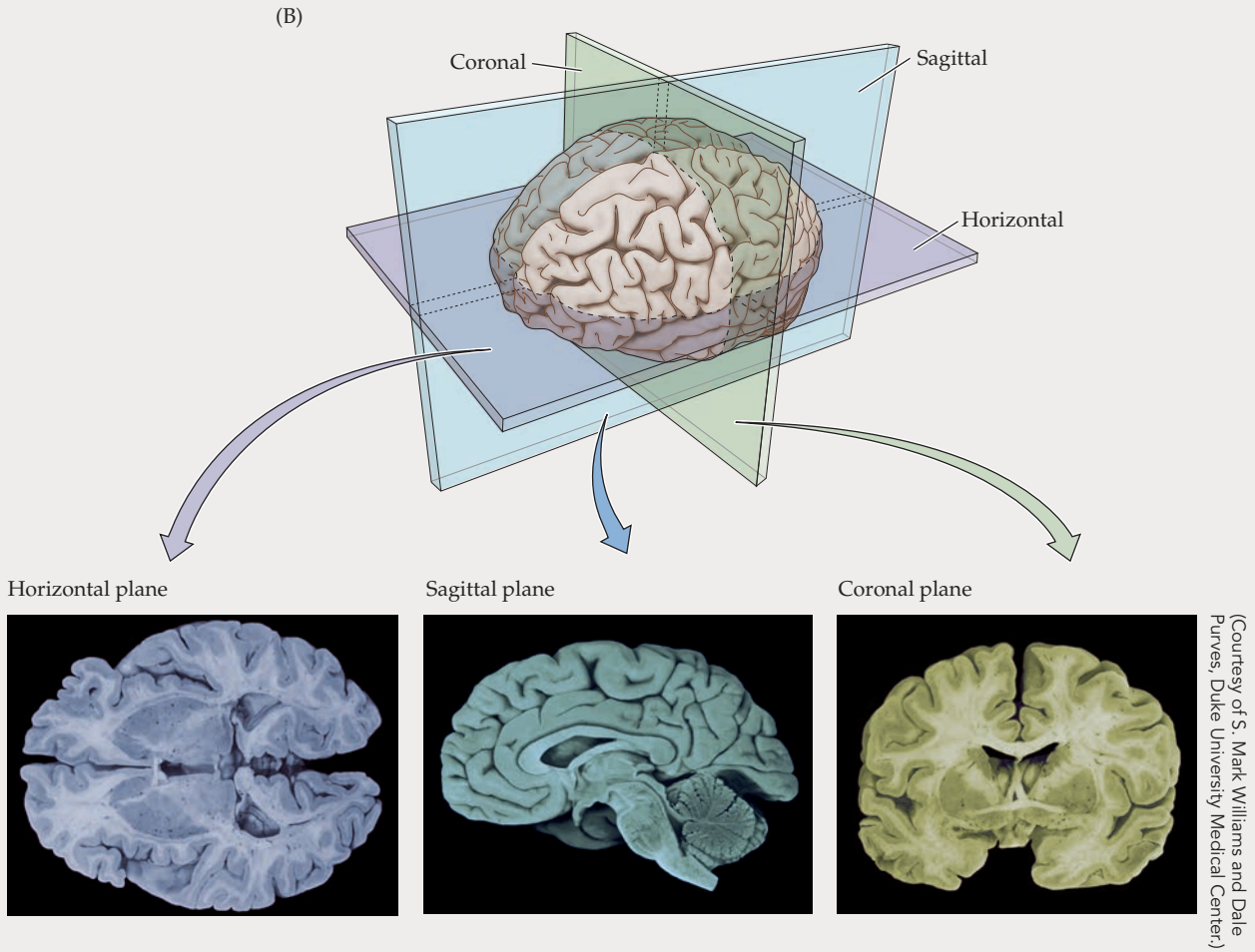
Identifying specific structures in these different views takes a good deal of experience. However, computer-assisted evaluation allows us to visualize

(*Continued*)



BOX 2.3 The Cutting Edge (*continued*)

the brain of a living human in far greater detail than was previously possible. Magnetic resonance imaging (MRI) and computerized tomography (CT) not only provide detailed anatomical images of brain slices but also reconstruct three-dimensional images of the brain using mathematical techniques. Positron emission tomography (PET) and functional MRI (fMRI) provide a view of the functioning brain by mapping blood flow or glucose utilization in various disease states, after drug administration, and during other experimental manipulations. These visualization techniques are described in Chapter 4, the chapter on methods in research.



(Courtesy of S. Mark Williams and Dale Purves, Duke University Medical Center.)

The nervous system comprises the central and peripheral divisions

The nervous system includes the central nervous system, or CNS (the brain and spinal cord), and the peripheral nervous system, or PNS (all nerves outside the CNS) (**FIGURE 2.16A**). The PNS in turn can be further divided into the somatic system, which controls voluntary muscles with both spinal nerves and cranial nerves, and the autonomic nervous system, which consists of autonomic nerves and some cranial nerves that control the function of organs and glands. The autonomic nervous system has both sympathetic and parasympathetic divisions, which help the organism to respond to changing energy demands. **FIGURE 2.16B** provides an overall view of the divisions of the nervous system. We begin by looking more closely at the peripheral nervous system.

SOMATIC NERVOUS SYSTEM Each spinal nerve consists of many neurons, some of which carry sensory information and others motor information; hence they are called *mixed nerves*. Within each mixed nerve, sensory information is carried from the surface of the body and from muscles into the dorsal horn of the spinal cord by neurons that have their cell bodies in the dorsal root ganglia (**FIGURE 2.17**). These signals going into the

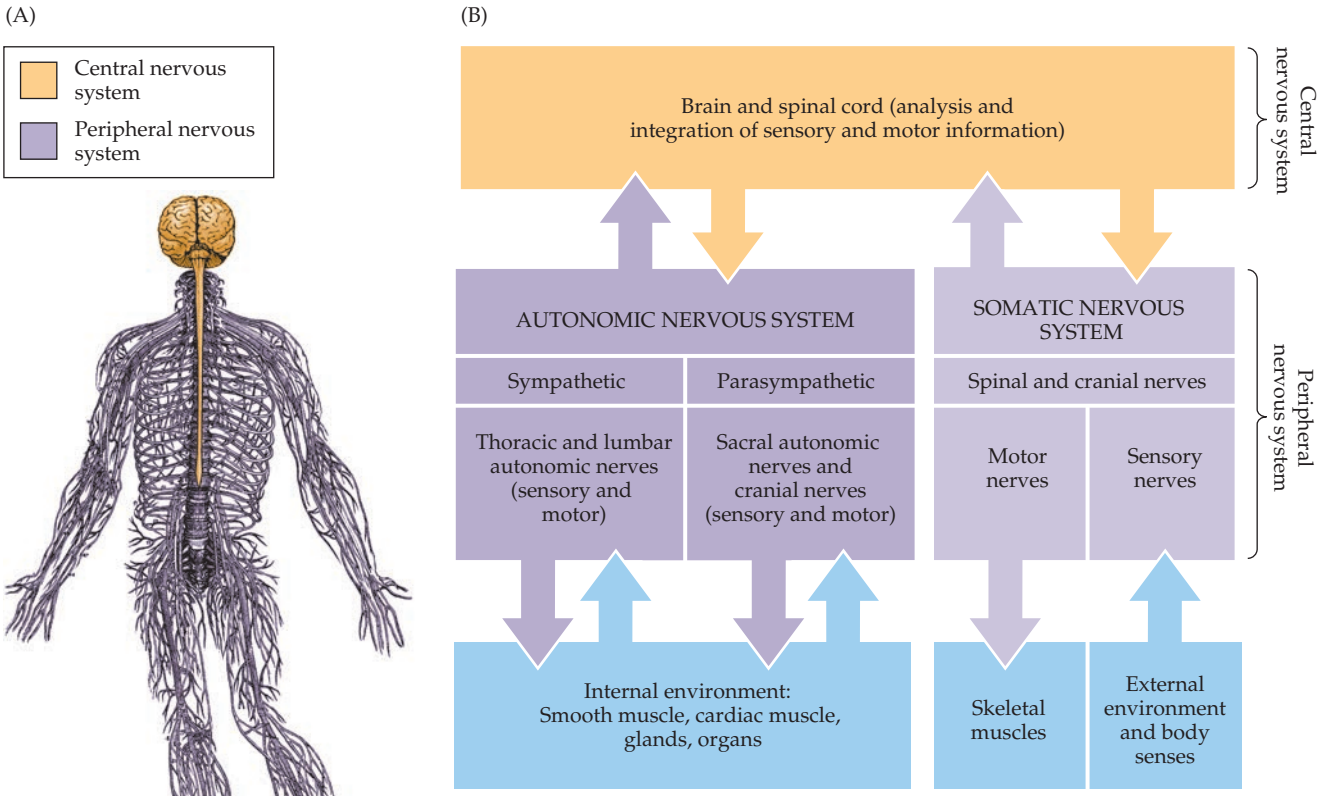


FIGURE 2.16 The central and peripheral nervous systems (A) The brain and spinal cord, shown in yellow, make up the CNS. The peripheral nervous system, shown in purple, connects all parts of the body to the CNS. (B) Organization of the nervous system: internal and external environments send sensory information by way of peripheral nerves to the CNS, where neural circuits analyze and integrate the information before sending signals to regulate muscle and internal organ function.

spinal cord are called **sensory afferents**. Mixed nerves also have motor neurons, which are cells beginning in the ventral horn of the spinal cord and ending on skeletal muscles. These are called **motor efferents** and are responsible for voluntary movements.

The 12 pairs of cranial nerves that project from the brain provide functions similar to those provided by the spinal nerves, except that they serve primarily the head and neck; hence they carry sensory information such as vision, touch, and taste into the brain and control muscle movement needed for things like chewing and laughing. They differ from the spinal nerves in that they are not all mixed nerves; several are dedicated to only sensory or only motor function. In addition, several of the cranial nerves innervate glands and organs rather than skeletal muscles; this means that they are part of the autonomic nervous system (see the next section). The vagus nerve (cranial nerve X) is unique among the

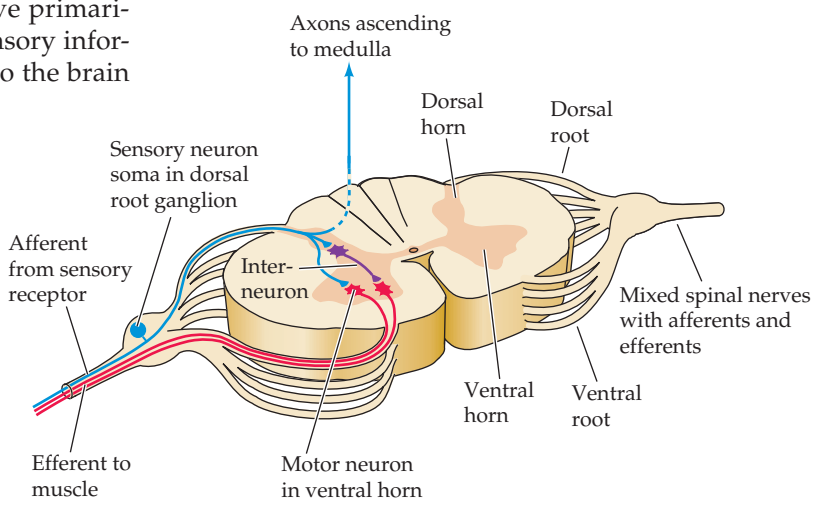


FIGURE 2.17 Spinal nerves of the peripheral nervous system Cross section of the spinal cord shows mixed spinal nerves with sensory afferents entering the dorsal horn and motor efferents leaving the ventral horn to innervate skeletal muscles. Note that the soma for the afferent neuron is in the dorsal root ganglion.

cranial nerves because it communicates with numerous organs in the viscera, including the heart, lungs, and gastrointestinal tract. The vagus consists of both sensory and motor neurons.

AUTONOMIC NERVOUS SYSTEM The autonomic nerves, collectively called the *autonomic nervous system* (ANS), regulate the internal environment by innervating smooth muscles such as those in the heart, intestine, urinary bladder, and glands, including the adrenal and salivary glands. The purpose of the ANS is to control digestive processes, blood pressure, body temperature, and other functions that provide or conserve energy appropriate to the environmental needs of the organism. The ANS is divided into two components, the sympathetic and parasympathetic divisions, and both divisions serve most organs of the body (**FIGURE 2.18**). Although their functions usually work in opposition to one another, control of our internal environment is not an all-or-none affair. Instead, activity of the **sympathetic** division predominates when energy expenditure is necessary, such as during times of stress, excitement, and exertion; hence its nickname is the "fight-or-flight" system. This system increases heart rate and blood pressure, stimulates secretion of adrenaline, and increases blood flow to skeletal muscles, among other effects. The **parasympathetic** division predominates at times when energy reserves can be conserved and stored for later use; hence this system increases salivation, digestion, and storage of glucose and other nutrients and also slows heart rate and decreases respiration.

In addition to contrasting functions, the two branches of the ANS have anatomical differences, including points of origin in the CNS. The cell bodies of the efferent sympathetic neurons are in the ventral horn of the spinal cord at the thoracic and lumbar regions (see Figure 2.18). Their axons project for a relatively short distance before they synapse with a cluster of cell bodies called *sympathetic ganglia*. Some of these ganglia are lined up very close to the spinal cord; others such as the celiac ganglion are located somewhat farther away. These preganglionic fibers release the neurotransmitter acetylcholine onto cell bodies in the ganglia. These postganglionic cells project their axons for a relatively long distance to the target tissues, where they release the neurotransmitter norepinephrine.

In contrast, the cell bodies of the efferent parasympathetic neurons are located either in the brain (cranial nerves III, VII, IX, and X) or in the ventral horn of the spinal cord at the sacral region. Preganglionic neurons travel long distances to synapse on cells in the parasympathetic ganglia that are not neatly lined up along the spinal cord but are close to individual target organs. The preganglionic fibers release acetylcholine, just as the sympathetic preganglionics do. However, parasympathetic postganglionic neurons, which are quite short, also release acetylcholine. Understanding the autonomic nervous system is especially important for psychopharmacologists because many psychotherapeutic drugs alter either norepinephrine or acetylcholine in the brain to relieve symptoms, but by altering those same neurotransmitters in the peripheral nerves, these drugs often produce annoying or dangerous side effects, such as elevated blood pressure, dry mouth, and urinary problems (all related to autonomic function). **TABLE 2.3** summarizes the differences between the two divisions of the ANS.

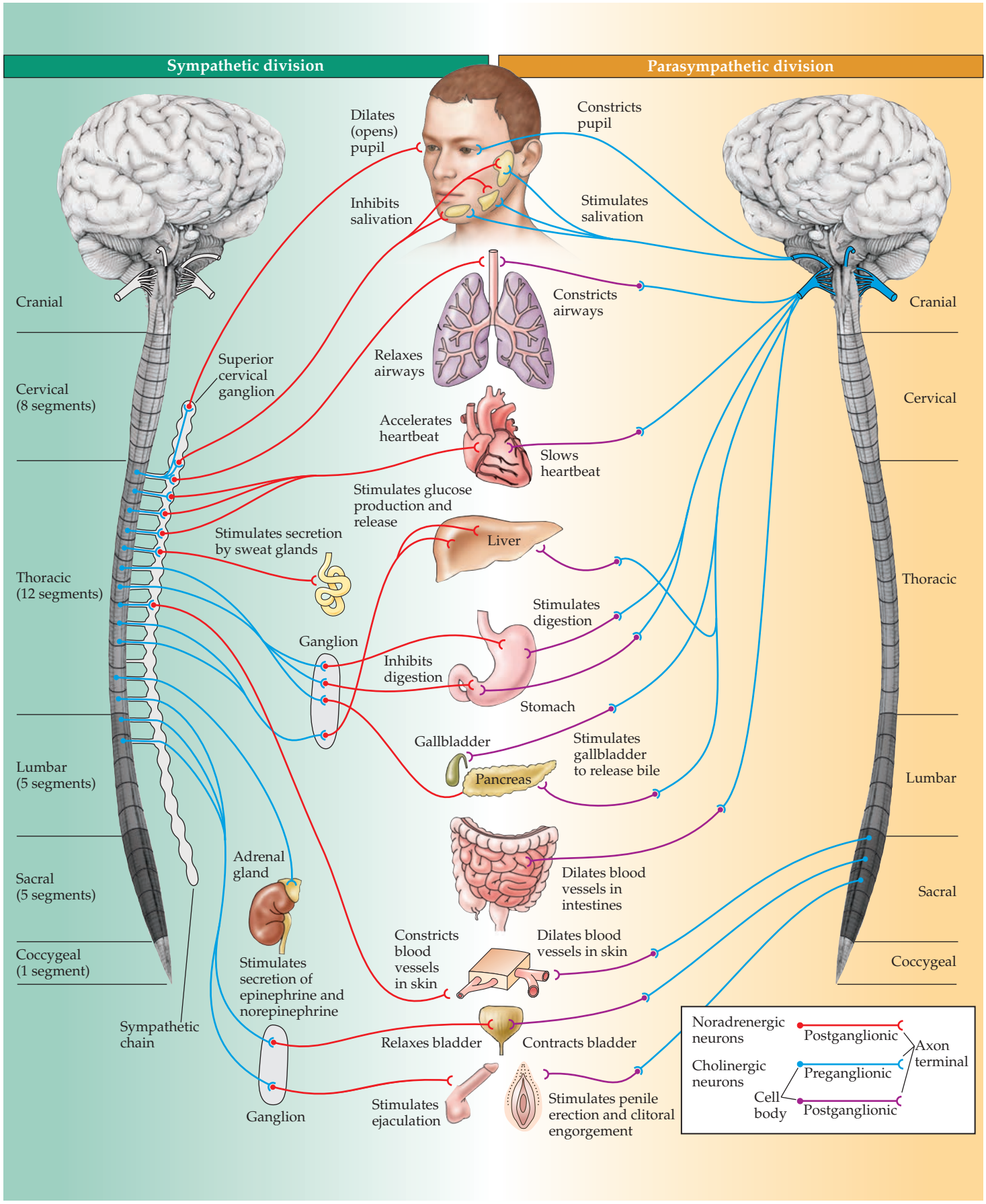
FIGURE 2.18 Autonomic nervous system (ANS) ▶
The internal organs, smooth muscles, and glands served by the ANS have both sympathetic and parasympathetic regulation. The two divisions have opposing effects on the organs; the sympathetic effects prepare the individual for action, and the parasympathetic effects serve to generate and store energy and reduce energy expenditure. Acetylcholine is the neurotransmitter released in all autonomic ganglia because preganglionic fibers are cholinergic neurons. At the target organs, the parasympathetic neurons release acetylcholine once again, while sympathetic neurons (noradrenergic neurons) release norepinephrine. Their anatomical and neurotransmitter differences are described in the text and are summarized in Table 2.3.

CNS functioning is dependent on structural features

The tough bone of the skull and vertebrae maintains the integrity of the delicate tissue of the brain and spinal cord. Three layers of tissue called **meninges** lie just within the bony covering and provide additional protection. The outermost layer, which is also the toughest, is the **dura mater**. The **arachnoid**, just below the dura, is a membrane with a weblike sublayer (subarachnoid space) filled with cerebrospinal fluid (CSF). Finally, the **pia mater** is a thin layer of tissue that sits directly on

TABLE **2.3** Characteristics of the Sympathetic and Parasympathetic Divisions of the ANS

Sympathetic	Parasympathetic
Energy mobilization	Energy conservation and storage
Origin in thoracic and lumbar spinal cord	Origin in the brain and sacral spinal cord
Relatively short preganglionic fibers; long postganglionics	Long preganglionic fibers ending near organs; short postganglionics
Releases acetylcholine in ganglia and norepinephrine at target	Releases acetylcholine at both ganglia and target


Sympathetic division
Parasympathetic division
Dilates (opens) pupil
Constricts pupil
Inhibits salivation
Stimulates salivation
Cranial
Constricts airways
Relaxes airways
Superior cervical ganglion
Cervical (8 segments)
Accelerates heartbeat
Slows heartbeat
Stimulates glucose production and release
Liver
Stimulates secretion by sweat glands
Thoracic (12 segments)
Stimulates digestion
Ganglion
Inhibits digestion
Stomach
Gallbladder
Stimulates gallbladder to release bile
Pancreas
Lumbar (5 segments)
Adrenal gland
Dilates blood vessels in intestines
Sacral (5 segments)
Constricts blood vessels in skin
Dilates blood vessels in skin
Coccygeal (1 segment)
Stimulates secretion of epinephrine and norepinephrine
Sympathetic chain
Relaxes bladder
Contracts bladder
Ganglion
Stimulates ejaculation
Stimulates penile erection and clitoral engorgement
Noradrenergic neurons
Postganglionic
Axon terminal
Cholinergic neurons
Preganglionic
Cell body
Postganglionic

the nervous tissue. Some readers may have heard of the type of cancer called meningioma. It should now be apparent to you that this cancer is a tumor that forms on these layers of tissue protecting the brain and spinal cord. The vast majority of tumors of this type are benign (i.e., nonmalignant) and slow growing and often require no treatment unless their growth causes significant symptoms such as headaches, double vision, loss of smell, or weakness. The symptoms that occur are dependent on their anatomic location and the compression of nearby structures.

The CSF not only surrounds the brain and spinal cord but also fills the irregularly shaped cavities within the brain, called **cerebral ventricles**, and the channel that runs the length of the spinal cord, called the **central canal**. CSF is formed by the choroid plexus within the lateral ventricle of each hemisphere and flows to the third and fourth ventricles before moving into the subarachnoid space to bathe the exterior of the brain and spinal cord (see Figure 1.7A). When this flow of CSF is impeded by a tumor, infection, or congenital abnormalities, the fluid builds up in the brain, causing compression of the delicate neural tissue surrounding the ventricles. This condition is called hydrocephalus. The brain compression produces a variety of symptoms, including nausea and vomiting, blurred vision, problems with balance and coordination, drowsiness, and memory loss. For a thorough description of the disorder, see the NIH *Hydrocephalus Fact Sheet* (NIH, 2013). A brief video from the Boston Children's Hospital describes hydrocephalus and the common neurosurgical treatment (Warf, 2011).

CSF not only protects the brain but also helps in the exchange of nutrients and waste products between the brain and the blood. This exchange is possible because the capillaries found in the choroid plexus do not have the tight junctions typical of capillaries in the brain. These tight junctions constitute the blood–brain barrier, a vital mechanism for protecting the delicate chemical balance in the CNS. Return to Chapter 1 for a review of the blood–brain barrier.

The CNS has six distinct regions reflecting embryological development

The six anatomical divisions of the adult CNS are evident in the developing embryo. It is important to know about the development of the brain because exposure to harmful events, including therapeutic and illicit drugs, environmental toxins, and stress, will have different outcomes depending on the timing of the insult and the developmental event occurring at that time. You will read more about this in later chapters on clinical disorders and in the chapter on alcohol. The CNS starts out as a fluid-filled tube that soon develops three enlargements at one end that become the adult hindbrain, midbrain, and forebrain, while the remainder of the neural tube becomes the spinal cord (**FIGURE 2.19A**). The structural organization of these regions reflects the hierarchical nature of their functions. Each level has overlapping functions, and higher levels partially replicate the functions of lower ones but provide increased behavioral complexity and refined nervous system control. The fluid-filled chamber itself becomes the ventricular system in the brain and the central canal in the spinal cord. Within 2 months of conception, further subdivisions occur: the hindbrain enlargement develops two swellings, as does the forebrain. These divisions, in ascending order, are the spinal cord, myelencephalon, metencephalon, mesencephalon, diencephalon, and telencephalon. Each region can be further subdivided into clusters of cell bodies, called **nuclei**, and their associated bundles of axons, called **tracts**. (In the PNS, they are called **ganglia** and **nerves**, respectively.) These interconnecting networks of cells will be the focus of much of the remainder of this book, because drugs that alter brain function, that is, psychotropic drugs, modify the interactions of these neurons. The principal divisions of the CNS are summarized in **FIGURE 2.19B,C**. You may be interested in seeing a brief animation of brain development on the Companion Website.

NEUROTROPHIC FACTORS Neurotrophic factors are proteins that act as neuron growth factors and influence not only neuron growth, but also cell differentiation and survival. Nervous system development and maintenance of synaptic connections over the life span are dependent on the presence of neurotrophic factors such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3, and neurotrophin-4/5. Although similar in structure and general function, neurotrophins show some specificity. For example, NGF, the first neurotrophic factor to be discovered, is synthesized and secreted by peripheral target organs and guides the development of axonal processes of nearby neurons to establish synaptic connections with the target organ. Additionally, the presence of neurotrophic factors determines which neuronal connections survive and which are unnecessary and are eliminated by cell death. Apparently, the large population of neurons competes for the limited amount of neurotrophic factor in the target tissue, and those that are not supported by access to neurotrophins die, while those that respond to NGF establish appropriate synaptic connections. This process ensures that the number of connections is appropriate for the target tissue. NGF guides the growth of sympathetic neurons and a subpopulation of sensory ganglion cells. A second neurotrophic factor, BDNF, in the periphery is released from skeletal muscles and guides motor neuron development and survival. Other neurotrophins aid the survival of other subsets of peripheral sensory neurons. It is also clear that glial Schwann cells, which myelinate neurons of the peripheral nervous system,

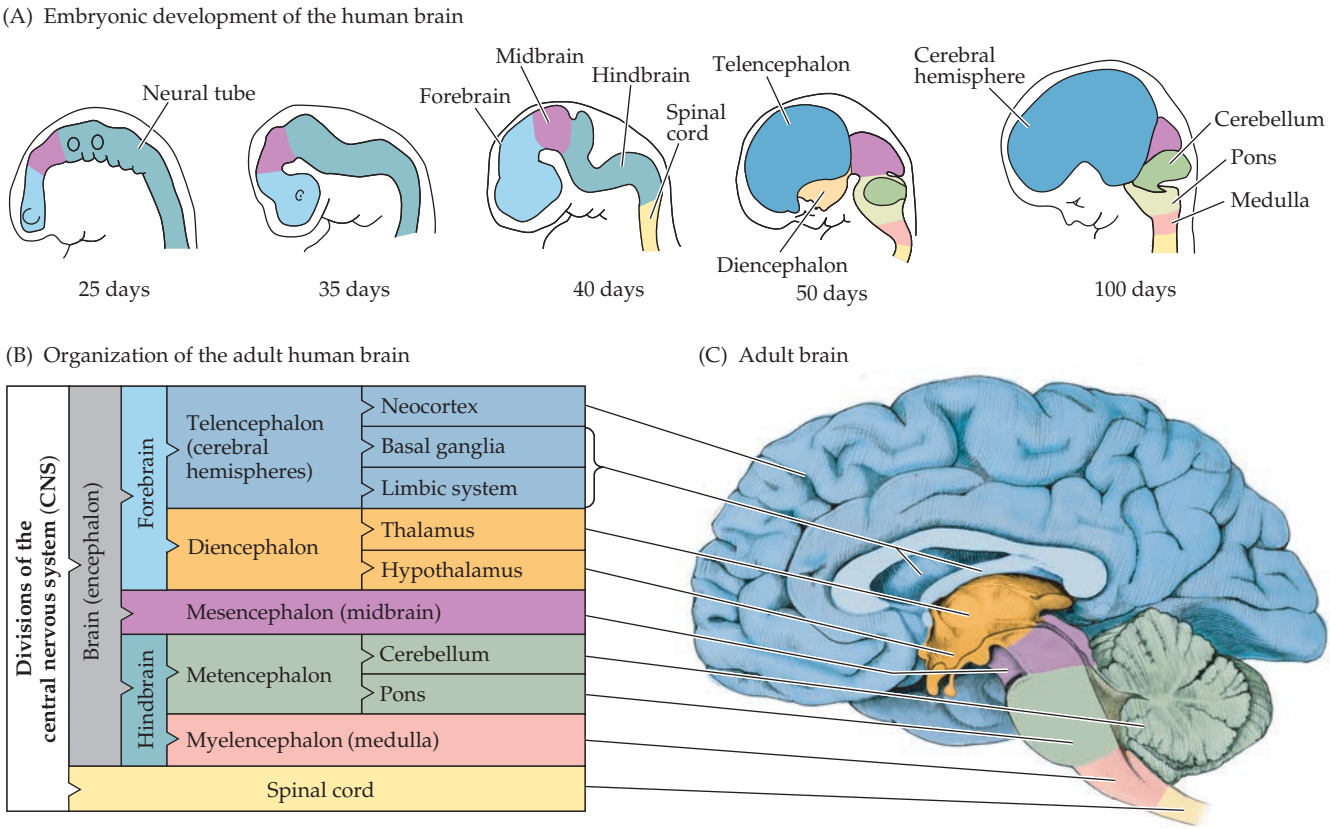


FIGURE 2.19 Divisions of the central nervous system (A) Beginning with the primitive neural tube in the human embryo, the CNS develops rapidly. By day 50 of gestation, the six divisions of the adult CNS are apparent in the fetus. (B) The organization of the CNS (brain and spinal cord) presented in the table is color-coded to match the divisions shown in the adult brain (sagittal section) (C).

release a growth factor when their axon is damaged. The growth factor in this case leads to regeneration of the damaged axon. In the CNS, neurotrophins like BDNF may be released by target neurons (rather than organs) to maintain appropriate synaptic connections, but in some cases, the neurotrophin acts on the same neuron that produces it for autoregulation. Additionally, in some cases, neurotrophic factors are transported from the soma to the axon terminals, where their release modifies nearby cell bodies or nerve terminals of other neurons. For example, neurotrophins determine which of the cell dendrites grow and which retract, a process that influences synaptic activity and plasticity, quite independent of their role in cell survival. Research into the involvement of neurotrophic factors in mood disorders has suggested the neurotrophic hypothesis of depression and other psychiatric disorders. The importance of neurotrophic factors as potential therapeutic agents for neurodegenerative diseases such as Alzheimer's disease (see Chapter 20) and psychiatric disorders (see Chapter 18) will be discussed further in later chapters.

SPINAL CORD The spinal cord is made up of gray and white matter. The former appears butterfly shaped in cross section (**FIGURE 2.20A**) and is called *gray matter* because the large numbers of cell bodies in this region appear dark on histological examination. The cell bodies in the dorsal horn receive information from sensory afferent neurons entering the spinal cord and cell bodies of motor neurons in the ventral horn send efferents to skeletal muscles. The white matter surrounding the butterfly-shaped gray matter is made up of myelinated axons of ascending pathways that conduct sensory information to the brain, as well as myelinated axons of descending pathways from higher centers to the motor neurons that initiate muscle contraction (**FIGURE 2.20B**).

As we move up the spinal cord and enter the skull, the spinal cord enlarges and becomes the **brainstem**. Examination of the ventral surface of the brain (**FIGURE 2.21A**) shows that the brainstem with its three principal parts—medulla, pons, and midbrain—is clearly visible. The brainstem contains the reticular formation, a large network of cells and interconnecting fibers that extends up the core of the brainstem for most of its length (described later in the section on the metencephalon). Additionally, the brainstem is the origin of numerous cranial nerves that receive sensory information from the skin and joints of the face, head, and neck, as well as providing motor control to the

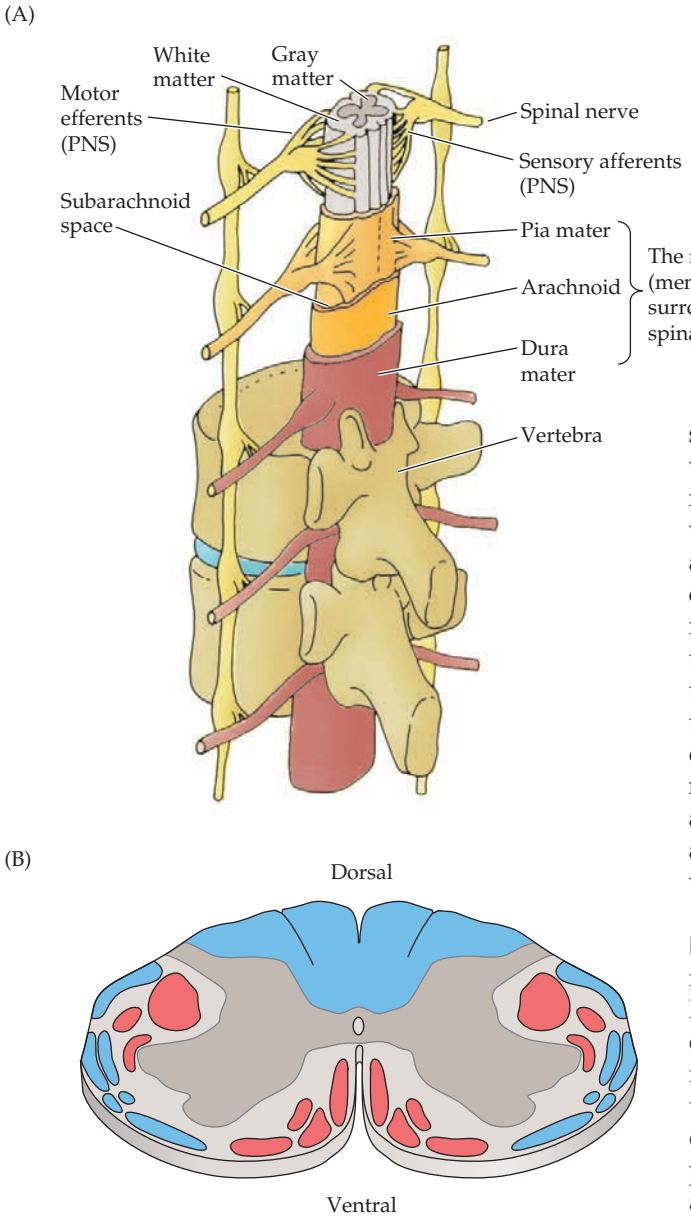


FIGURE 2.20 Spinal cord (A) A view of the spinal cord showing its relationship to the protective layers of meninges and the bony vertebrae. Note the clearly defined gray matter and white matter in cross section. (B) Schematic diagram of the ascending sensory tracts (blue) and the descending motor tracts (red).

muscles in that region. A significant volume of the brainstem is made up of ascending and descending axons coursing between the spinal cord and higher brain regions. The relationships of the structures of the brainstem are apparent in the midsagittal view (**FIGURE 2.21B**).

MYELENCEPHALON The first major structure of the brainstem that we encounter is the myelencephalon, or medulla. Within the **medulla**, multiple cell groups regulate vital functions, including heart rate, digestion, respiration, blood pressure, coughing, and vomiting. When an individual dies from a drug overdose, the cause is most often depression of the respiratory center in the medulla. Also located in the medulla is the **area postrema**, or the vomiting center, described in Chapter 1 as a cluster of cells with a reduced blood–brain barrier that initiates vomiting in response to toxins in the blood. Drugs in the opioid class such as morphine act on the area postrema and produce vomiting, a common unpleasant side effect of treatment for pain. The nuclei for cranial nerves XI and XII, which control the muscles of the neck and tongue, are also located in the medulla. One portion of the medulla contains the medullary pyramids. These pyramids (two ridgelike structures) contain motor fibers that start in the motor region of the cerebral cortex and descend to the spinal cord, so they are named the corticospinal tract. These neurons synapse on spinal motor neurons described earlier. From 80% to 90% of the corticospinal neurons cross to the other side of the brain in the pyramids, explaining why the left motor cortex controls all voluntary movement on the right side of the body while the right motor cortex controls the left.

METENCEPHALON Two large structures within the metencephalon are the pons and the cerebellum (see Figure 2.21). Within the central core of the pons and extending rostrally into the midbrain and caudally into the medulla is the **reticular formation**. The reticular formation is not really a structure but a collection of perhaps 100 small nuclei forming a network that plays an important role in arousal, attention, sleep, and muscle tone, as well as in some cardiac and respiratory reflexes. One nucleus, called the **locus coeruleus**, is of particular importance to psychopharmacology because it is a cluster of cell bodies that distribute their axons to many areas of the forebrain. These cells are the principal source of all neurons that release the neurotransmitter norepinephrine from their terminals. When active, these cells cause arousal, increased vigilance, and attention. Drugs such as amphetamine enhance their function, causing sleeplessness and enhanced alertness.

Other cell groups within the pons that also belong to the reticular formation are the **dorsal** and **median raphe nuclei**. These two clusters of cells are the source of most of the neurons in the CNS that use serotonin as their neurotransmitter. Together, cell bodies in the dorsal and median raphe send axons releasing serotonin to virtually all forebrain areas and function in the regulation of diverse processes, including sleep,

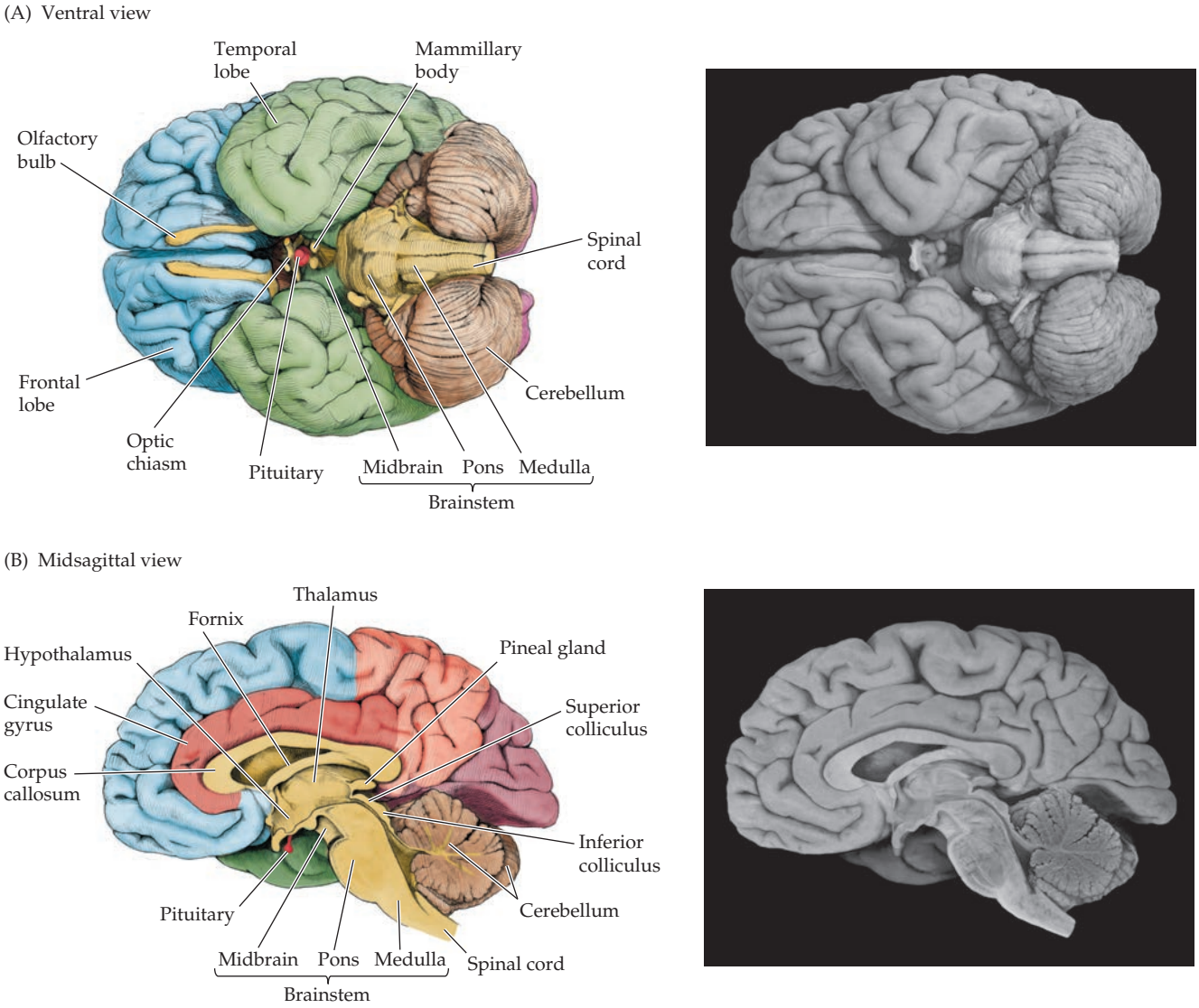


FIGURE 2.21 Two views of the human brain
The drawings on the left in each panel label the structural features that are visible on the ventral external surface (A) and the midsagittal (midline) section (B). The right side of each panel shows the same view of a human postmortem brain specimen. (Courtesy of S. Mark Williams and Dale Purves, Duke University Medical Center.)

aggression and impulsiveness, neuroendocrine functions, and emotion. Because it has a generally inhibitory effect on CNS function, serotonin may maintain behaviors within specific limits. Drugs such as LSD (lysergic acid diethylamide) produce their dramatic hallucinogenic effects by inhibiting the inhibitory functions of the raphe nuclei (see Chapter 15).

The **cerebellum** is a large foliated structure on the dorsal surface of the brain that connects to the pons by several large bundles of axons called **cerebellar peduncles**. The cerebellum is a significant sensorimotor center and receives visual, auditory, and somatosensory input, as well as information about body position and balance, from the vestibular system. By coordinating sensory information with motor information received from the cerebral cortex, the cerebellum coordinates and smoothes out movements by timing and patterning skeletal muscle contractions. In addition, the cerebellum allows us to make corrective movements to maintain our balance and posture. Damage to the cerebellum produces poor coordination and jerky movements. Drugs such as alcohol at moderate doses inhibit the function of the cerebellum and cause slurred speech and staggering.

MESENCEPHALON The midbrain has two divisions: the tectum and the tegmentum. The tectum, the dorsalmost structure, consists of the superior colliculi, which are part of the visual system, and the inferior colliculi, which are part of the auditory system (see Figure 2.21B). These nuclei are involved in reflexes such as the

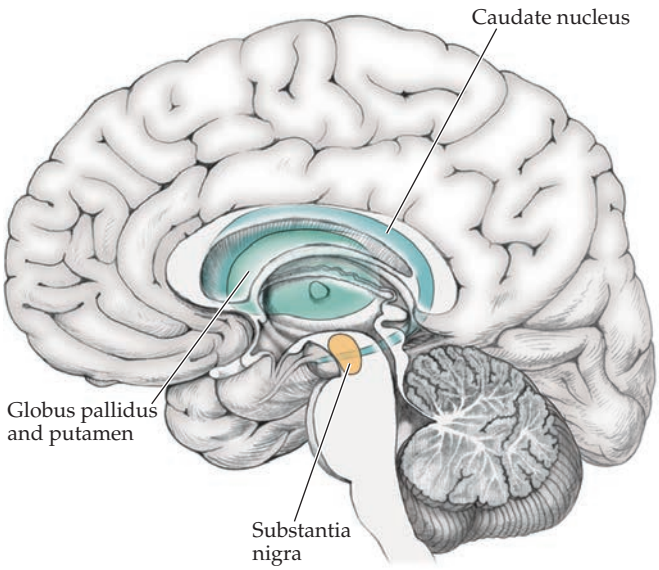


FIGURE 2.22 The basal ganglia These four structures form neural pathways that utilize dopamine as their neurotransmitter. These neural circuits constitute a system for motor control.

pupillary reflex to light, eye movement, and reactions to moving stimuli.

Within the **tegmentum** are several structures that are particularly important to psychopharmacologists. The first is the **periaqueductal gray** (**PAG**), which surrounds the cerebral aqueduct that connects the third and fourth ventricles. The PAG is one of the areas that are important for the modulation of pain. Local electrical stimulation of these cells produces analgesia but no change in the ability to detect temperature, touch, or pressure. The PAG is rich in opioid receptors, making it an important site for morphine-induced analgesia. Chapter 11 describes the importance of natural opioid neuropeptides and the PAG in pain regulation. The PAG is also important in sequencing species-specific actions, such as defensive rage and predation.

The **substantia nigra** is a cluster of cell bodies whose relatively long axons innervate the striatum, a component of the basal ganglia (**FIGURE 2.22**). These cells constitute one of several important neural pathways that utilize dopamine as their neurotransmitter. This pathway is called the nigrostriatal tract. (The names of neural pathways often combine the site of origin of the fibers with their termination site, hence *nigrostriatal*, meaning "substantia nigra to striatum.") This neural circuit is critical for the initiation and modulation of movement. Cell death in the substantia nigra is the cause of Parkinson's disease—a disorder characterized by tremor, rigidity, and inability to initiate movements. An adjacent cluster of dopaminergic cells in the midbrain is the **ventral tegmental area** (**VTA**). Some of these cells project axons to the septum, olfactory tubercle, nucleus accumbens, amygdala, and other limbic structures in the forebrain (see the section on the telencephalon below). Hence these cells form the mesolimbic tract (note that *meso* refers to "midbrain"). Other cells in the VTA project to structures in the prefrontal cortex, cingulate cortex, and entorhinal areas and are considered the mesocortical tract. All three of these dopamine pathways are of significance in our discussions of Parkinson's disease (see Chapter 20), drug addiction (see Chapter 9), and schizophrenia (see Chapter 19).

DIENCEPHALON The two major structures in the diencephalon are the thalamus and the hypothalamus. The **thalamus** is a cluster of nuclei that first process and then distribute sensory and motor information to the appropriate portion of the cerebral cortex. For example, the lateral geniculate nucleus of the thalamus receives visual information from the eyes before projecting it to the primary visual cortex. Most incoming signals are integrated and modified before they are sent on to the cortex. The functioning of the thalamus helps the cortex to direct its attention to selectively important sensory messages while diminishing the significance of others; hence the thalamus helps to regulate levels of awareness.

The second diencephalic structure, the **hypothalamus**, lies ventral to the thalamus at the base of the brain. Although it is much smaller than the thalamus, it is made up of many small nuclei that perform functions critical for survival (**FIGURE 2.23**). The hypothalamus receives a wide variety of information about the internal environment and, in coordination with closely related structures in the limbic system (see the section on the telencephalon below), initiates various mechanisms important for limiting the variability of the body's internal states (i.e., they are responsible for homeostasis). Several nuclei are involved in maintaining body temperature and salt–water balance. Other nuclei modulate hunger, thirst, energy metabolism, reproductive behaviors, and emotional responses such as aggression. The hypothalamus directs behaviors for adjusting to these changing needs by controlling both the autonomic nervous system and the endocrine system and organizing behaviors in coordination with other brain areas. Axons from nuclei in the hypothalamus descend into the brainstem to the nuclei of the cranial nerves that provide parasympathetic control. Additionally, other axons descend farther into the spinal cord to influence sympathetic nervous system function. Other hypothalamic nuclei communicate with the contiguous pituitary gland by two methods: neural control of the posterior pituitary and hormonal control of the anterior pituitary. By regulating the endocrine hormones, the hypothalamus has widespread and prolonged effects on body physiology. Of particular significance to psychopharmacology is the role of the paraventricular nucleus in regulating

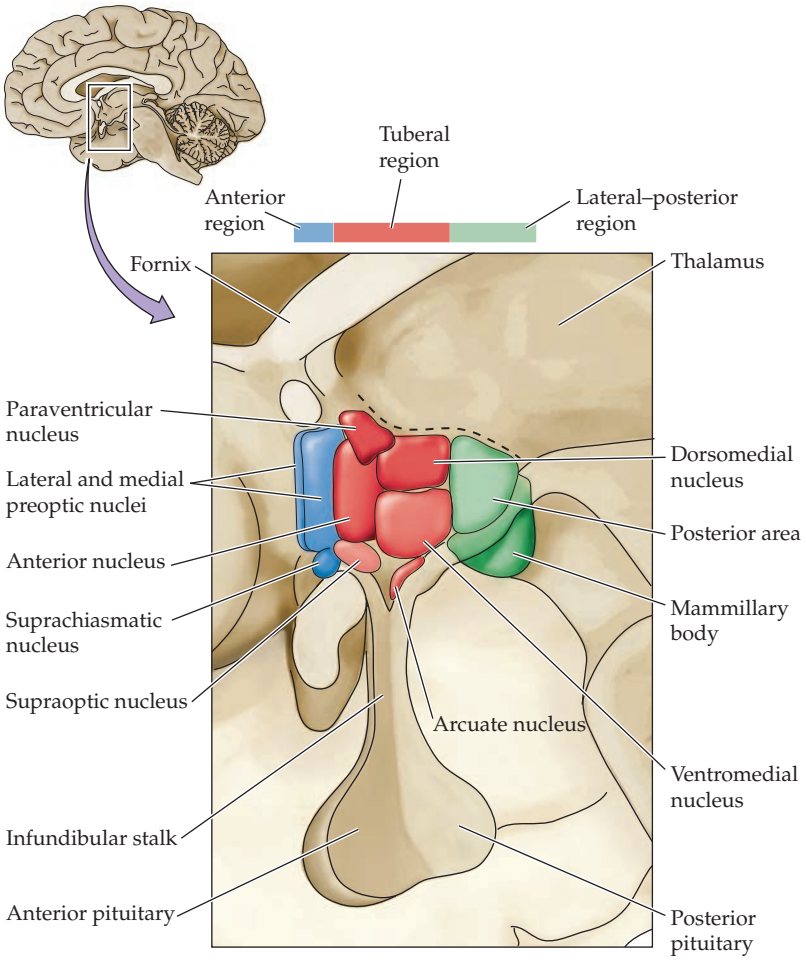


FIGURE 2.23 Hypothalamus The hypothalamus is a cluster of nuclei at the base of the forebrain that is often subdivided into three groups based on region: anterior, tuberal, and lateral–posterior. Each of the nuclei has its own complex pattern of neural connections and regulates one or several components of homeostatic function and motivated behavior.

the hormonal response to stress, because stress has a major impact on our behavior and vulnerability to psychiatric disorders. **BOX 2.4** describes the neuroendocrine response to stress and previews its significance for psychiatric ills and their treatment.

TELENCEPHALON The cerebral hemispheres make up the largest region of the brain and include the external cerebral cortex, the underlying white matter, and subcortical structures belonging to the basal ganglia and limbic system. The **basal ganglia** include the caudate, putamen, and globus pallidus and, along with the substantia nigra in the midbrain, comprise a system for motor control (see Figure 2.22). Drugs administered to control symptoms of Parkinson's disease act on this group of structures.

The **limbic system** is a complex neural network that is involved in integrating emotional responses and regulating motivated behavior and learning. The limbic system includes the limbic cortex, which is located on the medial and interior surface of the cerebral hemispheres and is transitional between allocortex (phylogenetically older cortex) and neocortex (the more recently evolved six-layer cortex). A significant portion of the limbic cortex is the cingulate. Chapter 11 describes the importance of the anterior portion of the cingulate in mediating the emotional component of pain. Some of the significant subcortical limbic structures are the amygdala, nucleus accumbens, and hippocampus, which is connected to the mammillary bodies and the septal nuclei by the fornix, the major tract of the limbic system (**FIGURE 2.24**). The **hippocampus** is most closely associated with the establishment of new long-term memories, spatial memory and contextual memory and has been the focus of research into Alzheimer's disease and its treatment, as you will read in Chapter 7. Additionally, the vulnerability of the hippocampus to high levels of stress hormones suggests its involvement in clinical depression and antidepressant drug treatment (see Chapter 18). The **amygdala** plays a central role in coordinating the various components of emotional

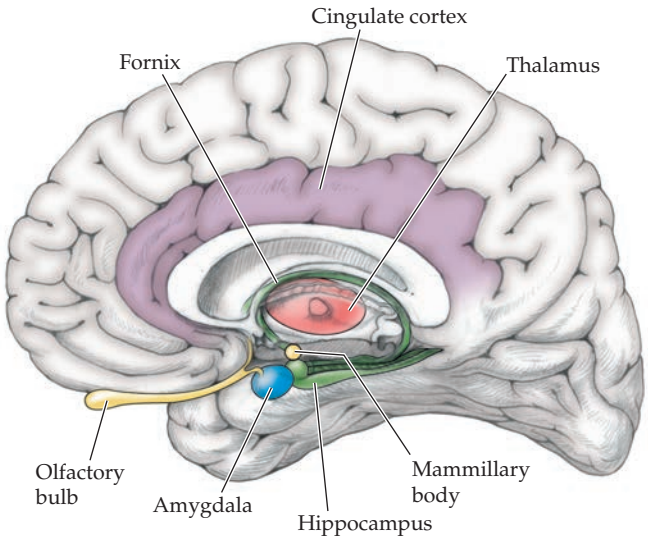


FIGURE 2.24 Limbic system Multiple subcortical structures interconnect to form the limbic system, which is critical for learning, memory, emotional responses, and motivation. Rich connections of limbic areas with association areas of the cortex contribute to decision making and planning.

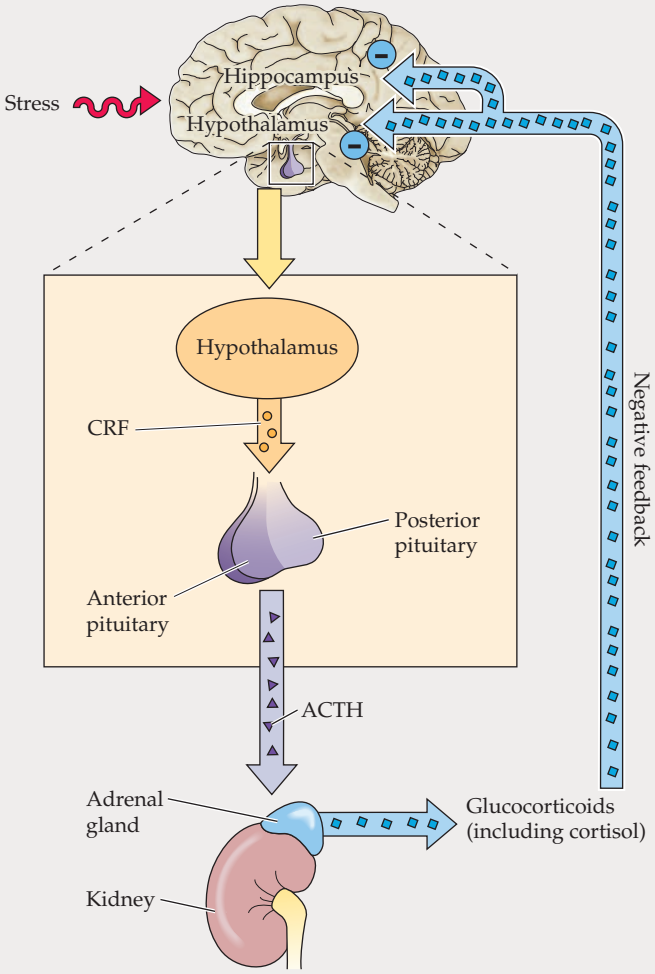
BOX 2.4 Of Special Interest

Neuroendocrine Response to Stress

The principal neuroendocrine response to stress is often referred to as the HPA axis because it depends on the interaction of the hypothalamus (H), the pituitary (P) gland, and the adrenal (A) gland. HPA axis activation is one part of complex emotional responses orchestrated by the amygdala. In essence, stress causes the secretion of corticotropin-releasing factor (CRF) by the paraventricular nucleus of the hypothalamus into the blood vessels ending in the anterior pituitary (see **Figure**). The binding of CRF in that gland causes the release of adrenocorticotropic hormone (ACTH) into the general blood circulation. ACTH subsequently binds to the adrenal cortex to increase the secretion of cortisol and other glucocorticoids, all of which contribute to the mobilization of energy to cope with stress or exertion. Under optimum conditions, cortisol feeds back to the hypothalamus (and hippocampus) to shut down HPA activation and return cortisol levels to normal. It also acts on the anterior pituitary (not shown) to reduce the production of ACTH.

Although HPA activity is critical for survival and adaptation to the environment, overuse of this adaptive mechanism leads to damaging changes to the brain and body. Damaging effects of prolonged cortisol response include such events as lower inflammatory response causing slower wound healing and immune system suppression. Stress has also been linked to an exacerbation of autoimmune diseases such as multiple sclerosis, gastric problems, diabetes, elevated blood pressure, premature aging, and many other disorders. In addition, stress affects neuron structure and brain function.

Several later chapters in this text will describe the relationship between stress and alcohol use disorder, the damaging effect of stress on cells in the hippocampus and its relationship to clinical depression, and the impact of early life traumas that alter the set point of the HPA axis, making it overly responsive to stressors later in life. The differential activation of stress response circuitry in men and women will also be addressed in the chapter on anxiety disorders. In Chapter 19, you will learn how stress-induced epigenetic events may alter the expression of a gene that is linked to the cognitive deficits characteristic of schizophrenia. All of these issues point to the critical need to evaluate more thoroughly the significant interaction between psychiatric and systemic medical disorders with the hope for potential new approaches to prevent and treat disabling conditions.



responses through its profuse connections with the olfactory system, hypothalamus (which is sometimes included in the limbic system, even though it is a diencephalic structure), thalamus, hippocampus, striatum, and brainstem nuclei, as well as portions of the neocortex, such as the orbitofrontal cortex. The amygdala and associated limbic areas play a prominent role in our discussions of antidepressants, alcohol, and antianxiety drugs. Chapters that describe the reinforcing value of abused substances also focus on limbic structures, notably the **nucleus accumbens**.

The cerebral cortex is divided into four lobes, each having primary, secondary, and tertiary areas

The cerebral cortex is a layer of tissue that covers the cerebral hemispheres. In humans, the cortex (or "bark") is heavily convoluted and has deep grooves called

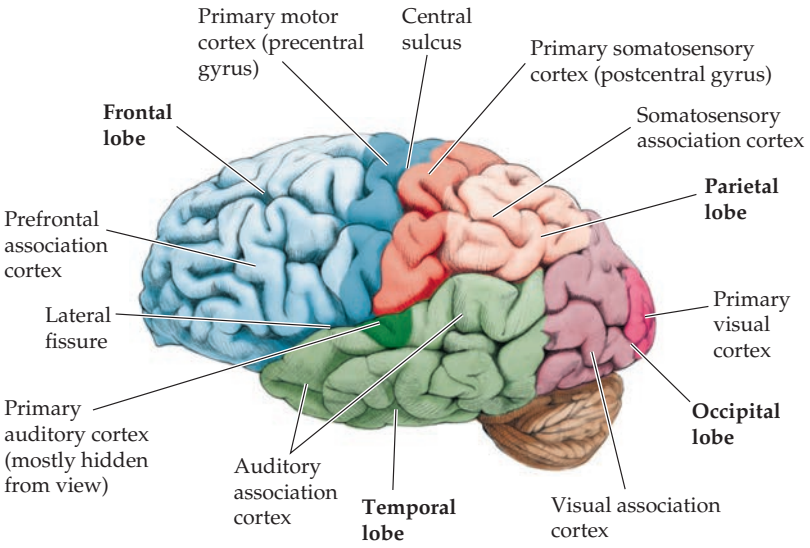


FIGURE 2.25 Lateral view of the exterior cerebral cortex The four lobes of the cerebral cortex are shown with distinct colors. Within each lobe is a primary area (darker in color) and secondary and tertiary association cortices. The caudal-most three lobes carry out sensory functions: vision (occipital), auditory (temporal), and somatosensory (parietal). The frontal lobe serves as the executive mechanism that plans and organizes behavior and initiates the appropriate sequence of actions.

fissures, smaller grooves called **sulci**, and bulges of tissue between called **gyri**. Thus the bulge of tissues immediately posterior to the central sulcus is the postcentral gyrus. The convolutions of the cortex greatly enlarge its surface area, to approximately 2.5 square feet. Only about one-third of the surface of the cortex is visible externally; the remaining two-thirds is hidden in the sulci and fissures. **FIGURE 2.25** shows some of the external features of the cerebral cortex. There may be as many as 100 billion cells in the cortex, arranged in six layers horizontal to the surface. Since these layers have large numbers of cell bodies, they appear gray; hence they are the gray matter of the cerebral cortex. Each layer can be identified by cell type, size, density, and arrangement. Beneath the six layers, the white matter of the cortex consists of millions of axons that connect one part of the cortex with another or connect cortical cells to other brain structures. One of the largest of these pathways is the **corpus callosum** (see Figure 2.21B). It connects corresponding areas in the two hemispheres, which are separated by a deep groove, the medial longitudinal fissure. In addition to the horizontal layers, the cortex has a vertical arrangement of cells that form slender vertical columns running through the entire thickness of the cortex. These vertically oriented cells and their synaptic connections apparently provide functional units for integration of information between various cortical regions.

The central sulcus and the lateral fissure (see Figure 2.25) visually divide the cortex into four distinct lobes in each hemisphere: the **parietal lobe**, **occipital lobe**, and **temporal lobe**, all of which are sensory in function, and the **frontal lobe**, which is responsible for movement and executive planning. Within each lobe is a small primary area, adjacent secondary cortex, and tertiary areas called *association cortex*. Within the occipital lobe is the primary visual cortex, which receives visual information from the thalamus that originates in the retina of the eye. The primary auditory cortex receives auditory information and is located in the temporal lobe; the primary somatosensory cortex, which receives information about body senses such as touch, temperature, and pain, is found in the parietal lobe just posterior to the central sulcus. Neither the gustatory cortex, which involves taste sensations, nor the primary olfactory area, which receives information regarding the sense of smell, is visible on the surface, but both lie within the folds of the cortex. The **primary cortex** of each lobe provides conscious awareness of sensory experience and the initial cortical processing of sensory qualities. Except for olfaction, all sensory information arrives in the appropriate primary cortex via projection neurons from the thalamus. In addition, except for olfaction, sensory information from the left side of the body goes to the right cerebral hemisphere first, and information from the right side goes to the left hemisphere. Visual information is somewhat different in that the left half of the visual field of each eye goes to the right occipital lobe and the right half of the visual field of each eye goes to the left occipital lobe.

Adjacent to each primary area is **secondary cortex**, which consists of neuronal circuits responsible for analyzing information transmitted from the primary area and providing recognition (or perception) of the stimulus. These areas are also the regions where memories are stored. Farther from the primary areas are association areas that lay down more-complex memories that involve multiple sensory systems such that our memories are not confined to a single sensory system but integrate multiple characteristics of the event. For example, many of us remember pieces of music from the past that automatically evoke visual memories of the person we shared it with, or the time in our lives when it was popular. These **tertiary association areas**

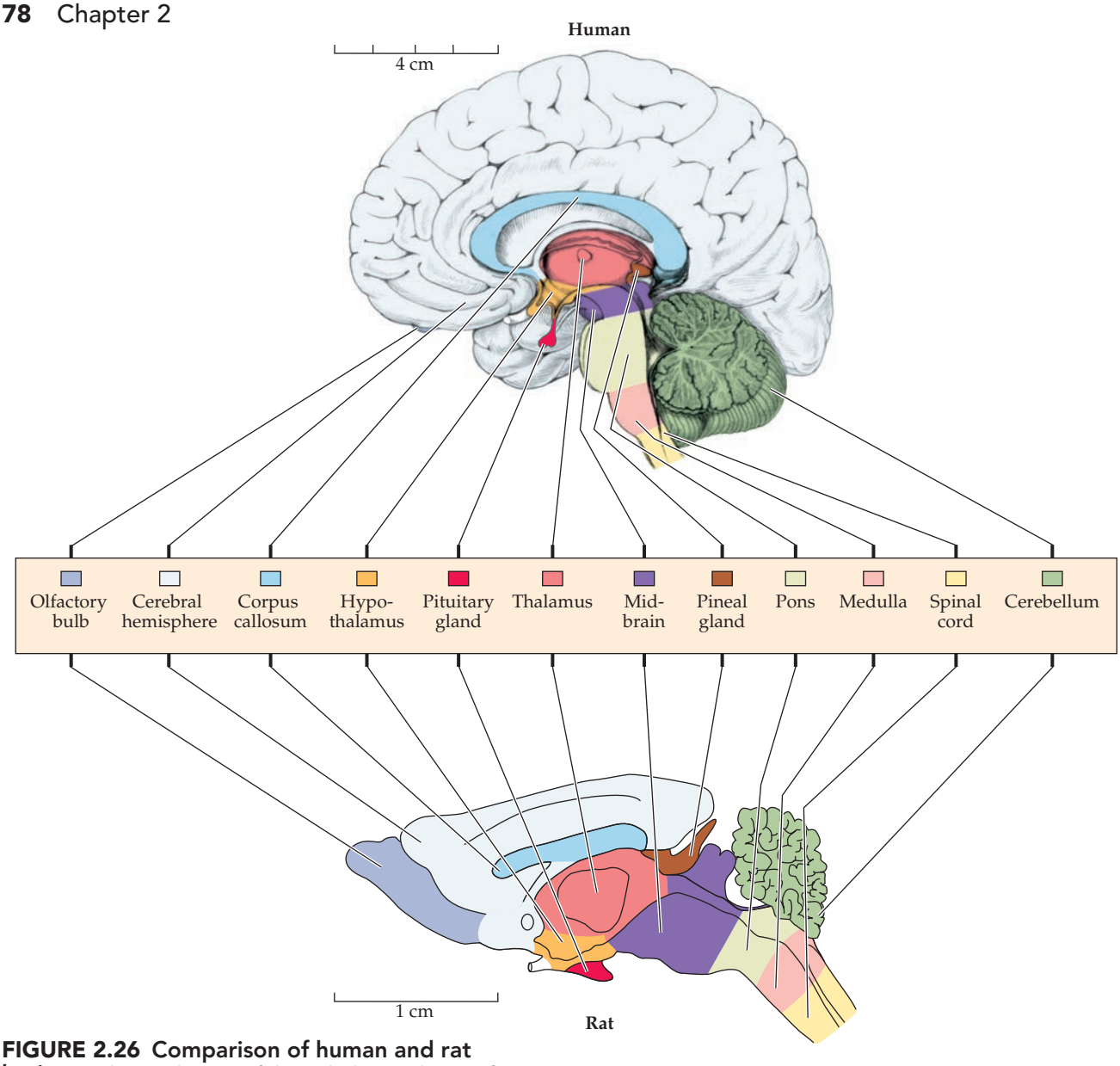


FIGURE 2.26 Comparison of human and rat brains Midsagittal views of the right hemispheres of human and rat brains show extensive similarities in brain structures and their relative topographic location. The brains differ in that the cerebral hemispheres are relatively much larger in the human brain, while the rat has a relatively larger midbrain and olfactory bulb. The rat brain has been enlarged about six times in linear dimensions relative to the human brain. (From Breedlove et al., 2010.)

are often called the parietal–temporal–occipital association cortex because they represent the interface of the three sensory lobes and provide the higher-order perceptual functions needed for purposeful action.

Within the frontal lobe, the primary motor cortex mediates voluntary movements of the muscles of the limbs and trunk. Neurons originating in primary motor cortex directly, or in several steps, project to the spinal cord to act on spinal motor neurons that end on muscle fibers. As was true for the sensory systems, the motor neurons beginning in the frontal cortex are crossed, meaning that areas of the right primary motor cortex control movements of limbs on the left side of the body, and vice versa. Adjacent to the primary motor cortex is the secondary motor cortex, where memories for well-learned motor sequences are stored. Neurons in this area connect directly to the primary motor cortex to direct movement. The rest of the frontal lobe comprises the prefrontal cortex, which receives sensory information from the other cortices via the large bundles of white matter running below the gray matter. Emotional and motivational input is contributed to the prefrontal cortex by limbic and other subcortical structures. The prefrontal cortex is critical for making decisions, planning actions, and evaluating optional strategies. Impaired prefrontal function is characteristic of several psychiatric disorders, including borderline personality disorder, memory loss after traumatic brain injury, attention deficit hyperactivity disorder, and others. The significance of this brain region for the symptoms and treatment of schizophrenia is discussed in Chapter 19.

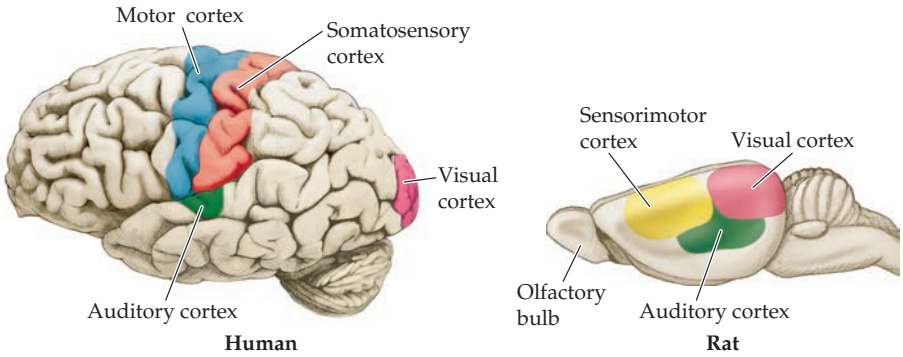


FIGURE 2.27 Lateral view of the left hemisphere of human and rat brains Note that the expanded human cortex does not involve the primary sensory or motor cortex (colored areas). Human cortical expansion involves the secondary and tertiary association areas that are responsible for higher-order perception and cognition. (After Bear et al., 2007.)

Rat and human brains have many similarities and some differences

Since the rat is one of the most commonly used animals in neuroscience and psychopharmacological research, it may be helpful to compare the neuroanatomy of the rat brain with that of the human brain. Overall, there has been great conservation of brain structures during the evolution of mammals. The basic mammalian brain plan draws on the common ancestry of mammals and is the model for the human brain. In fact, looking farther back in the evolutionary tree, all vertebrates show a striking similarity. Despite differences in absolute and relative sizes of the whole brain, all mammalian brains have the same major subdivisions, which are topographically organized in relatively the same locations with similar neural connections among structures. **FIGURE 2.26** shows the striking correspondence of brain structures in the brains of rats and humans. Extensive similarities can also be found for individual nuclei, fiber tracts, and types of cells, although human neurons are much larger than rat neurons and have more elaborate dendritic trees.

Despite the many similarities, there are also notable differences. What differs among mammals is the relative size of the brain regions, which likely reflects the environmental conditions encountered by each species and the importance of the functions of specific brain regions for adaptation of the species. For example, rats have relatively larger olfactory bulbs than humans, apparently because a very sensitive sense of smell provided evolutionary advantages for survival for these nocturnal rodents. More striking is the difference in cerebral cortex. The paired cerebral hemispheres occupy a much greater proportion of the brain in the human than in the rat. The six-layered nature of the neocortex (the outermost layers of the cerebral hemispheres) is characteristic of all mammals, and it is the newest part of the cerebral cortex to evolve. In humans, the surface area of the neocortex is approximately 2322 cm^2 (2.5 ft^2), which explains why the surface must bend and fold to fit within the skull, producing the extensively convoluted surface. As much as two-thirds of the neocortex is not visible on the surface but is buried in the grooves. In contrast, the surface of the rat cerebral cortex is smooth and has no gyri or fissures (**FIGURE 2.27**). It is also clear from Figure 2.27 that the expanded surface area of the human cortex does not involve the primary sensory areas (pink, green, and orange), which are the first cortical areas to receive input from ascending sensory pathways, nor does it involve the primary motor cortex (blue). Instead, one can see enlargement of the secondary sensory association areas responsible for the complex sensory processing, perception, and function (e.g., speech) of which humans are capable. Additionally, tertiary association areas of the cortex are greatly expanded, reflecting the human capacity for cognitive processing, reasoning, abstract thinking, and decision making.

Section Summary

- The central nervous system (CNS) includes the brain and the spinal cord. The remaining nerves of the body constitute the peripheral nervous system (PNS).
- The PNS is divided into the somatic nervous system, which includes spinal nerves that transmit sensory and motor information for skeletal muscles, and the autonomic nervous system, which serves smooth muscles, glands, and visceral organs.
- The autonomic nervous system has two divisions: the sympathetic, which serves to mobilize energy for times of "fight-or-flight"; and the parasympathetic, which reduces energy usage and stores reserves.
- The CNS is protected by a bony covering, three layers of meninges (dura, arachnoid, and pia), and cerebrospinal fluid.
- Neurotrophic factors are neuronal growth factors that guide the development of neurons, regulate dendritic growth and retraction, and aid in survival of neurons.
- The CNS can be divided into six regions containing multiple nuclei and their associated axons,

which form interconnecting neural circuits: spinal cord, myelencephalon, metencephalon, mesencephalon, diencephalon, and telencephalon.

- The gray matter of the spinal cord constitutes cell bodies that receive sensory information and cell bodies of motor neurons that serve muscles. The white matter consists of tracts of myelinated axons that carry signals in the ascending direction, to the brain, and the descending direction, for cortical control of the spinal cord.
- Continuous with the spinal cord is the myelencephalon, or medulla, which contains nuclei that serve vital functions for survival, such as respiration, heart rate, and vomiting.
- The metencephalon includes two major structures. The cerebellum functions to maintain posture and balance and provides fine motor control and coordination. The pons contains several nuclei that represent the origins of most of the tracts utilizing the neurotransmitters norepinephrine (the locus coeruleus) and serotonin (the raphe nuclei).
- Beginning in the medulla, running through the pons, and extending into the midbrain is the reticular formation—a network of interconnected nuclei that control arousal, attention, and survival functions.
- The mesencephalon, or midbrain, contains nuclei that control sensory reflexes such as pupillary constriction. Other nuclei (substantia nigra and ventral tegmental area) are the cell bodies of neurons that form three major dopaminergic tracts. The periaqueductal gray organizes behaviors such as defensive rage and predation and serves as an important pain-modulating center.
- The diencephalon contains the thalamus, which relays information to the cerebral cortex, and the hypothalamus, which is important for maintaining homeostasis of physiological functions and for modulating motivated behaviors, including eating, aggression, reproduction, and so forth. The many nuclei that constitute the hypothalamus control both the autonomic nervous system and the endocrine system.
- The telencephalon includes both the cerebral cortex and multiple subcortical structures, including the basal ganglia and the limbic system. The basal ganglia modulate movement.
- The limbic system is made up of several brain structures with perfuse interconnections that modulate emotion, motivation, and learning. Some of the prominent limbic structures are the amygdala, hippocampus, nucleus accumbens, and limbic cortex.
- The six-layered cerebral cortex is organized into four lobes: the occipital, temporal, and parietal, which are the sensory lobes involved in perception and memories, and the frontal, which regulates motor movements and contains the "executive mechanism" for planning, evaluating, and making strategies.
- Although there are differences in absolute and relative size, rat and human brains have the same major subdivisions, which are topographically organized in similar locations. Rat brains have relatively larger olfactory bulbs and midbrains. Human brains have expanded secondary and tertiary association areas of the cerebral cortex that serve higher-order sensory perception and cognitive functions.

STUDY QUESTIONS

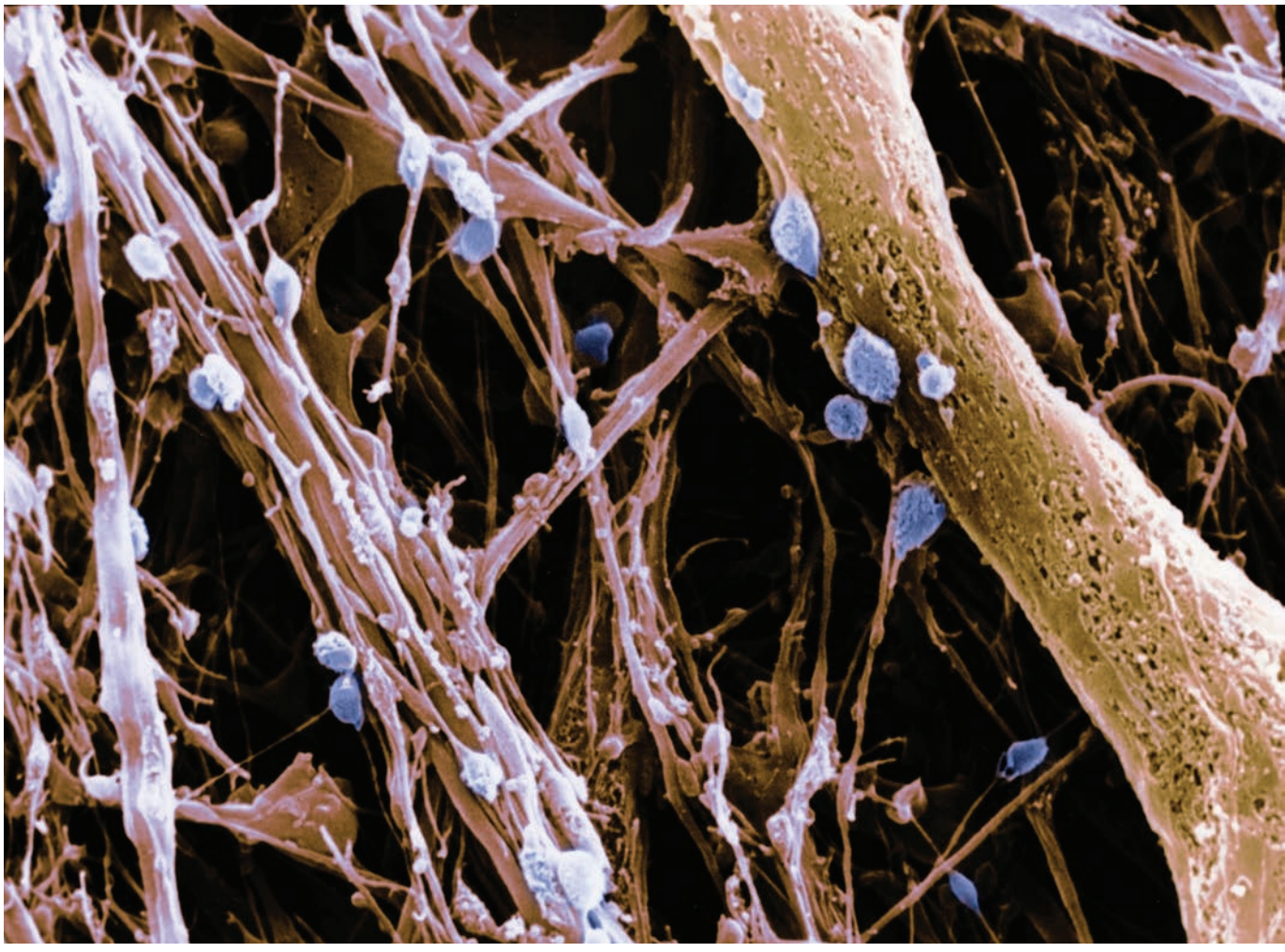
1. What are embryonic stem cells? Describe several potential benefits from stem cell research.
2. Name the three major external features of neurons and their basic functions.
3. What is myelin and why is it important to neuron function?
4. Briefly describe the two stages of protein synthesis as well as epigenetic modification of gene expression.
5. Describe the role of the cytoskeleton in normal axoplasmic transport and its contribution to Alzheimer's disease.
6. Compare and contrast ligand-gated and voltage-gated ion channels.
7. Name the four types of glial cells, and describe their basic functions.
8. What is the resting membrane potential, and what is responsible for it? Be sure to describe the establishment of the equilibrium potential for potassium.
9. How are local potentials generated? Why is summation or integration so important to neuronal signaling?

STUDY QUESTIONS *(continued)*

10. What happens at the axon hillock to generate an action potential? Describe the movement of ions during an action potential. What is responsible for the absolute refractory period and the relative refractory period?
11. Why is saltatory conduction so much more rapid and energy efficient than conduction on nonmyelinated axons?
12. Compare the characteristics of local potentials and action potentials.
13. Describe the somatic nervous system and its functions. Discuss the autonomic nervous system, and compare the sympathetic and parasympathetic divisions.
14. Describe the four protective features of the CNS: skull, meninges, cerebrospinal fluid, and blood–brain barrier.
15. What are the principal functions of neurotrophic factors?
16. Describe the basic functions and cell groups in the spinal cord, medulla, pons, and cerebellum.
17. What are the important functions of the periaqueductal gray, substantia nigra, and ventral tegmental area? How do the latter two nuclei control dopamine function?
18. What is the role of the hypothalamus in survival? Be sure to describe autonomic nervous system regulation as well as control of both anterior and posterior pituitary. How does it help us cope with stress?
19. What is the limbic system? How do the hippocampus and amygdala modify our behavior?
20. How is the cerebral cortex organized? What is the distinction between primary, secondary, and tertiary cortical regions?
21. What are the most significant differences between rat and human brains?

Go to the *Psychopharmacology* Companion Website at oup-arc.com/access/meyer-3e for animations, web boxes, flashcards, and other study aids.

CHAPTER 3



Scanning electron micrograph showing sites of axoaxonic and axodendritic synaptic contacts (in blue) within the hypothalamus. (David E. Scott / Medical Images.)

Chemical Signaling by Neurotransmitters and Hormones

IF YOU ARE READING THIS TEXTBOOK, you have probably taken one or more courses in psychology, biology, or neuroscience, in which you learned that the nerve cell (neuron) is the basic functional unit of the nervous system and that information is usually transmitted from one nerve cell to another by the release of a chemical substance called a **neurotransmitter**. Of course, these basic facts seem so obvious to us now, but they were not always obvious and had to be acquired over years of painstaking observation and experimentation, along with some good old-fashioned luck. Indeed, the discovery of chemical neurotransmission is marked by two great controversies involving some of the greatest pioneers in the relatively young history of neuroscience. The first controversy centered around two different views of nervous system structure: (1) the neuron doctrine, which proposed that the nervous system is composed of individual cells that are not physically connected and that information flow is unidirectional from one cell to another; and (2) the reticulum theory, which proposed instead that the nervous system consists of a series of vast continuous networks, in which all network elements are physically interconnected, and information can flow in any direction among these elements. The most distinguished proponents of these theories were two extraordinary late-nineteenth-century histologists—a reserved and humble Spaniard named Santiago Ramón y Cajal (sometimes considered the "father" of modern neuroscience), who strongly favored the neuron doctrine, and an outspoken and arrogant Italian named Camillo Golgi (discoverer of the remarkable silver staining method that bears his name), who was a fierce advocate of the reticulum theory and a bitter rival of Cajal. Despite working with what we now consider primitive tools, sometimes in a makeshift kitchen laboratory, and often in obscurity (particularly in the early stages of Cajal's career), the two scientists ultimately made such great contributions that they shared the 1906 Nobel Prize in Physiology or Medicine "in recognition of their work on the structure of the nervous system."[1] ■

[1]https://www.nobelprize.org/nobel_prizes/medicine/laureates/1906/

By 1906, the neuron doctrine controversy was already resolved in the minds of most researchers, with Golgi still a notable exception. Yet a second great controversy was starting to brew concerning the mechanism by which these separate nerve cells communicate with each other. Some researchers, particularly neurophysiologists like John Eccles, argued that communication between axon terminals and dendrites occurred by means of electrical currents that cross the gap between sending and receiving cells. In contrast, the work of various pharmacologists suggested that information might instead be transmitted by release of a chemical substance. Confirmation of chemical transmission was first obtained in the autonomic nervous system, which was much more amenable than the brain to this type of research. One of the defining studies was a brilliant experiment performed by the German pharmacologist Otto Loewi in 1920. Loewi stimulated the vagus (parasympathetic) nerve of a frog heart, causing slowing of the heartbeat. He then immediately transferred the fluid that had been bathing the first heart to a second heart, from which the vagus nerve had been removed. Remarkably, the second heart slowed down as well, proving that a chemical substance had been released into the fluid due to the vagal stimulation. Loewi and the British pharmacologist Henry Dale, who had been investigating both the autonomic and neuromuscular systems, were corecipients of the 1936 Nobel Prize in Physiology or Medicine for their respective contributions to the discovery of chemical neurotransmission. Still, it wasn't until the 1960s and later that researchers generally accepted this process as the primary basis for intercellular communication in the brain, not just in the peripheral nervous system.[2]

This historical summary has introduced you to one of the body's great systems of cellular communication—the system of synaptic transmission. Throughout the rest of this chapter, you will learn more about synapses, neurotransmitters, and the mechanisms of neurotransmitter action. The final section is devoted to another important communication system, the endocrine system, which is responsible for the secretion of hormones into the bloodstream.

[2]Students interested in learning more about these controversies and discoveries are referred to three excellent sources: *Nerve Endings: The Discovery of the Synapse*, by Richard Rapport, which recounts the stories of Cajal and Golgi; *The War of the Soups and the Sparks: The Discovery of Neurotransmitters and the Dispute Over How Nerves Communicate*, by Eliot Valenstein, which gives the history of the discovery of chemical neurotransmission; and Loewi's 1960 autobiographical sketch published in *Perspectives in Biology and Medicine*, in which he reminisces about his famous frog heart experiment and the nighttime dream that prompted it.

Chemical Signaling between Nerve Cells

The word **synapse** was coined in 1897 by the British physiologist Charles Sherrington. He derived the term from the Greek word *synapto*, which means "to clasp." Using only a light microscope, Sherrington could not see the actual point of communication between neurons, but physiological experiments had shown that transmission occurs in only one direction (from what we now call the **presynaptic cell** to the **postsynaptic cell**). The synapse was considered to be the specialized mechanism underlying this neuronal communication. Sherrington correctly inferred that sending (presynaptic) and receiving (postsynaptic) cells do not actually touch each other, as discussed above. Of course, the concept of chemical transmitter substances had barely been conceived, much less confirmed experimentally.

Our current knowledge of synaptic structure comes, in part, from the electron microscope. This and other modern imaging tools give us much greater magnification than the standard light microscope. The most common synapses in the brain are **axodendritic synapses**. In these synapses, an axon terminal from the presynaptic neuron communicates with a dendrite of the postsynaptic cell. An electron micrograph displaying this kind of synapse is shown in **FIGURE 3.1A** (see also **FIGURE 3.2A**). The dendrites of some neurons have short spines along their length, which are reminiscent of thorns growing out from a rosebush. **FIGURE 3.1B** shows a small segment of a dendrite from a **pyramidal neuron**[3] in the human cingulate cortex containing a high density of long-necked spines. This type of spiny dendrite is often found in human pyramidal neurons. When dendritic spines are present, they are important locations for synapses to form. You can see this directly in the electron micrograph shown in Figure 3.1A; although the staining method used for Figure 3.1B doesn't permit the visualization of presynaptic nerve terminals, we can be certain that synapses were present on those spines as well.

There is an exceedingly small (about 20 nm, which is 20×10^{-9} m) gap between presynaptic and postsynaptic cells that must be traversed by neurotransmitter molecules after their release. This gap is called the **synaptic cleft**. On the postsynaptic side, the area of the dendritic membrane facing the synaptic cleft appears dark and somewhat fuzzy looking. This structure, called the **postsynaptic density**, is rich in neurotransmitter receptors along with other proteins that help anchor the receptors in place. In the axon terminal, we can see many small saclike objects, termed **synaptic vesicles**, each of which is filled with several thousand molecules

[3]Pyramidal neurons are pyramid-shaped cells that function as the principal output neurons of the cerebral cortex.

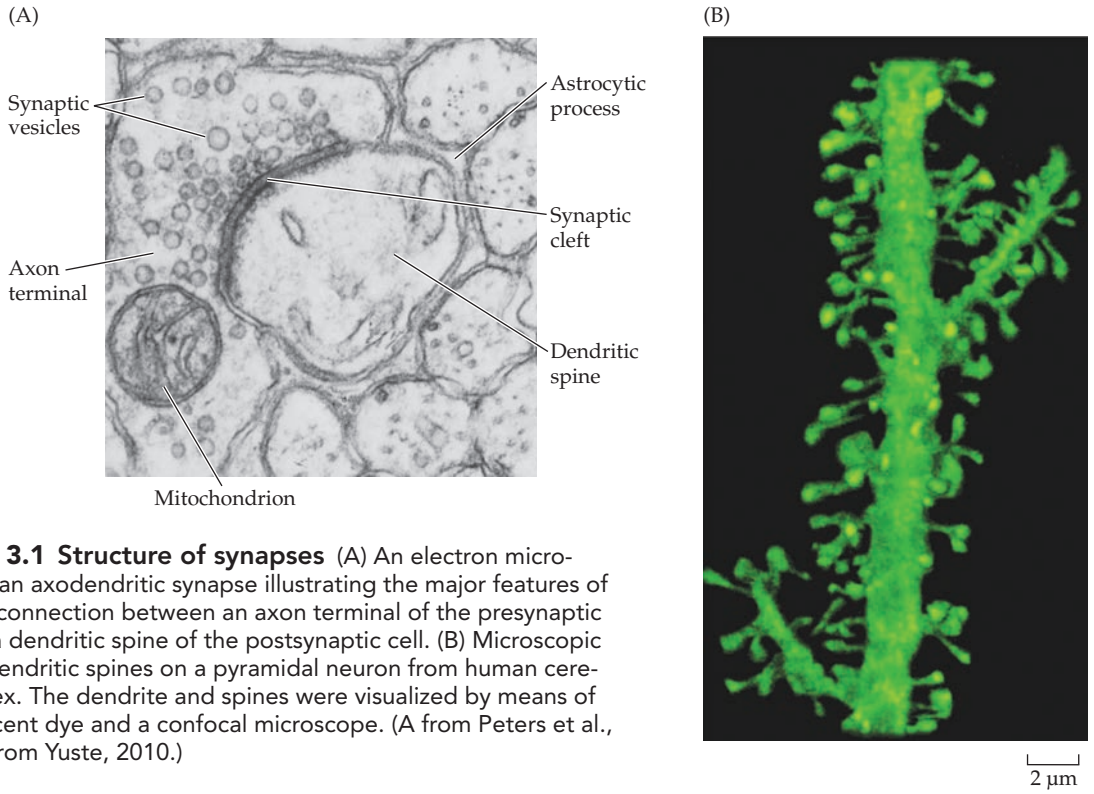


FIGURE 3.1 Structure of synapses (A) An electron micrograph of an axodendritic synapse illustrating the major features of a typical connection between an axon terminal of the presynaptic cell and a dendritic spine of the postsynaptic cell. (B) Microscopic view of dendritic spines on a pyramidal neuron from human cerebral cortex. The dendrite and spines were visualized by means of a fluorescent dye and a confocal microscope. (A from Peters et al., 1991; B from Yuste, 2010.)

of a neurotransmitter. As discussed later, these vesicles are the main source of transmitter release. A recent study found strong evidence that the sites of vesicular release on the presynaptic side are tightly aligned with areas of receptor concentration on the postsynaptic side (Tang et al., 2016). This finding led the authors to hypothesize the existence of a protein structure they termed a "nanocolumn" that spans the synaptic cleft and maintains the close alignment of neurotransmitter release and reception. A video animation based on this research can be found on YouTube (Blanpied, 2016).

Figure 3.1A shows additional features of a typical synapse, including the profile of a mitochondrion in the axon terminal. Mitochondria are the cellular organelles responsible for energy (adenosine triphosphate, or ATP) production. They are needed in large quantities in the terminals for various functions such as ion pumping and transmitter release. Finally, we see that the synapse is surrounded by processes (fibers) from astrocytes. In Chapter 8, we'll discuss an important role for these glial cells in regulating transmission by amino acid transmitters.

Other types of synapses are also present in the brain. For example, **axosomatic synapses** are synapses between a nerve terminal and a nerve cell body (**FIGURE 3.2B**). They function in a manner similar to axodendritic synapses. **Axoaxonic synapses** involve one axon synapsing on the terminal of another axon (**FIGURE 3.2C**). This unusual arrangement permits the presynaptic cell

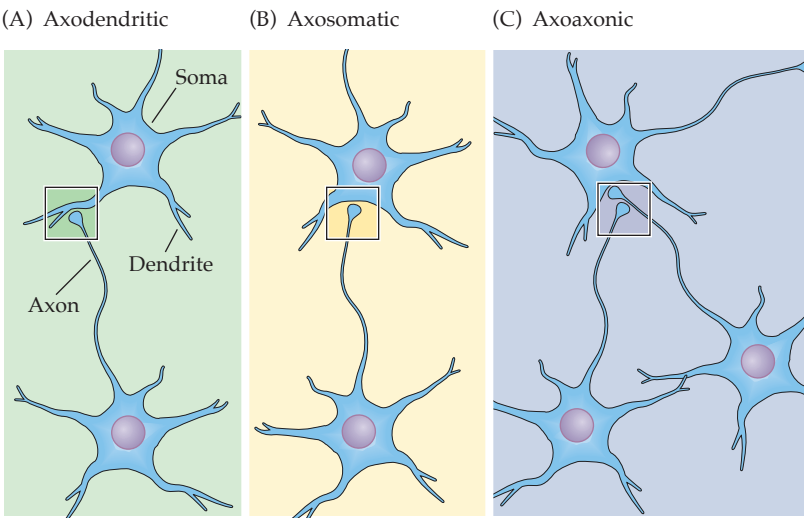


FIGURE 3.2 The three types of synaptic connections between neurons

to alter neurotransmitter release from the postsynaptic cell directly at the terminals. For example, activity at an axoaxonic synapse may reduce transmitter release from the terminal. This is called **presynaptic inhibition** of release. Enhanced release of transmitter, on the other hand, is called **presynaptic facilitation**.

In neuronal communication, the receiving cell may be another neuron, or it may be a muscle cell or a cell specialized to release a hormone or other secretory product. The connection point between a neuron and a muscle is called a **neuromuscular junction** instead of a synapse. A neuromuscular junction has many structural and functional similarities to a conventional synapse, and much has been learned about synaptic transmission by studying neuromuscular junctions.

Neurotransmitter Synthesis, Release, and Inactivation

As has been mentioned, neurotransmitters are chemical substances released by neurons to communicate with other cells. Scientists first thought that only a few chemicals were involved in neurotransmission, but more than 100 chemicals have now been identified. As there are many thousands of chemicals present in any cell, how do we know whether a particular substance qualifies as a neurotransmitter? Verifying a chemical's status as a neurotransmitter can be a difficult process, but here are some of the important criteria:

- The presynaptic cell should contain the proposed substance along with a mechanism for manufacturing it.
- A mechanism for inactivating the substance should also be present.
- The substance should be released from the axon terminal upon stimulation of the neuron.
- Receptors for the proposed substance should be present on the postsynaptic cell. (Receptors are discussed in greater detail later in the chapter.)
- Direct application of the proposed substance or of an agonist drug that acts on its receptors should have the same effect on the postsynaptic cell as stimulating the presynaptic neuron (which presumably would release the substance from the axon terminals).
- Applying an antagonist drug that blocks the receptors should inhibit both the action of the applied substance and the effect of stimulating the presynaptic neuron.

Note that researchers do not require that all of these criteria be met for a substance to be considered a neurotransmitter. We can argue that the most important criteria are that the substance is synthesized in a group of nerve cells, that the substance can be released by those cells, and that receptors for the substance have also been identified and have been shown to mediate a response by the receiving (postsynaptic) cells.

Neurotransmitters encompass several different kinds of chemical substances

Despite the great numbers of neurotransmitters, most of them conveniently fall into several chemical classes. The major types of transmitters and examples of each are shown in **TABLE 3.1**. A few neurotransmitters are categorized as **amino acids**.[4] Amino acids serve numerous functions: they are the individual building blocks of proteins, and they play other metabolic roles besides their role as neurotransmitters. In Chapter 8, we'll cover the two most important amino acid neurotransmitters, glutamate and γ-aminobutyric acid (GABA). Several other transmitters are **monoamines**, which are grouped together because each possesses a single (hence "mono") amine group. Monoamine transmitters are derived from amino acids through a series of biochemical reactions that include removal of the acidic part (–COOH) of the molecule. Consequently, we say that the original amino acid is a **precursor** because it precedes the amine in the biochemical pathway. In Chapters 5 and 6, we will discuss the best-characterized monoamine transmitters: dopamine (DA), norepinephrine (NE), and serotonin (5-HT). One important neurotransmitter that is neither an amino acid nor a monoamine is acetylcholine (ACh). This transmitter, which is covered in Chapter

[4] Amino acids are so named because they contain both an amino group ($–NH_2$) and a carboxyl group (–COOH), the latter of which releases a hydrogen ion (H^+) and thus acts as an acid.

TABLE 3.1 Major Categories of Neurotransmitters

Classical neurotransmitters	Nonclassical neurotransmitters
Amino acids	**Neuropeptides**
Glutamate	Endorphins and enkephalins
γ-aminobutyric acid (GABA)	Corticotropin-releasing factor (CRF)
Glycine	Orexin/hypocretin
Monoamines	Brain-derived neurotrophic factor (BDNF)
Dopamine (DA)	Many others
Norepinephrine (NE)	**Lipids**
Serotonin (5-HT)	Anandamide
Histamine (HA)	2-Arachidonoylglycerol
Acetylcholine (Ach)	**Gases**
Purines	Nitric oxide (NO)
Adenosine triphosphate (ATP)	Carbon monoxide (CO)
Adenosine	Hydrogen sulfide (H_2S)

Note: This is only a small sample of more than 100 substances known or suspected to be neurotransmitters in the brain.

7, serves key functions at the neuromuscular junction, in the autonomic nervous system, and in the brain. You may be surprised to learn that the purine-containing molecules ATP and its precursor adenosine have neurotransmitter activity in addition to their roles in energy metabolism. Like ACh, ATP is a transmitter both in the brain and in the autonomic nervous system, where it exerts multiple actions such as control of bladder function (see reviews by Burnstock, 2012; Cisneros et al., 2015; Kennedy, 2015). Adenosine signaling is discussed in Chapter 13 in conjunction with caffeine, a substance that blocks certain adenosine receptors. Acetylcholine, the amino acid and monoamine neurotransmitters, and to a lesser extent the purine neurotransmitters are sometimes called "classical" transmitters because they were generally discovered before the other categories and they follow certain principles of transmission that are discussed later in the chapter.

Besides the classical transmitters, there are several other types of neurotransmitters. The largest group of "nonclassical" neurotransmitters are the **neuropeptides**, whose name simply means "peptides found in the nervous system." Peptides are small proteins, typically made up of 3 to 40 amino acids instead of the 100+ amino acids found in most proteins. Neuropharmacologists are very interested in the family of neuropeptides called *endorphins* and *enkephalins*, which stimulate the same opioid receptors that are activated by heroin and other abused opioid drugs (see Chapter 11). Other neuropeptides relevant to neuropsychiatric illness and drug treatment include the following: corticotropin-releasing factor (CRF), which plays a role in anxiety, depression, and drug addiction (see Chapters 9, 17, and 18); brain-derived neurotrophic factor (BDNF), which has also been implicated in depression (see Chapter 18); vasopressin and oxytocin, two closely related substances that have been shown to regulate social and affiliative behaviors (Bachner-Melman and Ebstein, 2014; Baribeau and Anagnostou, 2015; Zimmerman et al., 2015); and orexin/hypocretin, which is involved in reward-seeking behaviors in sleep and wakefulness (**BOX 3.1**). A few transmitters are considered **lipids**, which is the scientific term for fatty substances. For example, in Chapter 14, we discuss two lipid transmitters that act on the brain like marijuana (or, more specifically, Δ^9-tetrahydrocannabinol [THC], which is the major active ingredient in marijuana). Finally, the most recently

BOX 3.1 Clinical Applications

Orexin-Based Medications: New Approaches to the Treatment of Sleep Disorders

Researchers have been discovering new neuropeptides over many years, and this process is still continuing today. One such discovery was reported independently by two research groups in 1998. In January of that year, Luis de Lecea and colleagues reported the discovery of two related peptides that they termed **hypocretin 1** and **hypocretin 2** (de Lecea et al., 1998). The name they chose reflected the expression of the peptides in the hypothalamus (hence *hypo*) and the structural similarity of the hypocretins to members of a family of metabolic hormones in the gut called incretins (hence *cretin*). Just a month later, a different research group headed by Takeshi Sakurai published the discovery of two peptides that they called **orexin-A** and **orexin-B** (Sakurai et al., 1998). This alternate naming system, which stems from the Greek word *orexis* meaning "appetite" or "desire," was based on the ability of either peptide to stimulate eating behavior when injected directly into the cerebral ventricles of rats. Following publication of the two papers, neuroscientists quickly grasped that the hypocretins and orexins were identical substances. Although both terminologies persist in the scientific literature, we will henceforth just use the term *orexin* for the sake of consistency.

Subsequent studies have thoroughly characterized the orexin neurotransmitter system and provided much insight into its functional roles (Mahler et al., 2014; Sakurai, 2014). There are roughly 70,000 orexin neurons in the human brain and approximately 3000 in the rat brain. In all species examined to date, orexin neurons are clustered in a small region within and around the hypothalamus that includes the lateral hypothalamic area, dorsomedial hypothalamus, posterior hypothalamus, and perifornical area. The initial studies by Sakurai and coworkers (1998) identified two different orexin receptors, OX1R and OX2R. Despite the relatively small number of orexin neurons in the brain, these cells receive many different inputs from various brain areas, and they also send their axons to a wide variety of areas that express OX1R, OX2R, or both. Not surprisingly, given the diverse projections of the orexin system, the two peptides together have been implicated in many different behavioral/physiological functions, including feeding behavior, regulation of body weight, reward, emotional responses, stress responses, and autonomic nervous system control (Sakurai, 2014). As most, if not all, of these functions have a motivational

(*Continued*)

BOX 3.1 Clinical Applications (*continued*)

component, some researchers have hypothesized a general motivational activation role for orexin (Mahler et al., 2014).

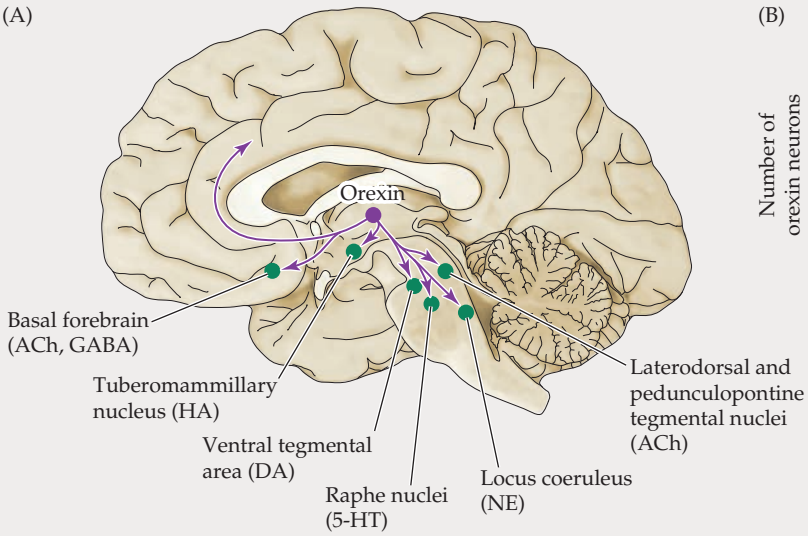
For purposes of the present discussion, however, we will focus on another key function of orexin—that of regulating arousal and the sleep–wake cycle (Alexandre et al., 2013; de Lecea and Huerta, 2014). Orexin neurons are part of a large circuit that controls this essential function. Within this circuit, orexin neurons project to acetylcholine and γ-aminobutyric acid neurons in the basal forebrain, histamine neurons in the tuberomammillary nucleus, dopamine neurons in the ventral tegmental area, serotonin neurons in the raphe nuclei, acetylcholine neurons in the laterodorsal and pedunculopontine tegmental nuclei, and norepinephrine neurons in the locus coeruleus (**Figure A**). Most of these brain areas and their corresponding neurotransmitters are discussed in detail in later chapters. Orexin axons additionally project directly to the cerebral cortex where they exert excitatory effects on the postsynaptic cells. Researchers have shown that orexin neurons fire more rapidly during waking than during sleep and that administration of orexin-A directly into the brain of rats (orexin peptides do not readily cross the blood–brain barrier) increases periods of wakefulness and reduces the amount of time spent sleeping (Alexandre et al., 2013; de Lecea and Huerta, 2014). In addition, activation of orexin neurons in sleeping mice using a technique called optogenetics (see Chapter 4 for a discussion of this technique) often causes the mice to wake up in less than a minute (de Lecea, 2015).

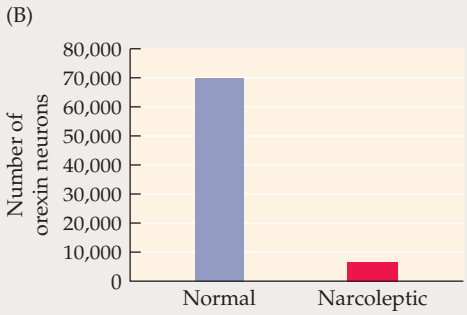
Many kinds of sleep disorders have been documented, ranging in severity from mild insomnia to an extremely disruptive disorder called **narcolepsy**. The symptoms of this disorder were first described in 1880 by the French physician Jean-Baptiste-Édouard Gélineau. His patient, a wine cask maker, was excessively sleepy during the daytime and also suffered from periodic episodes during which he suddenly lost all muscle control and fell to the ground. Gélineau named this unusual disorder *narcolepsie* (later Anglicized to narcolepsy) from the Greek words *narke*, meaning "numbness" or "stupor," and *lepsis*, meaning "attack" or "seizure" (as in epilepsy). The loss of muscle control in patients with narcolepsy is called **cataplexy** and is usually triggered by a strong emotion such as laughing or becoming angry or frustrated. A cataplexy episode may be minor, consisting of a few seconds of muscle weakness resulting in symptoms such as head dropping, knee buckling, or slurred speech. Alternatively, the patient may experience complete paralysis of the antigravity muscles that can last for several minutes. The patient remains conscious throughout the attack and is aware of his condition. Most, though not all, patients with narcolepsy exhibit cataplexy. A smaller number of patients additionally report unusual symptoms that may occur during the transition from wakefulness to sleep. These symptoms are **hypnagogic hallucinations** (vivid dreamlike sensations) and **sleep paralysis** (loss of muscle tone leading to a feeling of paralysis). Narcolepsy occurs in about 0.03% to 0.05% of the population (Leschziner, 2014; Scammell, 2015).

The first clue that orexin plays a role in narcolepsy came from studies in dogs. The occurrence of dogs exhibiting narcoleptic symptoms had been reported for many years, although the cause was unknown. However, because the disorder in this species seemed to have a genetic basis, researchers were able to breed lines of narcoleptic Doberman pinschers and Labrador retrievers, with the goal of identifying the culprit gene. This goal was finally reached with the discovery that canine narcolepsy is caused by a mutation in the *OX2R* gene, thereby resulting in synthesis of an abnormal OX2R protein and a loss of normal postsynaptic responsiveness to orexin (Lin et al., 1999). Within 2 weeks of publication of the canine study, a different group of researchers reported that mice that had been genetically engineered to "knock out" the gene for orexin itself (a single gene is responsible for both orexin-A and orexin-B) exhibited behavioral and EEG characteristics strikingly similar to those of both human patients with narcolepsy and dogs suffering from canine narcolepsy (Chemelli et al., 1999). Together, these findings strongly support a key role for orexin in narcolepsy; however, it turns out that human narcolepsy is not caused by mutations either in the *orexin* or *OX2R* genes. Instead, researchers who performed postmortem examinations of the brains of patients with narcolepsy observed a nearly complete loss of orexin neurons throughout the hypothalamic areas where these cells are normally present (Thannickal et al., 2000; **Figure B**). Due to the massive reduction of orexin input into the sleep–waking circuit described earlier, patients with narcolepsy exhibit intrusions of rapid-eye-movement (REM) sleep during the daytime. Attacks of cataplexy occur because skeletal muscle tone is suppressed during episodes of REM sleep. We don't yet know why orexin neurons have died in patients with narcolepsy, but one theory is that narcolepsy is an autoimmune disorder (i.e., the person's own immune system has attacked and killed the orexin neurons) (Kumar and Sagili, 2014; Liblau et al., 2015).

Current treatments for narcolepsy help reduce the symptoms but do not cure the disorder. Some of these treatments, such as methylphenidate, amphetamine, and modafinil are stimulants that reduce

BOX 3.1 Clinical Applications (*continued*)





(A) Orexin pathways involved in arousal and wakefulness Excitatory orexin projections to the depicted brain areas play an important role in arousal and the maintenance of wakefulness. ACh, acetylcholine; DA, dopamine; GABA, γ-aminobutyric acid; HA, histamine; 5-HT, serotonin; NE, norepinephrine. (From Alexandre et al., 2013.)

(B) Loss of orexin neurons in the brains of patients with narcolepsy and cataplexy The graph illustrates the mean number of orexin neurons within the hypothalamus and surrounding area in postmortem brain tissues obtained from patients with narcolepsy with cataplexy compared to controls without narcolepsy. (After Thannickal et al., 2000.)

daytime drowsiness but do not always prevent attacks of cataplexy (see Chapter 12). Moreover, these drugs have adverse side effects and, in the case of amphetamine and methylphenidate, have significant abuse potential. A very different medication that reduces both daytime sleepiness and episodes of cataplexy is sodium oxybate (trade name Xyrem), which is the sodium salt of γ-hydroxybutyrate (GHB). GHB exerts agonist effects on the GABA system, and in healthy individuals the drug has sedating effects (see Chapter 16). For this reason, GHB has been used as a "date rape" drug, and in recreational users GHB can be habit forming. We don't yet know whether the GABAergic effects of GHB are responsible for its therapeutic efficacy in narcolepsy.

Considering the limitations and problems associated with current narcolepsy treatments, it is not surprising that researchers are looking for alternative treatment approaches, especially ones that directly target the orexin system (Black et al., 2017). For example, a small study by Weinhold and colleagues (2014) found that intranasal orexin-A reduced daytime transitions from waking to REM sleep.[1] There was no attempt to determine whether the incidence of cataplexy was also affected by the treatment. In the long run, the most promising approach may be to use gene transfer methods to replace the missing orexin in patients with narcolepsy. The possibility of such an approach is supported by recent research using mouse models of narcolepsy (Blanco-Centurion et al., 2013; Kantor et al., 2013). There are also ongoing studies to produce orexin-synthesizing cells from human pluripotent stem cells, with the ultimate aim of transplanting the differentiated cells into patients (Black et al., 2017). However, routine application of any such methods to human patients with narcolepsy is undoubtedly some years away.

Although orexin-based medications for narcolepsy are still in the experimental stage, the orexin system has successfully been targeted in the treatment of insomnia. Historically, most prescription medications developed for treating insomnia have acted on the GABA system. Such medications include barbiturates, benzodiazepines like Valium, and related compounds that work like benzodiazepines but have a different chemical structure (see Chapter 17). Over-the-counter medications for insomnia include antihistamine drugs (compounds that block histamine receptors), as histaminergic neurons are part of the wake-promoting system described earlier. More recently, the discovery of

[1]Note that the orexin-A was not given orally, because it would likely be metabolized before reaching the brain. On the other hand, there is evidence that peptides can gain access to the brain when administered intranasally.

(*Continued*)

BOX 3.1 Clinical Applications *(continued)*

the role of orexin in arousal prompted a search for orexin receptor antagonists that could safely and effectively treat insomnia (Equihua et al., 2013). This search led to FDA approval in 2014 of **suvorexant** (trade name **Belsomra**), an antagonist at both OX1R and OX2R (Bennett et al., 2014). It seems likely that other orexin antagonists will also be released as insomnia medications. We note, however, that patients should exercise caution in using suvorexant or any other orexin antagonist, because the diverse functions of this neurotransmitter system raise the possibility of adverse side effects when orexin transmission is impaired.

discovered and intriguing group of neurotransmitters are the **gaseous transmitters** (sometimes shortened to gasotransmitters; Mustafa et al., 2009; Wang, 2014), which include nitric oxide (Cassenza et al., 2014), carbon monoxide (Levitt and Levitt, 2015), and hydrogen sulfide (Kimura, 2015). Later in this chapter, we will further discuss the lipid and gaseous transmitters and see that they break some of the rules followed by classical transmitter molecules.

When scientists first discovered the existence of neurotransmitters, it was natural to assume that each neuron made and released only one transmitter substance,[5] suggesting a simple chemical coding of cells in the nervous system. Much research over the past several decades has shattered that initial assumption. We now know that many neurons make and release two, three, and occasionally even more, different transmitters. In some cases, multiple small molecule neurotransmitters are released from the same neuron. In Chapter 8, we will touch on an unusual example of cells that corelease the excitatory transmitter glutamate and the inhibitory transmitter GABA at the same synapses. Other instances of transmitter coexistence within the same cell involve one or more neuropeptides, along with a classical transmitter (van den Pol, 2012). In such cases, the neuron has two different types of synaptic vesicles: small vesicles, which contain only the classical transmitter, and large vesicles, which contain the neuropeptide along with the classical transmitter (**FIGURE 3.3**).

Neuropeptides are synthesized by a different mechanism than other transmitters

How and where in the nerve cell are neurotransmitters manufactured? Except for the neuropeptides, transmitters are synthesized by enzymatic reactions that can occur anywhere in the cell. Typically, the enzymes required for producing a neurotransmitter are shipped out in large quantities to the axon terminals, so the terminals are an important site of transmitter synthesis. The neuropeptides are different, however. Their precursors are protein molecules, within which the peptides are embedded. The protein precursor for each type of peptide must be made in the cell body, which is the site of almost all protein synthesis in the neuron. The protein is then packaged into large vesicles, along with enzymes that will break down the precursor and liberate the neuropeptide (**FIGURE 3.4**). These vesicles are transported to the axon terminals, so release occurs from the terminals,

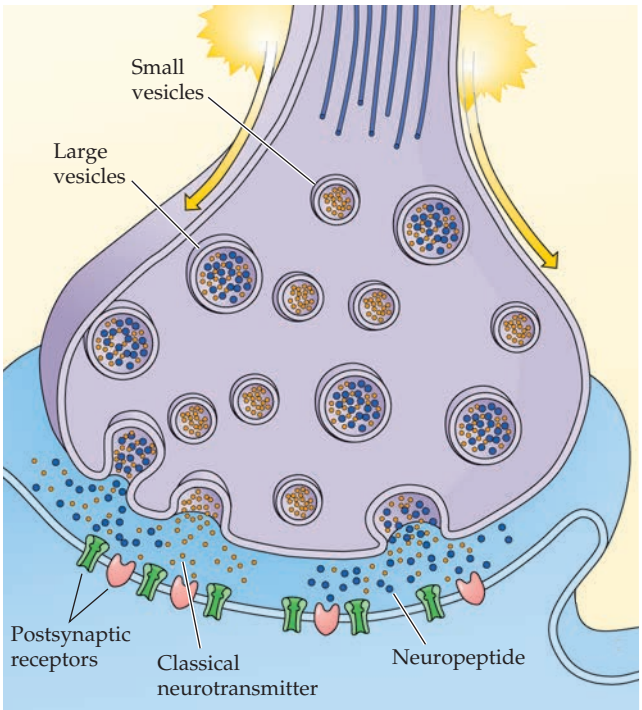


FIGURE 3.3 Axon terminal of a neuron that synthesizes both a classical neurotransmitter and a neuropeptide The small vesicles contain only the classical transmitter, whereas the large vesicles contain the neuropeptide and the classical neurotransmitter, which are stored and released together.

[5]This notion has sometimes been called "Dale's law," although what Henry Dale actually proposed is subtly different: that if one branch of a cell's axon were found to release a particular chemical substance when stimulated, then all axonal branches of that cell would also release that substance (notice that this statement doesn't preclude the cell from releasing multiple substances from its various branches).

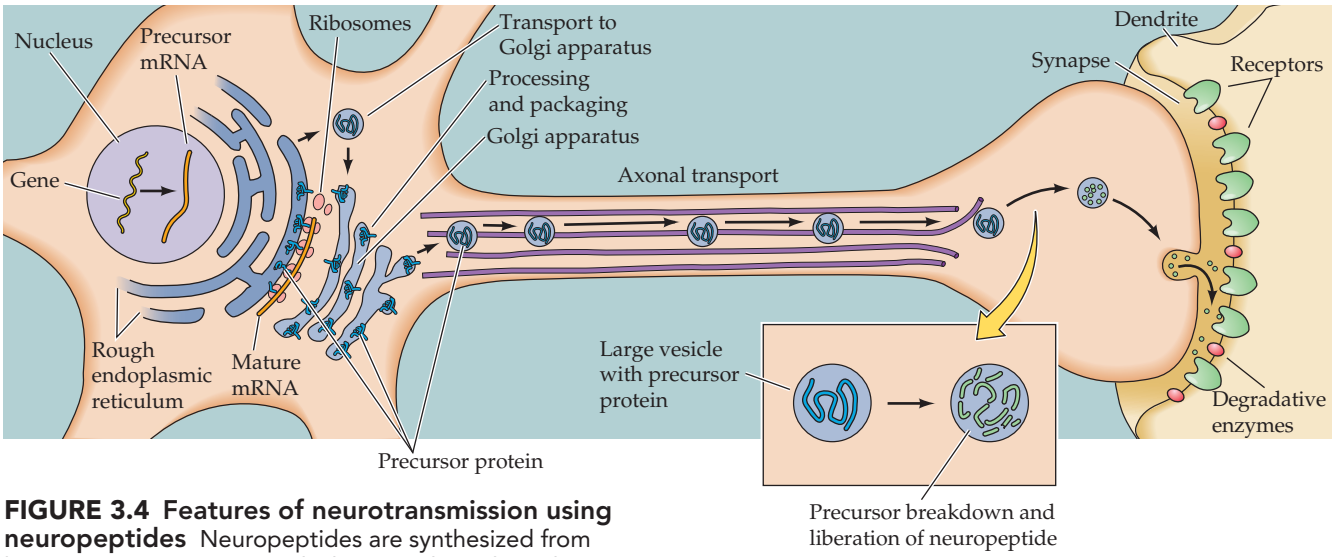


FIGURE 3.4 Features of neurotransmission using neuropeptides Neuropeptides are synthesized from larger precursor proteins, which are packaged into large vesicles by the Golgi apparatus. During transport from the cell body to the axon terminal, enzymes that have been packaged within the vesicles break down the precursor protein to liberate the neuropeptide. After it is released at the synapse and stimulates postsynaptic receptors, the neuropeptide is inactivated by degradative enzymes.

as with the classical transmitters. On the other hand, new neuropeptide molecules can be generated only in the cell body, not in the terminals. An important consequence of this difference is that replenishment of neuropeptides is slower than for small-molecule transmitters. When neurotransmitters are depleted by high levels of neuronal activity, small molecules can be resynthesized rapidly within the axon terminal. In contrast, neuropeptides cannot be replenished until large vesicles containing the peptide have been transported to the terminal from their site of origin within the cell body.

Neuromodulators are chemicals that don't act like typical neurotransmitters

Some investigators use the term **neuromodulators** to describe substances that don't act exactly like typical neurotransmitters. For example, a neuromodulator might not have a direct effect itself on the postsynaptic cell. Instead, it might alter the action of a standard neurotransmitter by enhancing, reducing, or prolonging the transmitter's effectiveness. Such effects may be seen when monoamine transmitters such as DA, NE, or 5-HT modulate the excitatory influence of another transmitter (e.g., glutamate) on the postsynaptic cell. Peptides that are coreleased with a classical transmitter may also function as neuromodulators. Yet another common property of neuromodulators is diffusion away from the site of release to influence cells more distant from the releasing cell than is the case at a standard synapse. This phenomenon has been termed **volume transmission** to distinguish it from the tight cell-to-cell synaptic interactions that constitute **wiring transmission** (Fuxe et al., 2010; 2013). No matter which criteria you use, though, the dividing line between neurotransmitters and neuromodulators is vague. For example, a particular chemical like serotonin may sometimes act within the synapse, but in other circumstances, it may act at a distance from its site of release. Therefore, we will refrain from talking about neuromodulators and instead will use the term *neurotransmitter* throughout the remainder of the book.

Classical transmitter release involves exocytosis and recycling of synaptic vesicles

As shown in **FIGURE 3.5**, synaptic transmission involves a number of processes that occur within the axon terminal and the postsynaptic cell. We will begin our discussion of these processes with a consideration of neurotransmitter release from the terminal. When a neuron fires an action potential, the depolarizing current sweeps down the length of the axon and enters all of the axon terminals. This wave of depolarization has a very important effect within the terminals: it opens large numbers of voltage-sensitive calcium (Ca^{2+}) channels, causing a rapid influx of Ca^{2+} ions into the terminals. The resulting increase in Ca^{2+} concentration within the terminals is the direct trigger for neurotransmitter release.

EXOCYTOSIS You already know that for most neurotransmitters, the transmitter molecules destined to be released are stored within synaptic vesicles, yet these molecules must somehow make their way past the membrane of the axon terminal and into the synaptic cleft. This occurs through a remarkable process known as **exocytosis**. Exocytosis is a fusion of the

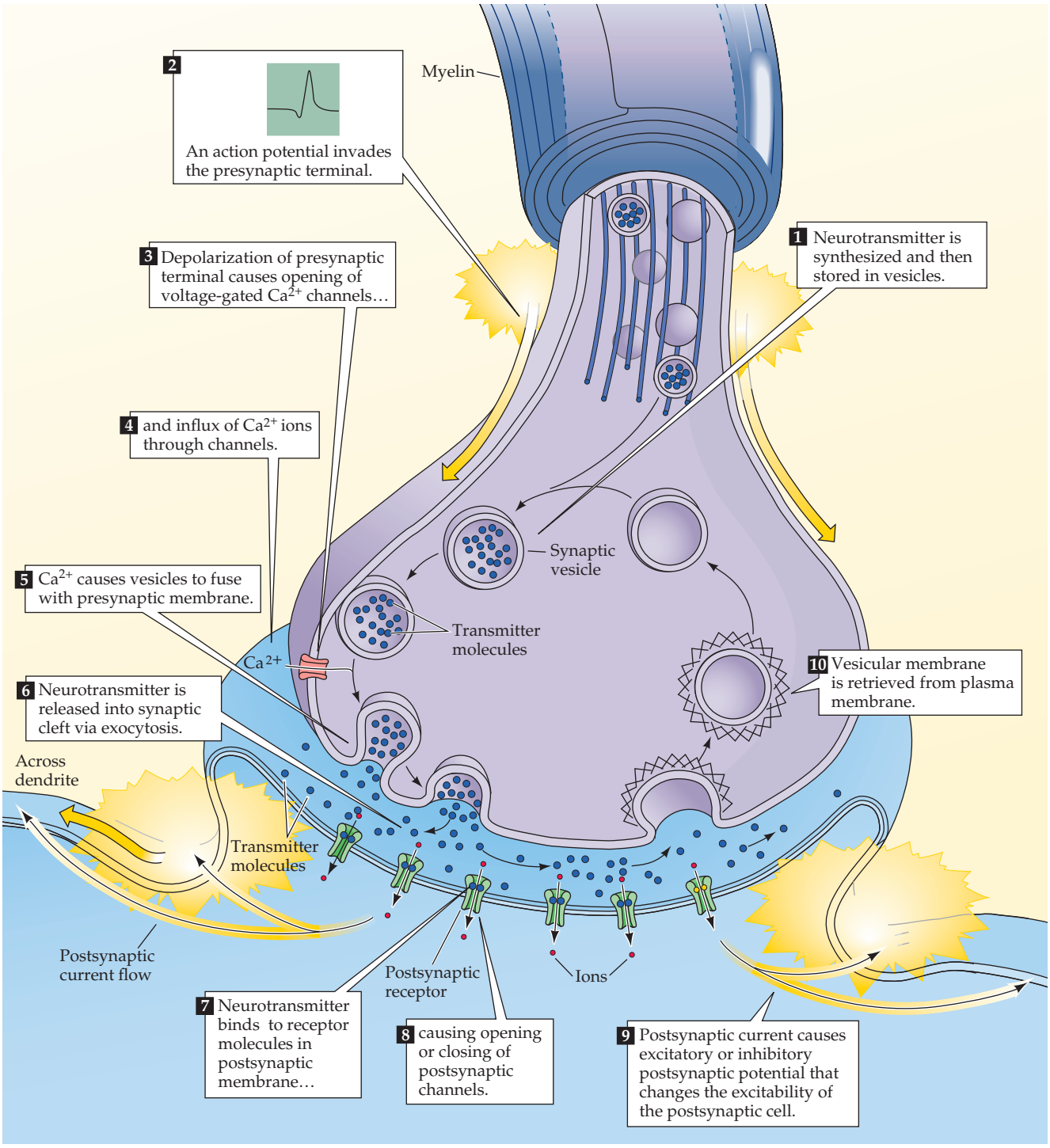


FIGURE 3.5 Processes involved in neurotransmission at a typical synapse using a classical neurotransmitter

vesicle membrane with the membrane of the axon terminal, which exposes the inside of the vesicle to the outside of the cell. In this way, the vesicle is opened, and its transmitter molecules are allowed to diffuse into the synaptic cleft. If you look back at the synapse shown in Figure 3.1A, you can see that some vesicles are very close to the terminal membrane, whereas others are farther away. In fact, transmitter release doesn't occur just anywhere along the terminal, but only at specialized regions near the postsynaptic cell, which stain darkly on the electron micrograph. These release sites are called **active zones**. For exocytosis to take place, a vesicle must be transported to an active zone by a mechanism that isn't yet fully understood.

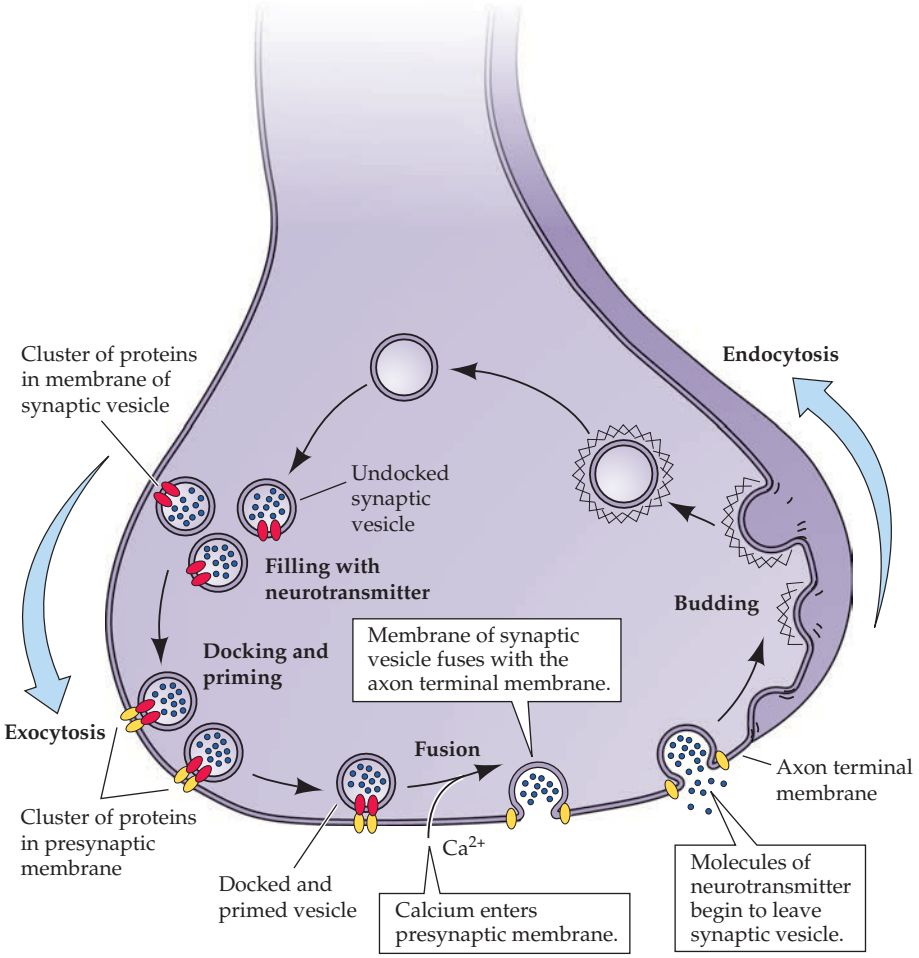


FIGURE 3.6 The life cycle of the synaptic vesicle Small vesicles containing classical neurotransmitters are constantly being recycled in the axon terminal. Vesicles are filled with neurotransmitter molecules, after which they are transported to the vicinity of release sites in the terminal and undergo processes of docking and priming. Ca^{2+}-dependent fusion with the axon terminal membrane, called exocytosis, permits release of the contents of the vesicle into the synaptic cleft. The vesicle membrane is retrieved by a process of invagination (budding) from the terminal membrane, endocytosis, and transmitter refilling, thus completing the cycle.

There, the vesicle must "dock" at the active zone, much like a boat docking at a pier. This docking step is carried out by a cluster of proteins—some located in the vesicle membrane and others residing in the membrane of the axon terminal. Docking is followed by a step called priming, which readies the vesicle for exocytosis once it receives the Ca^{2+} signal. Indeed, Ca^{2+} channels that open in response to membrane depolarization are concentrated in active zones near the sites of vesicle docking, so the protein machinery is exposed to particularly high concentrations of Ca^{2+} when the channels open. One or more proteins that are sensitive to Ca^{2+} then cause the vesicle and terminal membranes to fuse, and this allows the vesicle to open and the transmitter to be released. This process is illustrated in **FIGURE 3.6**.

In addition to the vesicle membrane proteins required for exocytosis, other proteins are required for other processes: some are required for pumping neurotransmitter molecules from the cytoplasm of the nerve terminal into the interior of the vesicle, whereas others are required for vesicle recycling, discussed in the next section. **FIGURE 3.7** shows a cutaway model of a typical synaptic vesicle that highlights the variety of proteins present in the vesicle membrane. One of these is **synaptobrevin**, which plays a key role in helping the vesicle fuse with the axon terminal membrane during the process of exocytosis. A detailed discussion of these proteins is beyond the scope of this book, but it's nevertheless important to note that some of them are targets for various drugs or naturally occurring toxins. For example, botulism poisoning results from a bacterial toxin (botulinum toxin) that blocks transmitter release at neuromuscular junctions, thus causing paralysis. Researchers have found that this blockade of release is due to enzymes within the toxin that attack some of the proteins that are required for the exocytosis process. This topic is covered in greater detail in Chapter 7, where we will also see how botulinum toxin has come to be used therapeutically in a wide range of neuromuscular disorders. Various studies have also shown that either acute or chronic exposure to ethanol can affect neurotransmitter release by acting on presynaptic proteins involved in the exocytosis process (Lovinger and Roberto, 2013). Indeed, these actions may contribute to both the intoxicating effects of ethanol and the development of ethanol dependence (i.e., alcoholism).

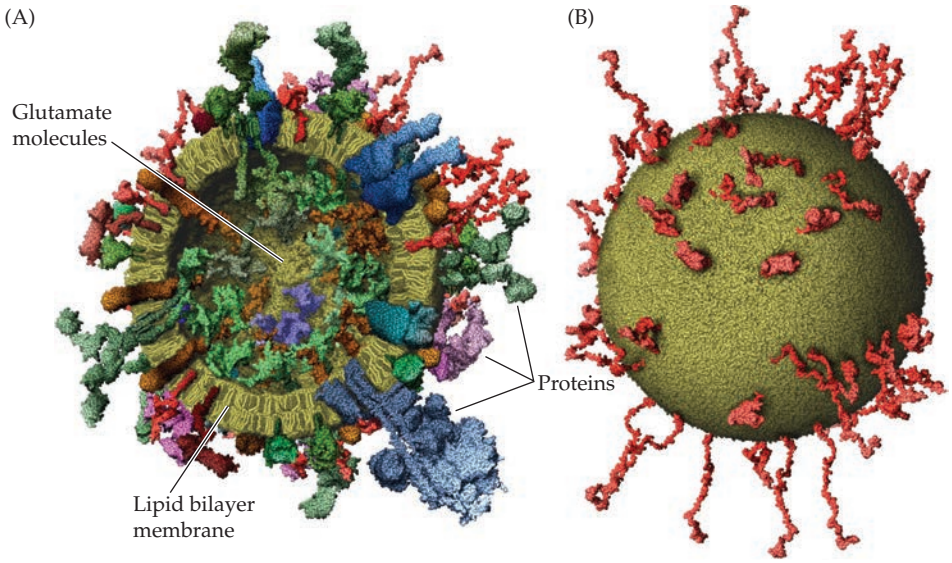


FIGURE 3.7 Molecular model of a glutamate synaptic vesicle This model depicts a synaptic vesicle used to store and release the neurotransmitter glutamate from nerve terminals. The model can be considered representative of all classical transmitter vesicles. (A) A cutaway view of the vesicle shows the lipid bilayer membrane in yellow along with many different proteins (drawn with different shapes and colors) inserted into or passing completely through the vesicle membrane. The small particles in the interior of the vesicle depict molecules of glutamate, several thousand of which are packed tightly within that space. (B) A simplified view of the exterior of the vesicle only shows a protein called synaptobrevin. This protein is the most abundant one found in synaptic vesicles. It helps vesicles to fuse with the axon terminal membrane during exocytosis. (From Takamori et al., 2006.)

VESICLE RECYCLING When a synaptic vesicle fuses with the axon terminal to release its transmitter contents, the vesicle membrane is temporarily added to the membrane of the terminal. If this process were never reversed, the terminal membrane would grow larger and larger as more and more vesicle membrane was added to it. In fact, we know that synaptic vesicles undergo a process of **vesicle recycling** that serves the dual functions of (1) preventing depletion of synaptic vesicles (especially when the neuron is firing rapidly and many vesicles are releasing their contents into the synaptic cleft), and (2) preventing an accumulation of vesicle membrane within the membrane of the nerve terminal.

Despite many years of research, scientists still disagree about the details of vesicle recycling, because of differing experimental results (Wu et al., 2014; Kononenko and Haucke, 2015). Three of the currently proposed models are depicted in **FIGURE 3.8**. The first two models, shown in Figure 3.8A and B, involve a full fusion of the vesicle membrane with the nerve terminal followed (after neurotransmitter release) by a flattening of the vesicle membrane. The semicircular figures illustrate a vesicle in the process of flattening. The model shown in Figure 3.8A is the most widely accepted model of vesicle recycling and is the one depicted previously in Figures 3.5 and 3.6. In this model, a protein called **clathrin** forms a coating on the membrane that is needed for membrane invagination and vesicle retrieval. For this reason, the mechanism is called **clathrin-mediated endocytosis**. Note that in this model, endocytosis is a relatively slow process that occurs in an area of the nerve terminal away from the release site. Figure 3.8B illustrates a more recently proposed model called **ultrafast endocytosis** in which vesicle retrieval occurs extremely quickly in an area close to the release site. The retrieved vesicles are thought to join with intracellular organelles called **endosomes** (membranous compartments inside the nerve terminal), after which the recycling process is completed when new synaptic vesicles bud off from the endosomes using a clathrin-dependent mechanism. The third model, which is somewhat controversial, is called **kiss-and-run** (Figure 3.8C). This model proposes that the vesicle fuses with the nerve terminal membrane merely for an extremely brief period of time to allow the neurotransmitter molecules to escape from the vesicle interior. The temporary pore formed during fusion then closes, and the original vesicle remains intact for refilling with neurotransmitter molecules and subsequent participation in synaptic transmission. Clathrin protein is not required for any of the steps involved in the kiss-and-run mechanism. Watanabe (2015) likens kiss-and-run versus the two endocytosis models to the recycling of glass bottles: kiss-and-run would be like refilling the same bottle, whereas recycling via endocytosis would be like combining each bottle with many others at a recycling plant before remolding a new bottle from the mixture.

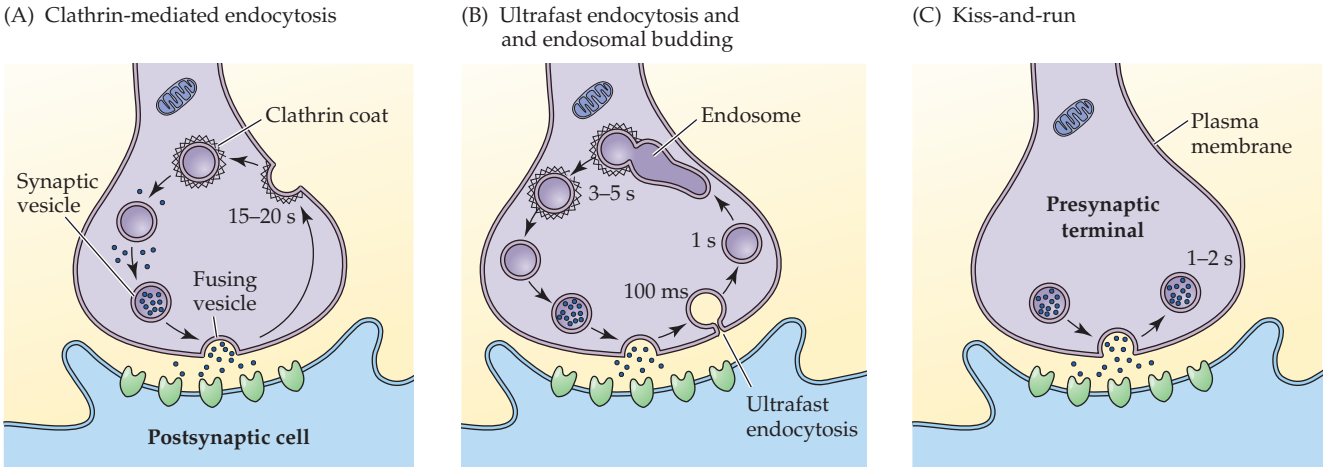


FIGURE 3.8 Models of synaptic vesicle recycling (A) In the well-established clathrin-mediated endocytosis model, the vesicle fully collapses after fusing with the plasma membrane. The vesicle membrane is subsequently retrieved at a point distant from the release site, using a process that requires the protein clathrin. (B) In the more recently proposed model called ultrafast endocytosis and endosomal budding, the vesicle membrane is retrieved extremely rapidly at a point near the release site. Clathrin is not required for membrane retrieval according to this model. The vesicle membrane fuses with an endosome, after which new vesicles bud off from the endosome in a clathrin-dependent mechanism. (C) In the kiss-and-run model, the vesicle membrane briefly fuses with the plasma membrane of the nerve terminal without collapsing. After the vesicle has released its contents into the synaptic cleft, it reforms and detaches from the plasma membrane. Neither clathrin nor endosomes play a role in this model. (After Watanabe, 2015.)

Two additional points need to be added before concluding this discussion. First, the three mechanisms just described occur under typical conditions of low to moderate neuronal activity. However, when neurons are stimulated very strongly, yet another mechanism called **bulk endocytosis** is used to retrieve the large amounts of vesicle membrane that have fused with the nerve terminal membrane. This process, which is not shown, has a mixture of properties shared with the other mechanisms. Like clathrin-mediated endocytosis, bulk endocytosis is slow and takes place at a distance from the release site. But like ultrafast endocytosis, bulk endocytosis makes use of endosomes and only requires clathrin for budding of new vesicles off of the endosome. The second point is that these recycling mechanisms only occur with the small vesicles containing classical transmitters, not with the larger neuropeptide-containing vesicles. You'll recall that neuropeptide precursor proteins must be packaged into the large vesicles in the cell body; therefore, recycling of such vesicles cannot occur at the axon terminal.

Lipid and gaseous transmitters are not released from synaptic vesicles

We mentioned earlier that lipid and gaseous transmitters break some of the normal rules of neurotransmission. One of the reasons is that these substances readily pass through membranes. Consequently, these substances cannot be stored in synaptic vesicles like classical and peptide transmitters. So nerve cells must make these substances "on demand" when needed. Moreover, as lipid and gaseous transmitters are not present in vesicles, they are not released by exocytosis but simply diffuse out of the nerve cell through the cell membrane. Once the transmitter molecules reach the extracellular fluid, they may not be confined to the synapse but may travel far enough to reach other cells in the vicinity of the release point. This is particularly true for the gaseous transmitters. Finally, lipid and gaseous transmitters typically are released by the postsynaptic rather than the presynaptic cell in the synapse. Molecules such as these that signal information from the postsynaptic to the presynaptic cell are called **retrograde messengers** (in Chapter 14 we describe an example of retrograde signaling by endogenous cannabinoids). **FIGURE 3.9** illustrates how signaling by gaseous and lipid transmitters differs from classical neurotransmitter signaling.

As with many different neurotransmitters, lipids and gases have signaling functions outside of the CNS. A well-known example of this pertains to nitric oxide (NO), the first gaseous transmitter to be discovered. The discovery of NO came about unexpectedly from the study of smooth muscle cells that surround the walls of arteries, regulating the rate of arterial blood flow. A number of chemical substances, including the neurotransmitter ACh, were known to relax these smooth muscle cells, thus causing vasodilation (widening

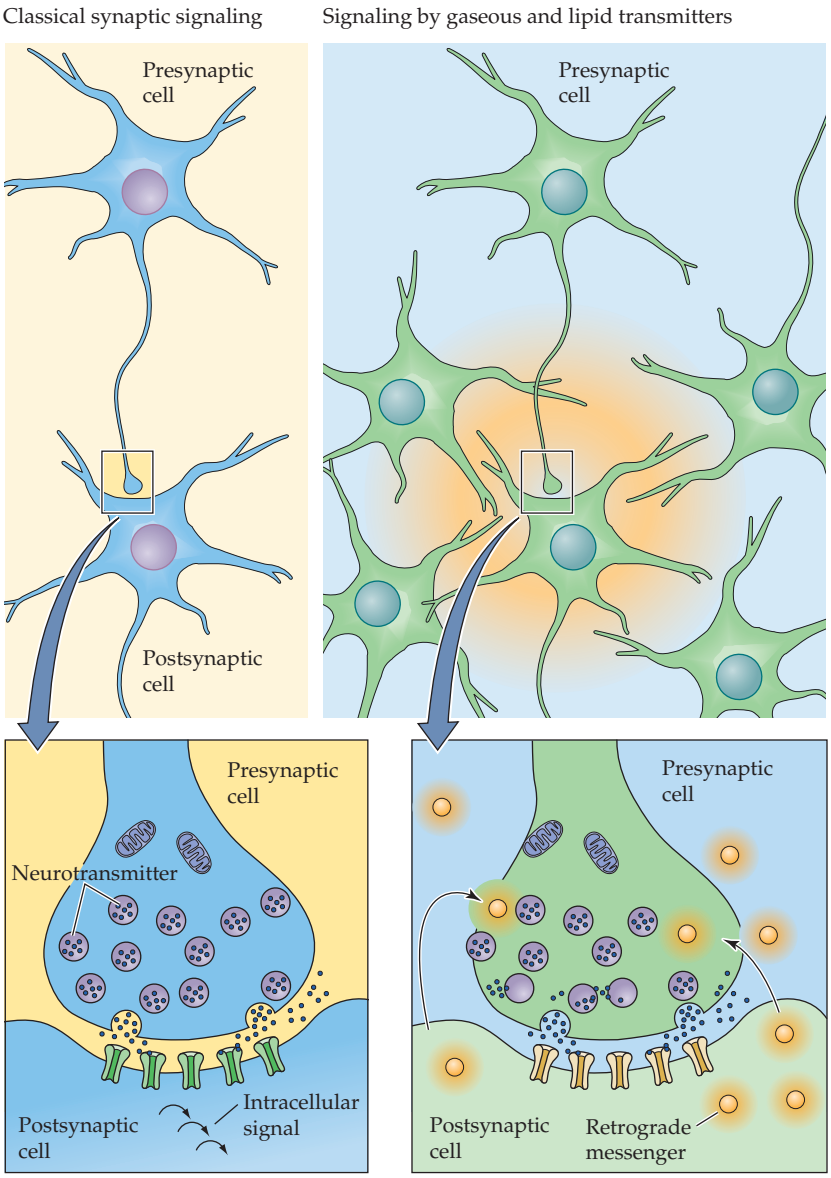


FIGURE 3.9 Signaling by classical versus gaseous and lipid transmitters In contrast to signaling by classical neurotransmitters (left), gaseous and lipid transmitters (right) are not released from synaptic vesicles. Gases and lipids cannot be stored within vesicles and, therefore, must be synthesized enzymatically when needed. Synthesis of gaseous and lipid transmitters usually occurs in the postsynaptic cell following release of a classical transmitter from the presynaptic cell and postsynaptic receptor activation. The newly formed gas or lipid subsequently diffuses across the membrane and acts as a retrograde messenger on the presynaptic cell as well as other neurons close enough to receive the signal.

of the blood vessels) and increased blood flow. However, the mechanism by which this occurred was unclear until the early 1980s, when researchers showed that endothelial cells were necessary for the relaxant effects of ACh on the muscle. In addition, they showed that ACh stimulated the endothelial cells to produce a chemical factor that traveled to nearby muscle cells and caused them to relax. This chemical factor was subsequently shown to be NO. The discovery of NO and of its role in regulating cardiovascular function was recognized by the awarding of the 1998 Nobel Prize in Physiology or Medicine to the three key pioneers in these discoveries: Robert Furchgott, Louis Ignarro, and Ferid Murad.

Several mechanisms control the rate of neurotransmitter release by nerve cells

Neurotransmitter release is regulated by several different mechanisms. The most obvious is the rate of cell firing. When a neuron is rapidly firing action potentials, it will release much more transmitter than when it is firing at a slow rate. A second factor is the probability of transmitter release from the terminal. It might seem odd that an action potential could enter a terminal and open Ca^{2+} channels without releasing any transmitter. Yet many studies have shown that synapses in different parts of the brain vary widely in the probability that even a single vesicle will undergo exocytosis in response to an action potential. Estimated probabilities range from less than 0.1 (10%) to 0.9 (90%) or greater for different populations of synapses. We don't yet know why these probabilities can vary so much, but it is clearly an important factor in the regulation of neurotransmitter release.

A third factor in the rate of transmitter release is the presence of **autoreceptors** on axon terminals or cell bodies and dendrites (**FIGURE 3.10**). An autoreceptor on a particular neuron is a receptor for the same neurotransmitter released by that neuron (*auto* in this case means "self"). Neurons may possess two different types of autoreceptors: **terminal autoreceptors** and **somatodendritic autoreceptors**. Terminal autoreceptors are so named because they are located on axon terminals. When they are activated by the neurotransmitter, their main function is to inhibit further transmitter release. This function is particularly important when the cell is firing rapidly and high levels of neurotransmitter are present in the synaptic cleft. Think of

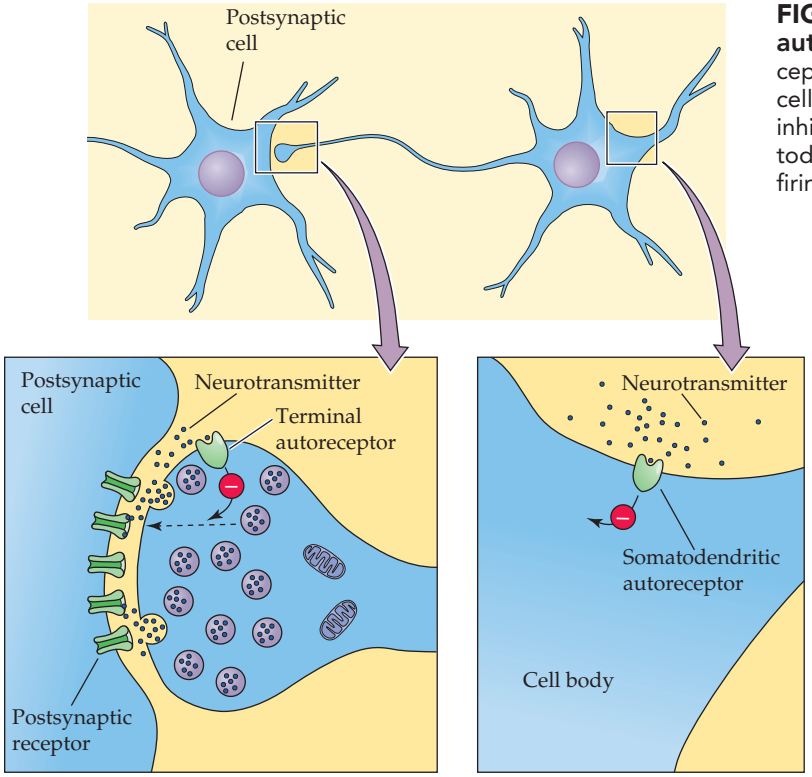


FIGURE 3.10 Terminal and somatodendritic autoreceptors Many neurons possess autoreceptors on their axon terminals and/or on their cell bodies and dendrites. Terminal autoreceptors inhibit neurotransmitter release, whereas somatodendritic autoreceptors reduce the rate of cell firing.

the thermostat ("autoreceptor") in your house, which shuts off the furnace ("release mechanism") when the level of heat ("neurotransmitter") gets too high. Somatodendritic autoreceptors are also descriptively named, in that they are autoreceptors found on the cell body (soma) or on dendrites. When these autoreceptors are activated, they slow the rate of cell firing, which ultimately causes less neurotransmitter release because fewer action potentials reach the axon terminals to stimulate exocytosis.

Researchers can use drugs to stimulate or block specific autoreceptors, thereby influencing the release of a particular neurotransmitter for experimental purposes. For example, administration of a low dose of the drug apomorphine to rats or mice selectively activates the terminal autoreceptors for DA. This causes lessened DA release, an overall reduction in dopaminergic transmission, and reduced locomotor activity among animals. A different drug, whose name is abbreviated 8-OH-DPAT, activates the somatodendritic autoreceptors for 5-HT and powerfully inhibits the firing of serotonergic neurons. The behavioral effects of administration of 8-OH-DPAT include increased appetite and altered responses on several tasks used to assess anxiety.

Finally, you'll recall from our earlier discussion that in addition to autoreceptors, axon terminals may have receptors for other transmitters released at axoaxonic synapses. Such receptors have come to be known as **heteroreceptors**, to distinguish them from autoreceptors. Heteroreceptors also differ from autoreceptors in that they may either enhance or reduce the amount of transmitter being released from the axon terminal.

Neurotransmitters are inactivated by reuptake and by enzymatic breakdown

Any mechanical or biological process that can be turned on must have a mechanism for termination (imagine the problem you would have with a car in which the ignition could not be turned off once the car had been started). Thus, it is necessary to terminate the synaptic signal produced by each instance of transmitter release, so the postsynaptic cell is free to respond to the next release. This termination is accomplished by removing neurotransmitter molecules from the synaptic cleft. How is this done?

Several different processes responsible for neurotransmitter removal are shown in **FIGURE 3.11**. One mechanism is enzymatic breakdown within or near the synaptic cleft. This mechanism is very important for the classical neurotransmitter ACh, for the lipid and gaseous transmitters, and also for the neuropeptide transmitters. An alternative mechanism involves removal of the neurotransmitter from the synaptic cleft by a transport process that makes use of specialized proteins called **transporters** located on the cell membrane. This mechanism is important for amino acid transmitters like glutamate and GABA and also for monoamine transmitters such as DA, NE, and 5-HT. Transport out of the synaptic cleft is sometimes accomplished by the same cell that released the transmitter, in which case it is called **reuptake**. In other cases, the transmitter may be taken up either by the postsynaptic cell or by nearby glial cells (specifically astrocytes). Some important psychoactive drugs work by blocking neurotransmitter transporters. Cocaine, for example, blocks the transporters for DA, 5-HT, and NE. Many antidepressant drugs block the 5-HT transporter, the NE transporter, or both. Since these transporters are so important for clearing the neurotransmitter from the synaptic cleft, it follows that

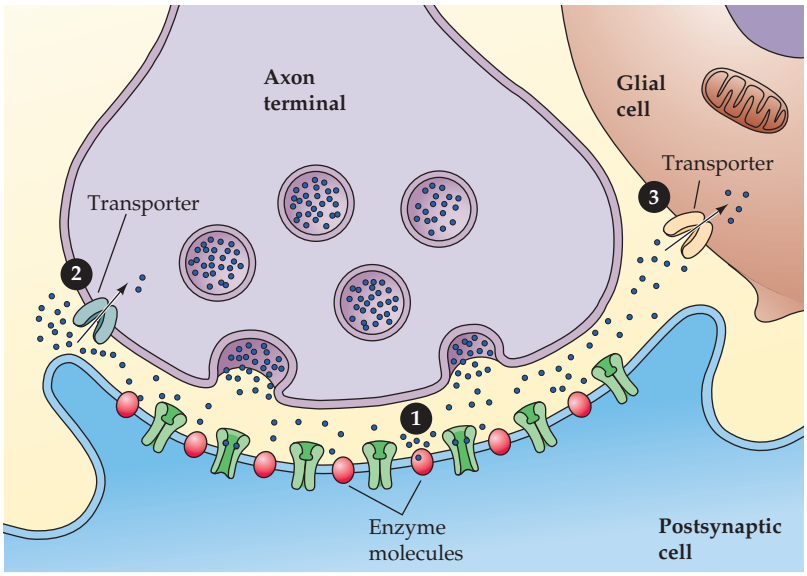


FIGURE 3.11 Neurotransmitter inactivation Neurotransmitter molecules can be inactivated by (1) enyzmatic breakdown, (2) reuptake by the axon terminal, or (3) uptake by nearby glial cells. Cellular uptake is mediated by specific membrane transporters for each neurotransmitter.

when the transporters are blocked, neurotransmitter molecules remain in the synaptic cleft for a longer time, and neurotransmission is enhanced at those synapses.

When neurotransmitter transporters are active, some transmitter molecules removed from the synaptic cleft are reused by being packaged into recycled vesicles. However, other transmitter molecules are broken down by enzymes present within the cell. Thus, uptake and metabolic breakdown are not mutually exclusive processes. Many transmitter systems use both mechanisms. Finally, it is important to keep in mind the distinction between autoreceptors and transporters. Even though both may be present on axon terminals, they serve different functions. Terminal autoreceptors modulate transmitter release, but they don't transport the neurotransmitter. Transporters take up the transmitter from the synaptic cleft, but they are not autoreceptors.

Section Summary

- Synapses may occur on the dendrite (axodendritic), cell body (axosomatic), or axon (axoaxonic) of the postsynaptic cell.
- Most neurotransmitters fall into one of the following categories (with acetylcholine as a notable exception): amino acid transmitters, monoamine transmitters, lipid transmitters, neuropeptide transmitters, and gaseous transmitters.
- Neurons commonly synthesize and release two or more neurotransmitters, often from different categories.
- Classical neurotransmitters (amino acids, monoamines, and acetylcholine) are mainly synthesized in the nerve terminal and then are transported into synaptic vesicles; neuropeptides are synthesized and are packaged into vesicles in the cell body.
- Neurotransmitters are released from nerve terminals by a Ca^{2+}-dependent process called exocytosis.
- Synaptic vesicles are replenished by recycling processes. Three models have been proposed to explain vesicle recycling at low to moderate rates of neuronal activity: clathrin-mediated endocytosis, ultrafast endocytosis, and kiss-and-run. At very high rates of activity, a fourth process called bulk endocytosis comes into play to retrieve and recycle large amounts of vesicle membrane that has fused with the nerve terminal membrane.
- Lipid and gaseous transmitters are synthesized upon demand, are not stored in synaptic vesicles, and often function as retrograde messengers by signaling from the postsynaptic to the presynaptic cell.
- Neurotransmitter release is controlled by the rate of cell firing, the release probability at a specific synapse, and inhibitory terminal and somatodendritic autoreceptors.
- Depending on the neurotransmitter, termination of transmitter action is accomplished by the processes of uptake (including reuptake by the presynaptic cell) and/or enzymatic breakdown.

Neurotransmitter Receptors and Second-Messenger Systems

Chemical signaling by neurotransmitters requires the presence of molecules on the membrane of the

postsynaptic cell called **receptors** that are sensitive to the neurotransmitter signal. As we shall see, there are several different mechanisms by which neurotransmitter receptors alter activity of the postsynaptic cell, some of which involve complex biochemical pathways known as **second-messenger systems**.

There are two major families of neurotransmitter receptors

In Chapter 1, you were introduced to the concept of a drug receptor. Many of the receptors for psychoactive drugs are actually receptors for various neurotransmitters. For this reason, it is very important to understand the characteristics of neurotransmitter receptors and how they function.

Virtually all neurotransmitter receptors are proteins, and in most cases, these proteins are located on the plasma membrane of the cell. As we saw earlier, the cell possessing the receptor may be a neuron, a muscle cell, or a secretory cell. The neurotransmitter molecule binds to a specific site on the receptor molecule, which activates the receptor and produces a biochemical alteration in the receiving cell that may affect its excitability. For example, postsynaptic receptors on neurons usually influence the likelihood that the cell will generate an action potential. The effect of receptor activation may be either excitatory (increasing the probability of an action potential) or inhibitory (decreasing the probability of an action potential), depending on what the receptor does to the cell (see following sections). Recall that if a particular drug mimics the action of the neurotransmitter in activating the receptor, we say that the drug is an agonist at that receptor (see Chapter 1). If a drug blocks or inhibits the ability of the neurotransmitter to activate the receptor, then the drug is called an antagonist.

Three key concepts are necessary for understanding neurotransmitter receptors. First, receptors *do not* act like the neurotransmitter transporters described above. Their role is to pass a signal (i.e., presence of released neurotransmitter molecules) from the presynaptic to the postsynaptic cell. Unlike transporters, receptors do not carry neurotransmitter molecules across the cell membrane. Instead, after a neurotransmitter molecule binds briefly to a receptor to activate it, the neurotransmitter physically disengages and is now available to activate another receptor, be taken up by a transporter, or become inactivated by a neurotransmitter metabolizing enzyme.

Second, almost all neurotransmitters discovered so far have more than one kind of receptor. Different varieties of receptors for the same transmitter are called **receptor subtypes** for that transmitter. The existence of subtypes adds complexity to the study of receptors, making the task of pharmacologists (as well as students!) more difficult. But this complexity has a positive aspect: if you can design a drug that stimulates or blocks just the subtype that you're interested in, you may be able to treat a disease more effectively and with fewer side effects. This is one of the central ideas underlying modern drug design and the continuing search for new pharmaceutical agents.

The third key concept is that most neurotransmitter receptors fall into two broad categories: **ionotropic receptors** and **metabotropic receptors**. A particular transmitter may use only receptors that fit one or the other of these general categories, or its receptor subtypes may fall into both categories. As shown in **TABLE 3.2**, ionotropic and metabotropic receptors differ in both structure and function, so we will discuss them separately.

IONOTROPIC RECEPTORS Ionotropic receptors work very rapidly, so they play a critical role in fast neurotransmission within the nervous system. Each ionotropic receptor is made up of several proteins called **subunits**, which are assembled to form the complete receptor before insertion into the cell membrane. Four or five subunits are needed, depending on the overall structure of the receptor (**FIGURE 3.12A**). At the center of every ionotropic receptor is a channel or pore through which ions can flow. The receptor also possesses one or more binding sites for the neurotransmitter. In the resting state with no neurotransmitter present, the receptor channel is closed, and no ions are moving. When the neurotransmitter binds

TABLE **3.2** Comparison of Ionotropic and Metabotropic Receptors

Characteristics	Ionotropic receptors	Metabotropic receptors
Structure	4 or 5 subunits that are assembled and then inserted into the cell membrane	1 subunit
Mechanism of action	Contain an intrinsic ion channel that opens in response to neurotransmitter or drug binding	Activate G proteins in response to neurotransmitter or drug binding
Coupled to second messengers?	No	Yes
Speed of action	Fast	Slower

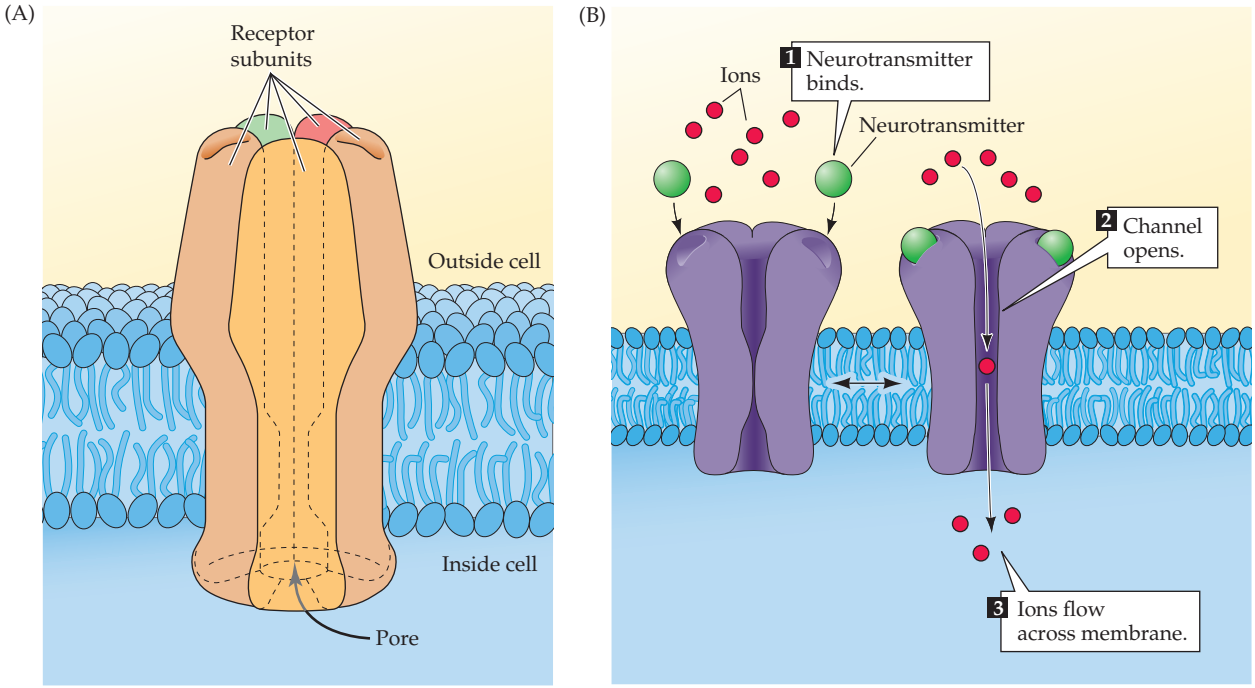


FIGURE 3.12 Structure and function of ionotropic receptors (A) Each receptor complex comprises either five (as shown) or four protein subunits that form a channel or a pore in the cell membrane. (B) Binding of the neurotransmitter to the receptor triggers channel opening and the flow of ions across the membrane.

to the receptor and activates it, the channel immediately opens and ions flow across the cell membrane (**FIGURE 3.12B**). When the neurotransmitter molecule leaves (dissociates from) the receptor, the channel quickly closes. Because of these features, a common alternative name for ionotropic receptors is **ligand-gated channel receptors**.[6] Another feature of ionotropic receptors is that they can undergo a phenomenon called **desensitization**, in which the channel remains closed even though there may be ligand molecules bound to the receptor (the details of this process are beyond the scope of the chapter). Once this occurs, the channel must resensitize before it can be activated once again.

Some ionotropic receptor channels allow sodium (Na^+) ions to flow into the cell from the extracellular fluid. Because these ions are positively charged, the cell membrane is depolarized, thereby producing an excitatory response of the postsynaptic cell. One of the best-known examples of this kind of excitatory ionotropic receptor is the nicotinic receptor for ACh, which we'll discuss further in Chapter 7. A second type of ionotropic receptor channel permits the flow of Ca^{2+} as well as Na^+ ions across the cell membrane. As we will see shortly, Ca^{2+} can act as a **second messenger** to trigger many biochemical processes in the postsynaptic cell. One important ionotropic receptor that functions in this way is the *N*-methyl-D-aspartate (NMDA) receptor for the neurotransmitter glutamate (see Chapter 8). Finally, a third type of receptor channel is selective for chloride (Cl^-) ions to flow into the cell. These ions are negatively charged, thus leading to hyperpolarization of the membrane and an inhibitory response of the postsynaptic cell. A good example of this kind of inhibitory ionotropic receptor is the $GABA_A$ receptor (see Chapter 8). From this discussion, you can see that the characteristics of the ion channel controlled by an ionotropic receptor are the key factor in determining whether that receptor excites the postsynaptic cell, inhibits the cell, or activates a second-messenger system.

METABOTROPIC RECEPTORS Metabotropic receptors act more slowly than ionotropic receptors. It takes longer for the postsynaptic cell to respond, but its response is somewhat more long-lasting than in the case of ionotropic receptors. Metabotropic receptors are composed of only a single protein subunit, which winds its way back and forth through the cell membrane seven times. Using the terminology of cell biology, we say that these receptors have seven transmembrane domains; in fact, they are sometimes abbreviated 7-TM receptors (**FIGURE 3.13**). It is important to note that metabotropic receptors do not possess a channel or pore. How, then, do these receptors work?

[6]This terminology distinguishes such channels from voltage-gated channels, which are controlled by voltage across the cell membrane rather than by binding of a ligand such as a neurotransmitter or a drug.

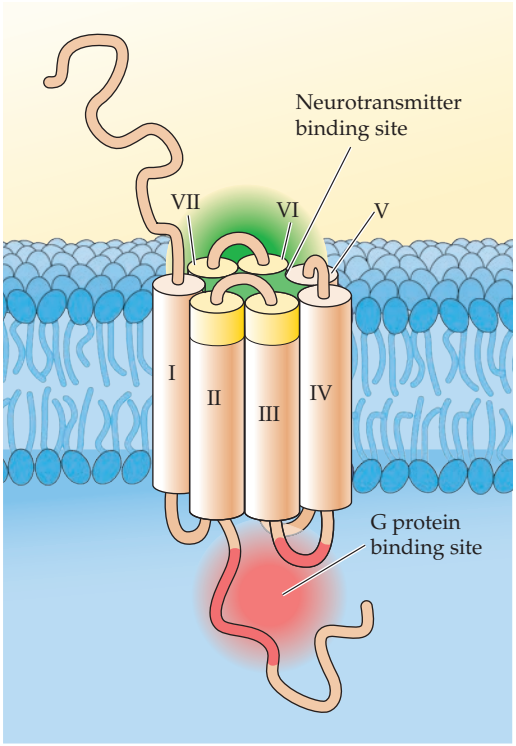


FIGURE 3.13 Structure of metabotropic receptors
Each receptor comprises a single protein subunit with seven transmembrane domains (labeled here by Roman numerals).

Metabotropic receptors work by activating other proteins in the cell membrane called **G proteins** (students interested in learning more about the structure and functioning of G proteins are referred to **Web Box 3.1**). Consequently, another name for this receptor family is **G protein–coupled receptors**. Many different kinds of G proteins have been identified, and how a metabotropic receptor influences the postsynaptic cell depends on which G protein(s) the receptor activates. However, all G proteins operate by two major mechanisms. One is by stimulating or inhibiting the opening of ion channels in the cell membrane (**FIGURE 3.14A**). Potassium (K^+) channels, for example, are stimulated by specific G proteins at many synapses. When these channels open, K^+ ions flow out of the cell, the membrane is hyperpolarized, and consequently the cell's firing is suppressed. This is a common mechanism of synaptic inhibition used by various receptors for ACh, DA, NE, 5-HT, and GABA and some neuropeptides like the endorphins. Note that the K^+ channels controlled by G proteins are not the same as the voltage-gated K^+ channels that work together with voltage-gated Na^+ channels to produce action potentials.

The second mechanism by which metabotropic receptors and G proteins operate is by stimulating or inhibiting certain enzymes in the cell membrane (**FIGURE 3.14B**). These enzymes are sometimes called **effector enzymes** because they produce biochemical and physiological effects in the postsynaptic cell. Most of the effector enzymes controlled by G proteins are involved in either the synthesis or the breakdown of small molecules called *second messengers*. Second messengers were first discovered in the 1960s and later found to play an important role in the chemical communication processes of both neurotransmitters and hormones. In these processes, the neurotransmitter or hormone was considered to be the "first messenger," and the "second messenger" within the receiving cell (the postsynaptic cell, in the case of a neurotransmitter) then carried out the biochemical change signaled by the first messenger. When everything is put together, this mechanism of metabotropic receptor function involves (1) activation of a G

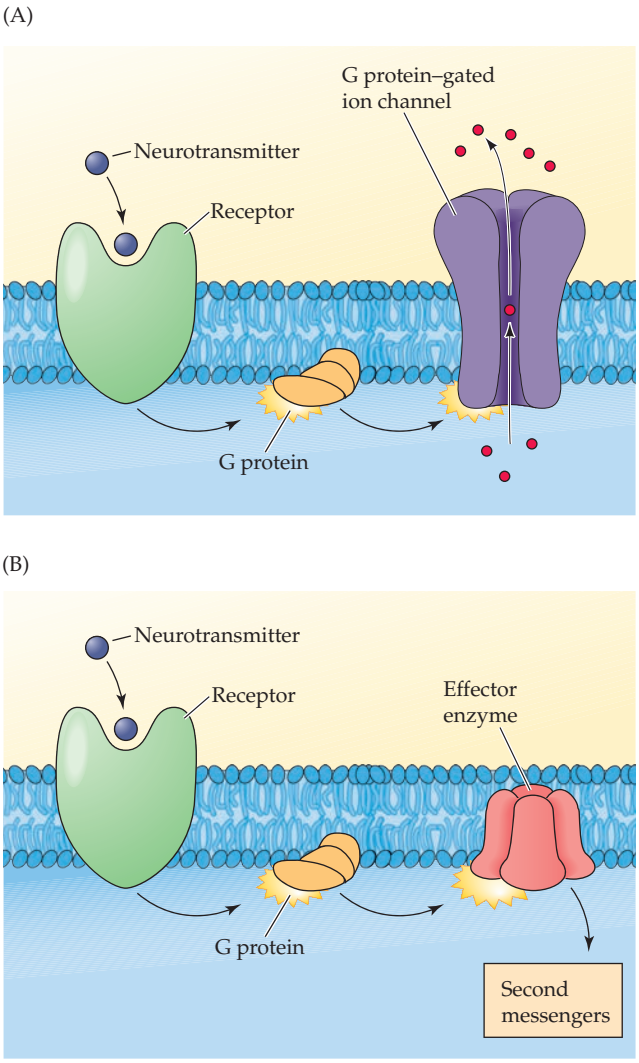


FIGURE 3.14 Functions of metabotropic receptors
Metabotropic receptors activate G proteins in the membrane, which may either (A) alter the opening of a G protein–gated ion channel or (B) stimulate or inhibit an effector enzyme that either synthesizes or breaks down a second messenger.

protein, followed by (2) stimulation or inhibition of an effector enzyme in the membrane of the postsynaptic cell, followed by (3) increased synthesis or breakdown of a second messenger, followed by (4) biochemical or physiological changes in the postsynaptic cell due to the altered levels of the second messenger (see Figure 3.14B). This sequence of events is an example of a biochemical "cascade."

BOTH TYPES OF RECEPTORS CAN BE AFFECTED BY ALLOSTERIC MODULATORS It may be surprising to learn that many ionotropic and metabotropic receptors possess additional binding sites besides the site(s) recognized by a typical agonist or antagonist (Melancon et al., 2012; Changeux, 2013). These additional binding sites are called **allosteric sites**, and molecules such as drugs that bind to such sites and alter the functioning of the receptor are called **allosteric modulators**. **FIGURE 3.15** illustrates the concept of allosteric modulation using a metabotropic receptor as an example. You can see that allosteric modulators can have either a positive or a negative effect on receptor signaling. Also shown is the important fact that allosteric modulators only modify the effects of an agonist; they have no effects when given alone. That key difference, along with the presence of a binding site separate from the agonist binding site, is why allosteric modulators are not simply receptor agonists or antagonists.

Allosteric modulators, particularly modulators of metabotropic receptors, are becoming increasingly important in the search for better drugs to treat psychiatric and neurological disorders. Among the many disorders being targeted in such searches are schizophrenia, depression, anxiety disorders, addiction, autism spectrum disorders, Alzheimer's disease, and Parkinson's disease (Nickols and Conn, 2014). Compared with traditional agonists or antagonists, allosteric modulators often possess greater receptor subtype selectivity, which would decrease the likelihood of adverse side effects when the drug is given to a patient. Moreover, when an allosteric modulator is used to alter the effect of a neurotransmitter agonist on its receptor, the modulator may "fine-tune" signaling by that neurotransmitter (either amplifying or diminishing such signaling) in a more controllable manner than would administering a direct agonist or antagonist for the receptor. For example, dysfunction of the glutamate neurotransmitter system has been implicated in the cognitive deficits associated with disorders like Alzheimer's disease and schizophrenia. Directly activating or blocking glutamate receptors in the brain either is difficult from a practical standpoint (e.g., the drugs may not pass the blood–brain barrier) or produces very troubling side effects. However, altering central glutamate activity using allosteric modulators of metabotropic glutamate receptors (see Chapter 8) has much more promise for improving cognitive function (Nickols and Conn, 2014).

Second messengers work by activating specific protein kinases within a cell

Second-messenger systems are too complex to be completely covered in this text. We will therefore highlight several of the most important systems and how they alter cellular function. One of the key ways in which second messengers work is by activating enzymes called **protein kinases** (**FIGURE 3.16**). Kinases are enzymes that **phosphorylate** another molecule, that is, they catalyze the addition of one or more phosphate groups ($-PO_4^{2-}$) to the molecule. As the name suggests, a protein kinase phosphorylates a protein. The substrate protein might be an ion channel, an enzyme involved in neurotransmitter synthesis, a neurotransmitter receptor or transporter, a structural protein, or almost any other kind of protein. The phosphate groups added by the kinase then alter functioning of the protein in some way. For example, an ion channel might open, a neurotransmitter-synthesizing enzyme might be activated, a receptor might become more sensitive to the neurotransmitter, and so forth. Furthermore, kinases can phosphorylate proteins in the cell nucleus that turn on or turn off specific genes in that

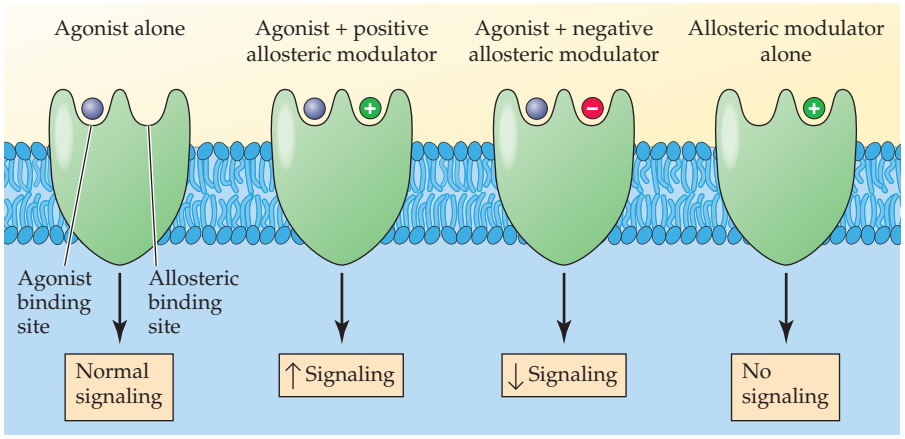


FIGURE 3.15 Allosteric modulation of neurotransmitter receptor signaling Using metabotropic receptors as an example, the figure shows how allosteric modulators can either positively or negatively influence receptor signaling. In the absence of an agonist, including the neurotransmitter itself, the allosteric modulator produces no receptor signaling. (After Wootten et al., 2013.)

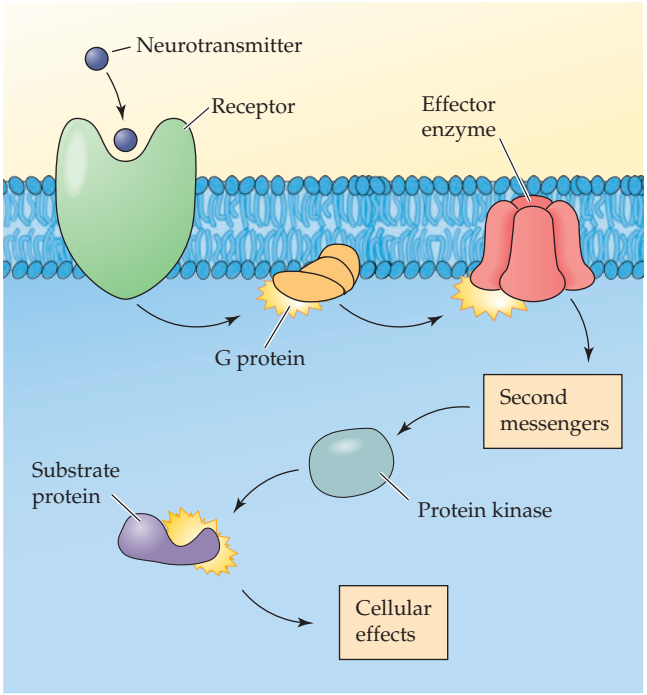


FIGURE 3.16 The mechanism of action of second messengers Second messengers work by activating protein kinases to cause phosphorylation of substrate proteins within the postsynaptic cell.

cell. You can see that protein kinases activated by second messengers are capable of producing widespread and profound changes in the postsynaptic cell, even including long-lasting changes in gene expression.

Now let us consider several specific second messengers and their protein kinases. The first second messenger to be discovered was **cyclic adenosine monophosphate** (**cAMP**). Levels of cAMP are controlled by receptors for several different neurotransmitters, including DA, NE, 5-HT, and endorphins. Cyclic AMP stimulates a protein kinase called **protein kinase A** (**PKA**). A related second messenger is **cyclic guanosine monophosphate** (**cGMP**). One of the key regulators of cGMP is the gaseous messenger nitric oxide, which activates the enzyme that synthesizes this second messenger. Cyclic GMP has its own kinase known as **protein kinase G** (**PKG**). A third second-messenger system is sometimes termed the **phosphoinositide second-messenger system**. This complex system, which works by breaking down a phospholipid in the cell membrane, actually liberates two second messengers: **diacylglycerol** (**DAG**) and **inositol trisphosphate** (**IP_3**). Working together, these messengers cause an elevation of the level of Ca^{2+} ions within the postsynaptic cell and activation of **protein kinase C** (**PKC**). The phosphoinositide system is controlled by receptors for several neurotransmitters, including ACh, NE, and 5-HT. Finally, Ca^{2+} itself is a second messenger. Calcium levels in the cell can be increased by various mechanisms, including the phosphoinositide second-messenger system, voltage-sensitive Ca^{2+} channels, and, as mentioned earlier, certain ionotropic receptors like the NMDA receptor. The nervous system contains a family of protein kinases activated by Ca^{2+} that require the participation of an additional protein known as *calmodulin*. At the synapse, the most important of these kinases is **calcium/calmodulin kinase II** (**CaMKII**). Finally, another function of Ca^{2+} in second-messenger signaling is to help activate PKC. **TABLE 3.3** summarizes these second-messenger systems and their associated protein kinases.

The second messengers cAMP and cGMP are inactivated by enzymes called phosphodiesterases (PDEs). Eleven different PDE families have been discovered, some of which are specific for cAMP (PDEs 4, 7, and 8), others of which are specific for cGMP (PDEs 5, 6, and 9), and the remainder of which break down both cAMP and cGMP (PDEs 1, 2, 3, 10, 11) (Francis et al., 2011). Although this plethora of PDEs may seem like "overkill," it's important to realize that a given tissue or organ may express only one or a few of the forms of this enzyme. Of relevance for pharmacology is that various compounds have been developed that selectively target particular forms of PDE. The best-known examples of this concern PDE5 inhibitors like sildenafil (Viagra), tadalafil (Cialis), and vardenafil (Levitra), which are used to treat erectile dysfunction. Cyclic GMP causes relaxation of smooth muscles surrounding the penile blood vessels, thereby leading to dilation of the vessels and production of an erection. Inhibitors of PDE5 facilitate this process by increasing the levels and prolonging the action of cGMP in the penile smooth muscles. PDE inhibitors are also currently being used to treat cardiac failure (PDE3), chronic obstructive pulmonary disease (PDE4), and pulmonary hypertension (PDE5 again) (Francis et al., 2011). Most relevant for psychopharmacologists, researchers are actively working to assess the potential usefulness of PDE inhibitors for treating neurodegenerative diseases, traumatic brain or spinal cord injury, schizophrenia, and other CNS disorders (for more information, see reviews by Heckman et al., 2015; Paterniti et al., 2014; Titus et al., 2015; Wang et al., 2015; Wilson and Brandon, 2015).

TABLE 3.3 Second-Messenger Systems and Protein Kinases

Second-messenger system	Associated protein kinase
Cyclic AMP (cAMP)	Protein kinase A (PKA)
Cyclic GMP (cGMP)	Protein kinase G (PKG)
Phosphoinositide	Protein kinase C (PKC)
Calcium (Ca^{2+})	Calcium/calmodulin kinase II (CaMKII)

Tyrosine kinase receptors mediate the effects of neurotrophic factors

There is one more family of receptors that you need to learn about: the **tyrosine kinase receptors**. These receptors mediate the action of **neurotrophic factors**, proteins that stimulate the survival and growth of neurons during early development and that are also involved in neuronal signaling. Nerve growth factor (NGF) was the first neurotrophic factor to be discovered, but many others are now known, including BDNF, which was mentioned earlier, neurotrophin-3 (NT-3), and NT-4.

Three specific tyrosine kinase receptors are used by these neurotrophic factors: trkA (pronounced "track A") for NGF, trkB for BDNF and NT-4, and trkC for NT-3. The trk receptors are activated through the following mechanism. After the neurotrophic factor binds to its receptor, two of the resulting complexes come together in the cell membrane, which is a process that is necessary for receptor activation (**FIGURE 3.17**). When the two trk receptors are activated, they phosphorylate each other on tyrosine residues[7] (hence the "tyrosine kinase receptor") located within the cytoplasmic region of each receptor. This process then triggers a complex sequence involving additional protein kinases, including some that differ from those described in the previous section. Tyrosine kinase receptors and the neurotrophic factors they serve generally participate more in regulation of long-term changes in gene expression and neuronal functioning than in rapid synaptic events that determine the rate of cell firing.

[7]Proteins are long chains of amino acids. When amino acids are strung together in the synthesis of a protein, each adjacent pair of amino acids loses a water molecule (an H from one amino acid and an OH from the other). What remain are called "amino acid residues." Each residue is named for the specific amino acid from which it was derived from, such as tyrosine in this case. Tyrosine kinases are differentiated from the kinases mentioned earlier (e.g., PKA), because those kinases phosphorylate proteins on residues of the amino acids serine and threonine instead of tyrosine. This difference, in turn, is important because it influences how phosphorylation affects the functioning of the target protein.

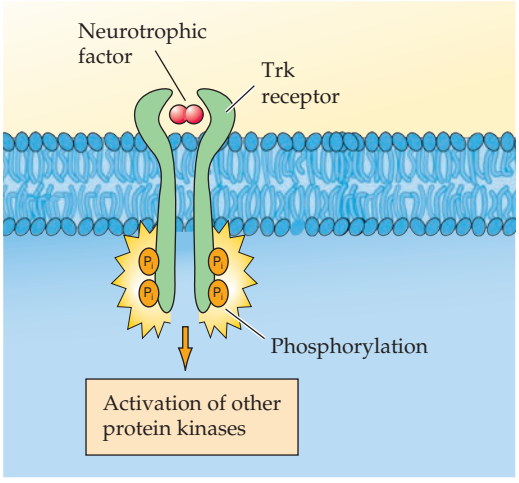


FIGURE 3.17 Activation of trk receptors Neurotrophic factors stimulate trk receptors by bringing two receptor molecules into close proximity in the cell membrane, which then leads to reciprocal phosphorylation of tyrosine residues and activation of other protein kinases.

Pharmacology of Synaptic Transmission

Drugs can either enhance or interfere with virtually all aspects of synaptic transmission. Synaptic effects form the basis of almost all of the actions of psychoactive drugs, including drugs of abuse, as well as those prescribed for the treatment of serious mental disorders such as depression and schizophrenia. **FIGURE 3.18** illustrates the major ways in which such drugs can alter the neurotransmission process.

Drugs may either increase or decrease the rate of transmitter synthesis. If the drug is a chemical precursor to the transmitter, then the rate of transmitter formation may be increased. Two examples of this approach involve L-dihydroxyphenylalanine (L-DOPA), which is the precursor to DA, and 5-hydroxytryptophan (5-HTP), which is the precursor to 5-HT. Because patients with Parkinson's disease are deficient in DA, the primary treatment for this neurological disorder is L-DOPA (see Chapter 5 for more information). Alternatively, a drug decreases levels of a neurotransmitter by inhibiting a key enzyme needed for transmitter synthesis. Alpha-methyl-*para*-tyrosine inhibits the enzyme tyrosine hydroxylase, which helps manufacture both DA and NE, and *para*-chlorophenylalanine inhibits the 5-HT–synthesizing enzyme tryptophan hydroxylase.

Besides administering a precursor substance, you can also enhance the action of a neurotransmitter by reducing its inactivation. This can be accomplished in two ways. First, levels of the transmitter can be increased by blocking the enzyme involved in its breakdown. Physostigmine blocks the enzyme acetylcholinesterase, which breaks down ACh, and phenelzine blocks monoamine oxidase (MAO), an enzyme that is important in the breakdown of DA, NE, and 5-HT. As we will see in Chapter 18, phenelzine and other MAO-inhibiting drugs are sometimes used to treat patients with depression. For neurotransmitters that use transporters for reuptake out of the synaptic cleft, a second way to reduce neurotransmitter inactivation is to block those transporters. This increases the amount and prolongs the presence of the transmitter in the synaptic cleft, thereby enhancing its effects on the postsynaptic cell. As described previously, cocaine blocks the transporters for DA, NE, and 5-HT, and drugs that more selectively prevent reuptake of 5-HT are commonly used as antidepressant medications (see Chapters 12 and 18).

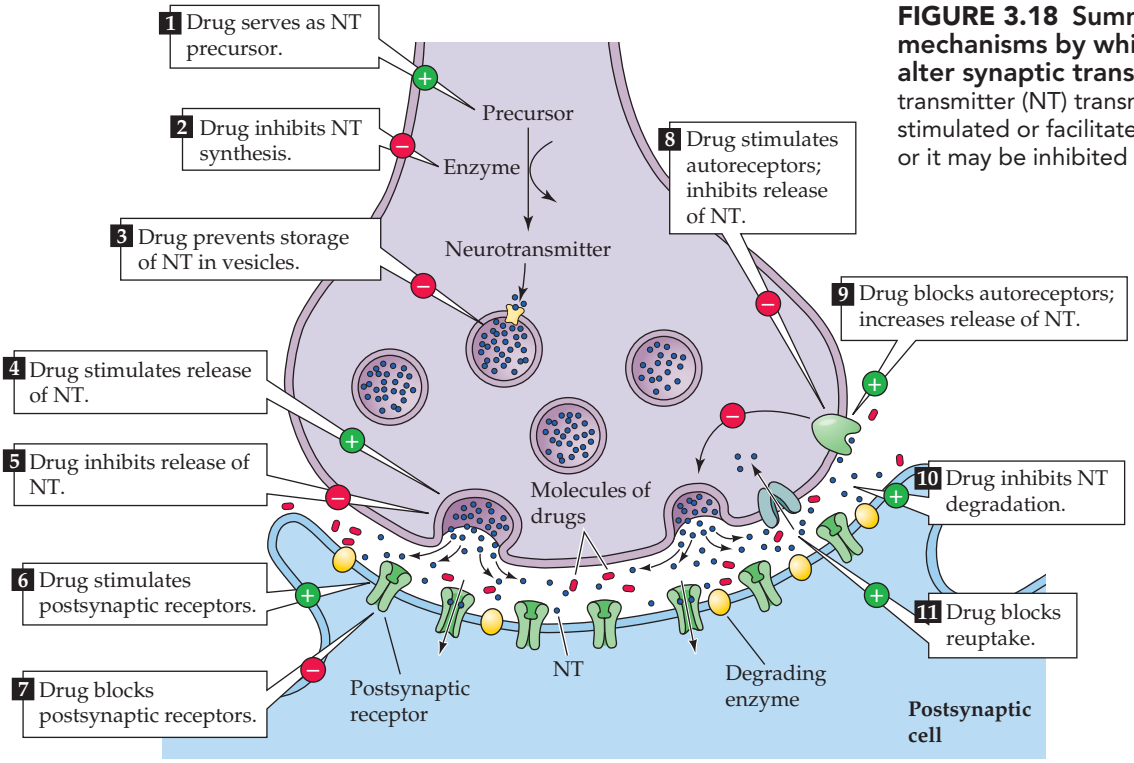


FIGURE 3.18 Summary of the mechanisms by which drugs can alter synaptic transmission Neurotransmitter (NT) transmission may be stimulated or facilitated (denoted by +), or it may be inhibited (denoted by –).

Other drugs affect neurotransmitter storage or release. For example, reserpine blocks the storage of DA, NE, and 5-HT in synaptic vesicles. Reserpine treatment initially causes a burst of neurotransmitter release as the vesicles empty out, but this is followed by a period of extremely low transmitter levels because storage in vesicles is necessary to prevent breakdown of transmitter molecules by enzymes present in the axon terminal. Amphetamine stimulates the release of DA and NE from the cytoplasm of the axon terminal, and a related substance called fenfluramine produces the same effect on 5-HT. These releasing agents work by reversing the effects of the neurotransmitter transporters. That is, instead of the transporters taking up transmitter molecules into the neuron from the synaptic cleft, they work in the reverse direction to carry the transmitter out of the neuron and into the synaptic cleft. As we saw earlier, some drugs alter neurotransmitter release in a different way, by stimulating or inhibiting autoreceptors that control the release process. Clonidine and 8-OH-DPAT stimulate autoreceptors for NE and 5-HT, respectively. In both cases, such stimulation reduces release of the related transmitter. Autoreceptor inhibition can be produced by yohimbine in the case of NE and pindolol in the case of 5-HT. Not surprisingly, administration of these compounds increases transmitter release.

One final mechanism of action can be seen in drugs that act on postsynaptic receptors for a specific neurotransmitter. If the drug is an agonist for a particular receptor subtype, it will mimic the effect of the neurotransmitter on that receptor. If the drug is a receptor antagonist, it will inhibit the effect of the transmitter on the receptor. Many psychoactive drugs, both therapeutic and recreational, are receptor agonists. Examples include benzodiazepines, which are agonists at benzodiazepine receptors and are used clinically as sedative and antianxiety drugs (see Chapter 17); opioids like heroin and morphine, which are agonists at opioid receptors (see Chapter 11); nicotine, which is an agonist at the nicotinic receptor subtype for ACh (see Chapter 7); and THC, which is an agonist at cannabinoid receptors (see Chapter 14). Receptor antagonists are likewise important in pharmacology. Most drugs used to treat patients with schizophrenia are antagonists at the D_2 receptor subtype for DA (see Chapter 19), but the widely ingested substance caffeine is an antagonist at receptors for the neurotransmitter adenosine (see Chapter 13).

Synaptic Plasticity

For many years after the first visualization of synapses in the 1950s using the electron microscope, most researchers believed that these structures were stable once they were formed. Since it was also thought (erroneously) that no new neurons could be generated in the mature brain, there was no obvious need for new synapses, because existing ones presumably could be strengthened or weakened as needed. Subsequent

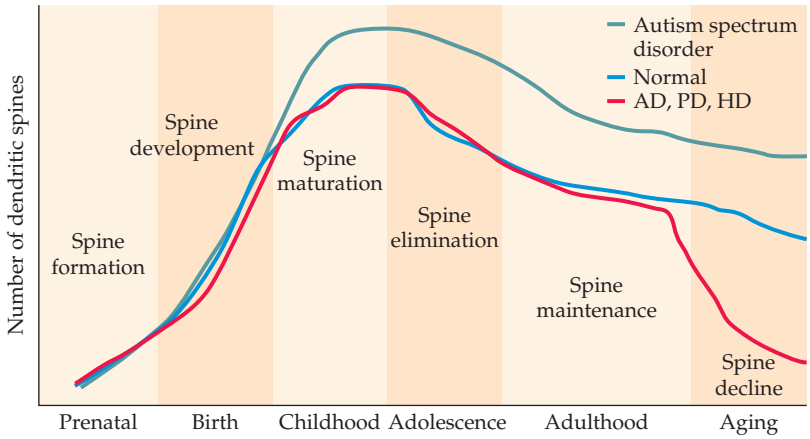


FIGURE 3.19 Changes in numbers of dendritic spines over a lifetime The number of dendritic spines in the brain changes during normal development, as it also does as a consequence of neurological disorders. AD, Alzheimer's disease; HD, Huntington's disease; PD, Parkinson's disease. (After Maiti et al., 2015.)

research has, indeed, confirmed that various kinds of experiences (e.g., learning) can alter the strength of synaptic connections activated by those experiences (see discussion of long-term potentiation and long-term depression in Chapter 8). But there is also abundant evidence that even in adulthood, axons can grow new terminals, dendrites can expand or contract their branches and/or gain or lose spines, and that synapses can be created or lost (Holtmaat and Svoboda, 2009). The term **synaptic plasticity** was coined to reflect the whole variety of synaptic changes, ranging from functional changes in the strength of existing synapses to structural changes involving the growth of new synapses or the loss of previously existing ones.

Synaptic plasticity can be studied at several levels, ranging from structural changes in synaptic components (for example, growth or loss of nerve terminals and/or dendritic spines) down to the molecular events that underlie the structural changes. At the molecular level, one of the major players in synaptic plasticity is a family of protein kinases that constitute the mitogen-activated protein kinase system (more commonly referred to as the **MAP kinase system**). MAP kinases respond to many different extracellular signaling molecules, typically molecules that affect cell growth, differentiation, and survival (Roux and Blenis, 2004). These functions of the MAP kinase system are carried out mainly by phosphorylating transcription factors and altering gene expression (see Chapter 2); however, the MAP kinase system can also be activated indirectly by synaptic input, leading to changes in synapse strength (Mao and Wang, 2016). Synaptic modulation by **ERK** (extracellular signal-related kinase), an element of the MAP kinase system, has now been implicated in the mechanisms of memory formation (Sweatt, 2001) and in the actions of various drugs of abuse (Cahill et al., 2014; Pascoli et al., 2014; Zamora-Martinez and Edwards, 2014).

Structural studies of synaptic plasticity have focused on alterations in dendritic structure, including dendritic spines. Neurons can rapidly change the size and shape of their dendritic spines, grow new spines, and also lose some of them. Naturally, the most profound changes in the number of dendritic spines occur during development of the brain, when most spines are formed. Spines and synapses are normally "pruned" during adolescence, a period when excess neuronal connections are eliminated. Nevertheless, dendritic spines retain their plasticity in the mature brain. For example, researchers believe that changes in dendritic spines may play an important role in the formation of long-term memories (Kasai et al., 2010). Furthermore, many neurological disorders are associated with either an abnormally low or abnormally high number of dendritic spines (Maiti et al., 2015; **FIGURE 3.19**). In addition to the disorders shown in the figure, loss of dendritic spines in the prefrontal cortex has been observed in postmortem studies of patients with schizophrenia (Glausier and Lewis, 2013). Finally, animal studies suggest that dendritic spine changes may be important in drug and alcohol dependence and withdrawal (Kyzar and Pandey, 2014; Spiga et al., 2014) and in the deleterious effects of stress on the brain (Leuner and Shors, 2013).

Section Summary

- Neurotransmitter receptors serve the purpose of signaling information from the presynaptic to the postsynaptic cell. Unlike transporters, they do not carry neurotransmitter molecules across the cell membrane.
- Most neurotransmitters make use of multiple receptor subtypes.
- Neurotransmitter receptors fall into two categories: ionotropic and metabotropic.
- Ionotropic receptors are composed of multiple subunits and form an intrinsic ion channel that is permeable either to cations such as Na^+ (and sometimes also Ca^{2+}) or to anions such as Cl^-. These receptors respectively mediate fast excitatory or fast inhibitory transmission.

- Metabotropic receptors are coupled to G proteins in the cell membrane and mediate slower transmission involving ion channel opening (e.g., inhibitory K^+ channels) or second-messenger synthesis or breakdown.
- Allosteric modulators are molecules that either increase or decrease receptor activity by binding to sites on the receptor protein separate from the agonist binding site.
- Second messengers work by activating protein kinases that phosphorylate target proteins in the postsynaptic cell.
- Some important second-messenger systems and their respective kinases are the cAMP (protein kinase A), cGMP (protein kinase G), Ca^{2+} (calcium/calmodulin kinase II), and phosphoinositide (protein kinase C) systems.
- The second messengers cAMP and cGMP are inactivated by enzymes called phosphodiesterases (PDEs). Inhibitors of specific PDEs are being tested for their potential efficacy in treating various CNS disorders.
- Neurotrophic factors like NGF and BDNF work by activating tyrosine kinase receptors.
- Psychoactive drugs usually exert their subjective and behavioral effects by modifying synaptic transmission in one or more of the following ways: (1) increasing or decreasing transmitter synthesis, (2) reducing transmitter inactivation by inhibiting enzymatic breakdown or blocking reuptake, (3) stimulating transmitter release, and (4) acting as agonists or antagonists at transmitter receptors on the postsynaptic or presynaptic (i.e., autoreceptors) cell.
- Synaptic plasticity refers to functional and structural changes in synaptic connectivity. Components of the MAP kinase system such as the extracellular signal-related kinase (ERK) play an important role in the molecular mechanisms of synaptic plasticity. Structurally, synaptic plasticity is manifested by changes in the size, shape, and/or number of dendritic spines, and such changes are found both in neuropsychiatric disorders and in drug and alcohol dependence and withdrawal.

The Endocrine System

As we have seen, neurotransmitters normally travel only a tiny distance before reaching their target at the other side of the synaptic cleft, or sometimes a little farther away. Another method of cellular communication, however, involves the release of chemical substances called **hormones** into the bloodstream. Hormones are secreted by specialized organs called **endocrine glands**. Upon reaching the circulation, hormones can travel long distances before reaching target cells anywhere in the body. To respond to a given hormone, a target cell must possess specific receptors for that hormone, just as a postsynaptic cell must respond to a neurotransmitter. Moreover, sometimes the same substances (e.g., norepinephrine, epinephrine) are used both as neurotransmitters within the brain and as hormones within the endocrine system. Thus, synaptic and endocrine communications are similar in many respects, although they differ in terms of the proximity of the cells involved and the anatomic features of the synapses described earlier (**FIGURE 3.20**).

Endocrine glands can secrete multiple hormones

As shown in **FIGURE 3.21**, numerous endocrine glands are located throughout the body. Some of these glands secrete more than one type of hormone. We'll now briefly describe each gland and its associated hormone(s), including the chemical classification and functions of that hormone.

The **adrenal glands** lie over the kidneys. An adrenal gland is actually two separate glands that have come together during embryonic development (**FIGURE 3.22**). The inner part of the gland, which is called the **adrenal medulla**, is derived from nervous system tissue. Like a sympathetic ganglion, it receives input from the preganglionic fibers of the sympathetic nervous system (see Chapter 2). Cells of the adrenal medulla, which are called **chromaffin cells**, secrete the hormones **epinephrine** (**EPI**) and **norepinephrine** (**NE**), both of which

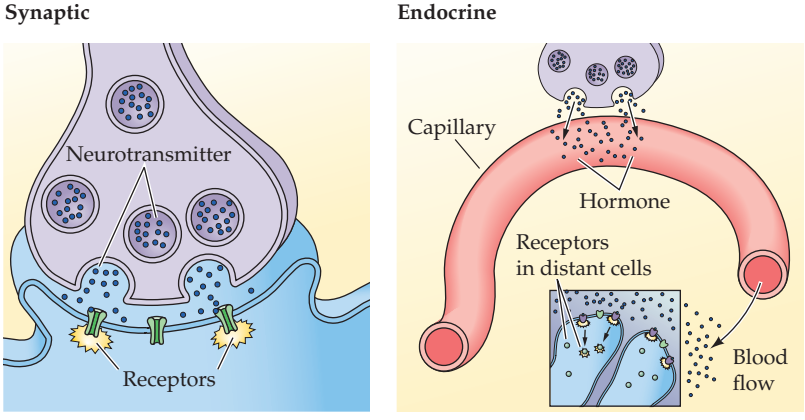


FIGURE 3.20 Comparison of synaptic and endocrine communication

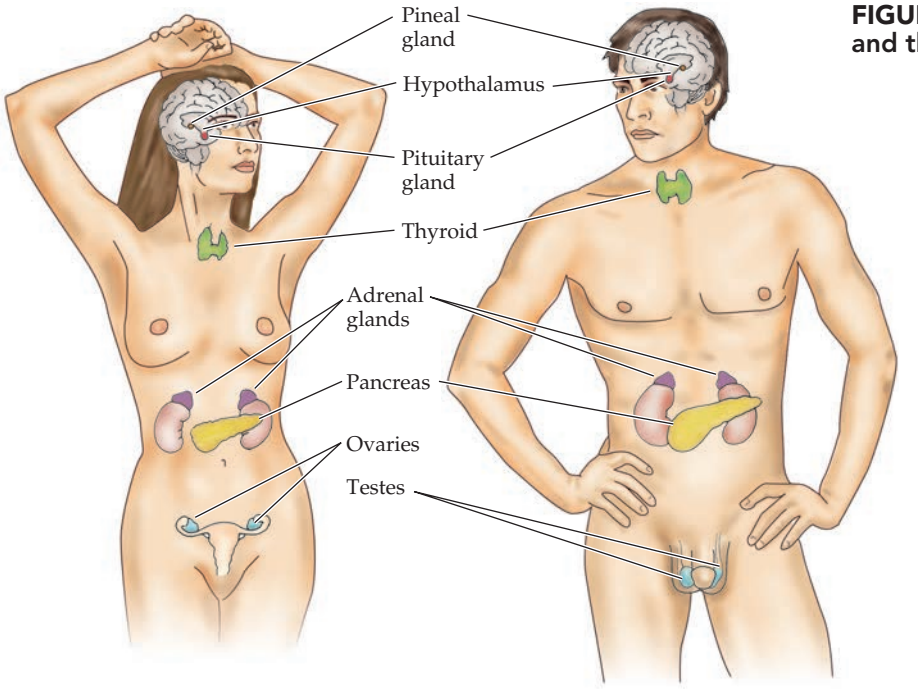


FIGURE 3.21 Major endocrine glands and their location in the body

are monoamines. Physical or psychological stressors stimulate the release of EPI and NE as part of the classic "fight-or-flight" response. Once in the bloodstream, these hormones mobilize glucose (sugar) from the liver to provide immediate energy, and they also divert blood from the internal organs (e.g., the organs of digestion) to the muscles, in case physical action is needed. Some of their effects contribute to the physical sensations that we experience when we're highly aroused or stressed, such as a racing heart and cold, clammy hands.

The outer part of the adrenal gland, the **adrenal cortex**, secretes hormones called **glucocorticoids**. Which glucocorticoid is present depends on the species: humans and other primates make **cortisol** (sometimes called *hydrocortisone*), whereas rats and mice make **corticosterone**. Glucocorticoids belong to a class of molecules known as **steroids**, all of which are derived from the precursor cholesterol. One of the main functions of glucocorticoids is to maintain normal blood glucose levels while helping to store excess glucose for future use. These hormones are also secreted in increased amounts during stress and normally help us cope with stressful experiences. However, there is substantial evidence that chronic stress may lead to serious consequences—including damage to certain parts of the brain—if high glucocorticoid levels persist for long periods of time (McEwen, 2012).

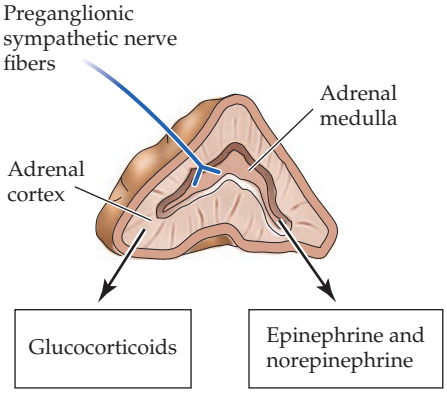


FIGURE 3.22 Structure of the adrenal gland, showing the outer cortex and the inner medulla

Other glands that secrete steroid hormones are the **gonads**: the **ovaries** in females and the **testes** in males. The ovaries secrete female sex hormones called **estrogens** (such as **estradiol**) and **progestins** (mainly **progesterone**), whereas the testes secrete male sex hormones called **androgens** (such as **testosterone**). These hormones determine some of the physical differences between males and females (the so-called secondary sex characteristics) that occur after puberty. Testosterone also has two other important roles. During early development, this hormone acts within the brain to produce neural changes important for determining later gender-based differences in behavior. Later on, it plays a significant role in stimulating sexual motivation in males and even in females (both genders possess some quantity of each other's sex hormones).

Within the pancreas is an endocrine gland known as the **islets of Langerhans**. Cells within this tissue secrete two hormones: **insulin** and **glucagon**. Insulin release is stimulated by food intake, and together with glucagon, it plays an important role in regulating glucose and other sources of metabolic energy. Lack of insulin gives rise to the serious disorder diabetes. Both insulin and glucagon are peptide hormones, similar to the neuropeptides discussed earlier but somewhat larger in size.

Residing in the throat is the **thyroid gland**, which secretes **thyroxine** (**T4**) and **triiodothyronine** (**T3**). These hormones are important for normal energy metabolism. Underactivity of the thyroid gland (hypothyroidism) causes feelings of weakness and lethargy

(even mimicking some of the symptoms of clinical depression), whereas thyroid overactivity (hyperthyroidism) leads to excessive energy and nervousness. These two thyroid hormones are made from the amino acid tyrosine, which is the same precursor used to make DA, NE, and EPI (see Chapter 5).

The **pineal gland** is situated just over the brainstem and is covered over by the cerebral hemispheres. This gland secretes the hormone **melatonin**, which is synthesized using the neurotransmitter 5-HT as a precursor. Melatonin has been implicated in the control of various rhythmic functions, which differ depending on the species. In humans and in many other vertebrates, most melatonin secretion occurs during the night, which suggests a possible role in controlling sleep rhythms. Tablets that contain small amounts of melatonin can be purchased over the counter in drug stores and supermarkets, and for some people, these tablets induce drowsiness and faster sleep onset.

The **pituitary gland** is sometimes called the "master gland" because it secretes several hormones that control other glands. The pituitary is found just under the hypothalamus and is connected to that brain structure by a thin stalk. Like the adrenals, the pituitary actually comprises two separate glands with different hormones that serve distinct functions. The **anterior pituitary** secretes **thyroid-stimulating hormone** (**TSH**; also known as thyrotropin), **adrenocorticotropic hormone** (**ACTH**), **follicle-stimulating hormone** (**FSH**), **luteinizing hormone** (**LH**), **growth hormone** (**GH**), and **prolactin** (**PRL**). TSH stimulates the thyroid gland, and ACTH promotes the synthesis and release of glucocorticoids from the adrenal cortex. FSH and LH together control the growth and functioning of the gonads. LH stimulates estrogen and androgen secretion by the ovaries and testes, respectively. GH stimulates the production of insulin-like growth factor I (IGF-I) from peripheral organs such as the liver; IGF-I is critical for skeletal growth during development. Finally, PRL promotes milk production by the mammary glands.

The pituitary stalk connecting the hypothalamus with the pituitary gland contains blood vessels that carry special **hypothalamic releasing hormones** (**FIGURE 3.23**). These hormones are mainly neuropeptides manufactured by various groups of neurons in the hypothalamus. Instead of forming normal synapses, these neurons release peptides into blood capillaries in a region called the **median eminence**. Blood vessels then carry releasing hormones to the hormone-secreting cells of the anterior pituitary. For example, **thyrotropin-releasing hormone** (**TRH**) is a hypothalamic peptide that stimulates the release of TSH, **corticotropin-releasing hormone** (**CRH**) (alternatively called *corticotropin-releasing factor*, or CRF) stimulates ACTH release (corticotropin is another name for ACTH), and **gonadotropin-releasing hormone** (**GnRH**) stimulates both FSH and LH. We can see that the endocrine system sometimes functions through the interactions of several glands, with one gland controlling another until the final hormone is secreted. For example, stress does not directly cause increased glucocorticoid secretion from the adrenal cortex. Instead, stress leads to enhanced CRH release from the hypothalamus, which provokes ACTH release from the anterior pituitary; ACTH travels through the bloodstream to the adrenal glands, where it stimulates the secretion of glucocorticoids. Because of this complicated control system, it may take a few minutes before the level of glucocorticoids in our blood is significantly increased. Thus the endocrine system works much more slowly than chemical communication by neurotransmitters.

In addition to blood vessels that connect the hypothalamus to the anterior pituitary, the pituitary stalk contains the axons of specialized secretory neurons located in the hypothalamus. These axons reach the **posterior pituitary**, where, like the hypothalamic neurons mentioned earlier, they form endings on blood vessels instead of other cells. Secretory neurons synthesize and release the peptide hormones **vasopressin** and **oxytocin** from the posterior pituitary into the bloodstream. Vasopressin (also called *antidiuretic hormone*) acts on the kidneys to increase water retention (i.e., make the urine more concentrated). Alcohol inhibits vasopressin secretion, which is one of the reasons why people urinate so frequently when they drink (it's not just the increased fluid consumption). Historically, oxytocin has been known mainly for two important physiological functions in female mammals: stimulation of uterine contractions during childbirth, and triggering of milk letdown from the breasts during lactation. In recent years, however, animal studies have shown that both oxytocin and vasopressin play important roles in pair-bonding, parenting, and other kinds of affiliative behavior in many different species (McCall and Singer, 2012; Baribeau and Anagnostou, 2015). These simple molecules additionally seem to participate in normal and abnormal social behaviors in humans, although much more research needs to be done before such a role is fully understood (Bachner-Melman and Ebstein, 2014).

Mechanisms of hormone action vary

As mentioned in Chapter 1, two broad types of receptors are used in cellular communication: extracellular (membrane) receptors and intracellular receptors. Earlier in the present chapter, we observed that most neurotransmitter receptors are located on the cell membrane. In contrast, hormones use various types of receptors, both extracellular and intracellular.

Peptide hormones function by means of membrane receptors (**FIGURE 3.24A**). Some of these are just like metabotropic neurotransmitter receptors, working through second-messenger systems. One example is

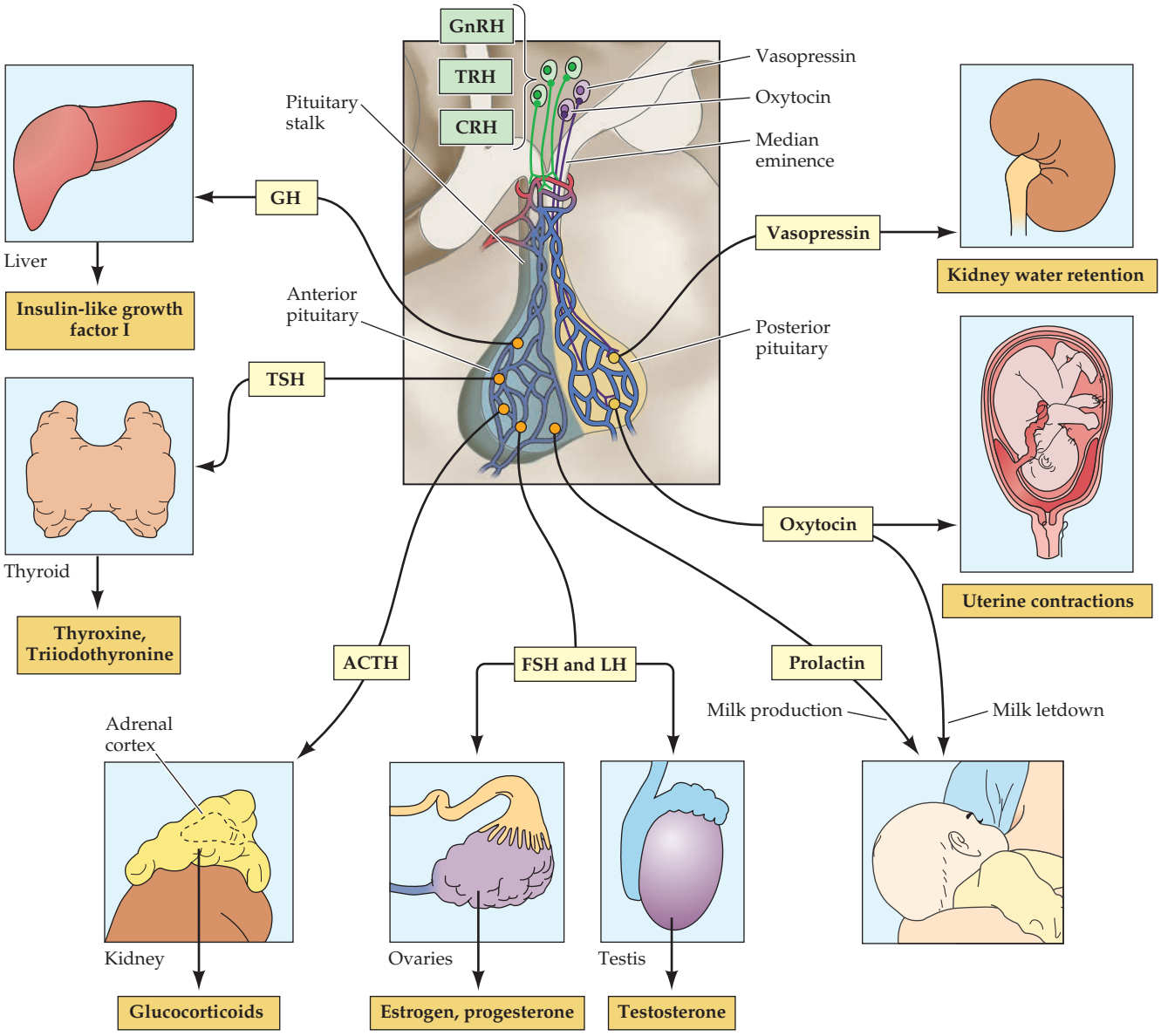


FIGURE 3.23 Organization of the hypothalamic–pituitary axis Note that the axon terminals of the hypothalamic releasing hormone neurons are located near blood capillaries in the median eminence, whereas oxytocin and vasopressin neurons send their axons all the way into the posterior lobe of the pituitary gland. For the purpose of simplicity, not all hypothalamic releasing hormones are shown. ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; FSH, follicle-stimulating hormone; GH, growth hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone.

the receptors for CRH, which stimulate formation of the second messenger cAMP. However, some hormones, such as insulin, use tyrosine kinase receptors that are similar to the trk receptors described earlier.

Steroid and thyroid hormones operate mostly through intracellular receptors (**FIGURE 3.24B**). These receptors are proteins just like the membrane receptors for neurotransmitters or peptide hormones, but they are generally located within the cell nucleus, where they function as **transcription factors** to either turn on or turn off the expression of specific genes within the cell. Since gene expression determines which proteins are made by the cell, the ultimate effects of steroid and thyroid hormones are seen in the altered synthesis of particular proteins. This process takes much longer (many minutes to a few hours or longer) than the rapid effects typically produced by membrane receptors. On the other hand, changes in gene expression and protein synthesis are also longer-lasting, thus allowing an animal or person to keep responding to a hormone long after it is released. In addition to this classical, relatively slow action of

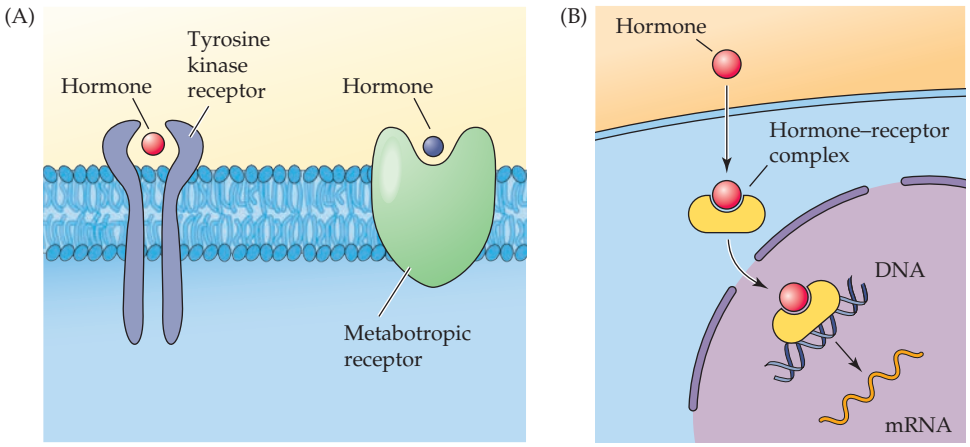


FIGURE 3.24 Hormonal signaling A variety of extracellular (A) and intracellular (B) receptors mediate hormone signaling.

steroid hormones, there is now good evidence that some of these hormones (e.g., glucocorticoids, estrogens) can exert rapid effects on neuronal excitability via nongenomic mechanisms (Roepke et al., 2011; Joëls et al., 2013). Membrane receptors mediating such effects have been identified for estrogens (Almey et al., 2015), but research on membrane glucocorticoid receptors is still ongoing (Strehl and Buttgereit, 2014).

Why is the endocrine system important to pharmacologists?

By this time, you may be wondering why pharmacologists are concerned about hormones and the endocrine system. Actually, there are a number of good reasons, four of which we'll briefly mention here:

1. Both therapeutic and abused drugs can alter the secretion of many hormones, causing physiological abnormalities. For example, chronic alcoholism can lead to reduced testosterone levels, testicular atrophy, and impotence in men. Alcoholic women may have menstrual disorders and at least temporarily may become infertile (see Chapter 10).
2. Hormones may alter subjective and behavioral responses to drugs (Terner and de Wit, 2006). This is illustrated by the important role of female sex hormones as well as glucocorticoids in modulating sensitivity to drugs of abuse such as cocaine, amphetamine, and nicotine (**BOX 3.2** and Web Box 3.2).
3. Hormones themselves sometimes have psychoactive properties like those of certain drugs. We mentioned earlier that melatonin has a sedative effect on many people. In addition, thyroid hormones are occasionally prescribed along with an antidepressant drug to enhance the therapeutic response.
4. The secretion of pituitary hormones and other hormones dependent on the pituitary is controlled by neurotransmitter systems in the brain. This fact sometimes enables us to use the endocrine system as a "window to the brain" that tells us whether a particular neurotransmitter system has been altered by disease (such as a psychiatric or neurological disorder), injury, or the effect of a psychoactive drug.

Let's look at a more detailed example that relates to both the first and fourth reasons to study the interactions between psychoactive drugs and hormones. Secretion of the hormone prolactin is inhibited by the neurotransmitter DA acting on D_2 receptors expressed by the prolactin-synthesizing cells in the pituitary gland (see Chapter 5 for more information on the DA system). The DA that regulates this system under normal conditions comes from neurons located at the base of the hypothalamus. Several drugs that are used to treat psychiatric disorders, particularly schizophrenia, are D_2 receptor antagonists. The therapeutic benefit derived from such compounds depends on their blocking of D_2 receptors in the brain; however, because the D_2 receptors in the pituitary gland are also blocked, the inhibitory effect of DA is removed, and this results in a rise in blood prolactin levels.

Studies of this phenomenon have revealed that more than 50% of brain dopamine D_2 receptors must be occupied for prolactin levels to increase (**FIGURE 3.25**) and that a similar (if not greater) degree of receptor blockade seems to be necessary for symptom improvement in patients with schizophrenia (Nordstrom and Farde, 1998). For many years, elevated circulating prolactin was one of the few biomarkers available that could serve as an index of central D_2 receptor occupancy (Green and Brown, 1988). However, chronically high prolactin levels (termed *hyperprolactinemia*) can have adverse consequences, including menstrual abnormalities in women, as well as breast abnormalities, sexual dysfunction, and mood disturbances in both women and men (Madhusoodanan et al., 2010). Fortunately, newer antipsychotic drugs have been developed that are less likely to produce this potentially serious side effect (Hamner, 2002), and modern neuroimaging methods have supplanted measurement of plasma prolactin as the best way to assess drug binding to central D_2 receptors (see Chapter 19).

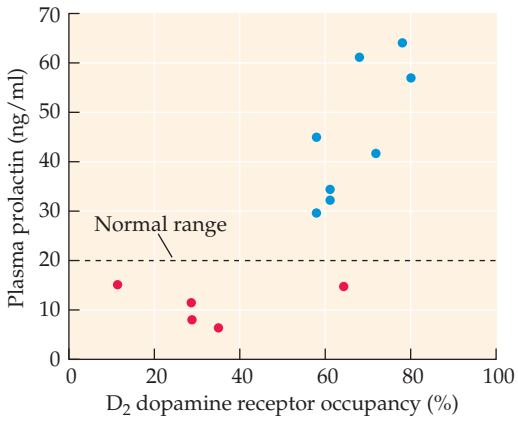


FIGURE 3.25 Relationship between estimated dopamine D_2 receptor occupancy and plasma prolactin concentrations in patients with schizophrenia Patients were treated for 4 weeks with 2, 6, or 12 mg daily of the antipsychotic drug raclopride. At the end of the treatment period, D_2 receptor occupancy in the putamen was measured using PET, and plasma prolactin concentrations were measured by radioimmunoassay in each patient. All three patients who received the lowest dose of raclopride and two of six patients who received the intermediate dose exhibited plasma prolactin levels within the normal range (red circles; the dashed line depicts the upper limit of this range) and, in almost all cases, showed a low percentage of estimated D_2 receptor occupancy. The remaining patients (blue circles) had >50% estimated D_2 receptor occupancy and correspondingly elevated prolactin levels. (After Nordstrom and Farde, 1998.)

BOX 3.2 Pharmacology in Action

Sex Hormones and Drug Abuse

Surveys have shown that use and abuse of illicit drugs are generally more common in men than in women. This gender difference can be seen, for example, in the results of the yearly National Survey on Drug Use and Health, which is carried out every year by the federal government's Substance Abuse and Mental Health Services Administration (2017). Nevertheless, even though there are more male than female drug users, other findings suggest greater vulnerability of females to drug abuse. Such findings include a greater rate of escalation of drug use and greater difficulty quitting among women compared with men (Becker and Hu, 2008).

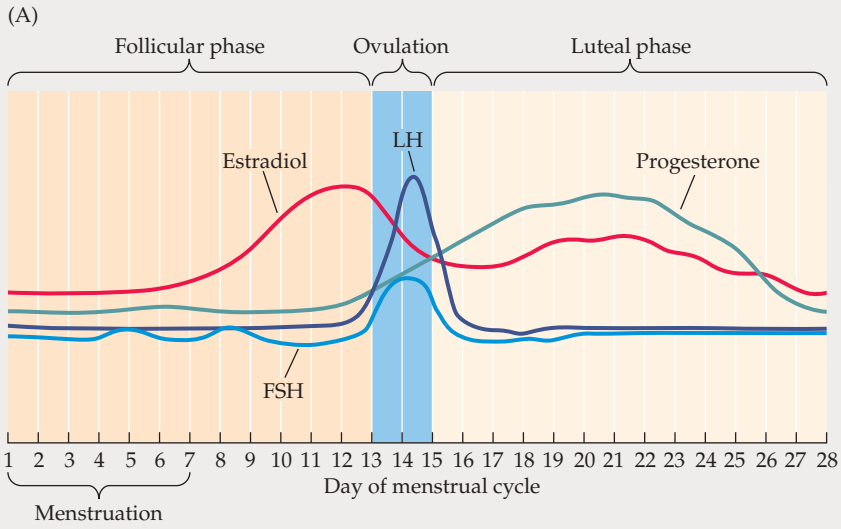
Gender differences in drug use and abuse are likely related to a variety of different factors, some cultural and others biological. Particularly relevant to this chapter is the potential role of sex hormones in drug sensitivity. We focus here on the effects of the female gonadal hormones estradiol and progesterone on responses to cocaine and nicotine, as this has been a very active area of research for many years.

Fewer women than men are users of cocaine or tobacco (Substance Abuse and Mental Health Services Administration, 2017), which is consistent with the overall gender difference in drug use mentioned previously. However, Evans and Foltin (2010) note that despite the difference in the sheer numbers of women and men who become cocaine-dependent, there is evidence that women who do take cocaine progress to dependence more rapidly than men and also relapse more quickly after treatment. Could this greater vulnerability in women be related to sex hormones? This issue has been addressed in various studies examining the subjective effects of cocaine administered in a laboratory setting to experienced cocaine users recruited for research purposes. The first and most basic question concerns whether men and women differ in the subjective effects produced by taking the drug. The answer is mainly no; women overall respond to cocaine in a similar manner to men (Evans and Foltin, 2010). However, when we say "overall," we mean that there is no gender difference when the stage of the menstrual cycle is not controlled in the experiment. Before going further, it is important to briefly discuss the stages of the menstrual cycle and the hormonal changes that occur at each stage.

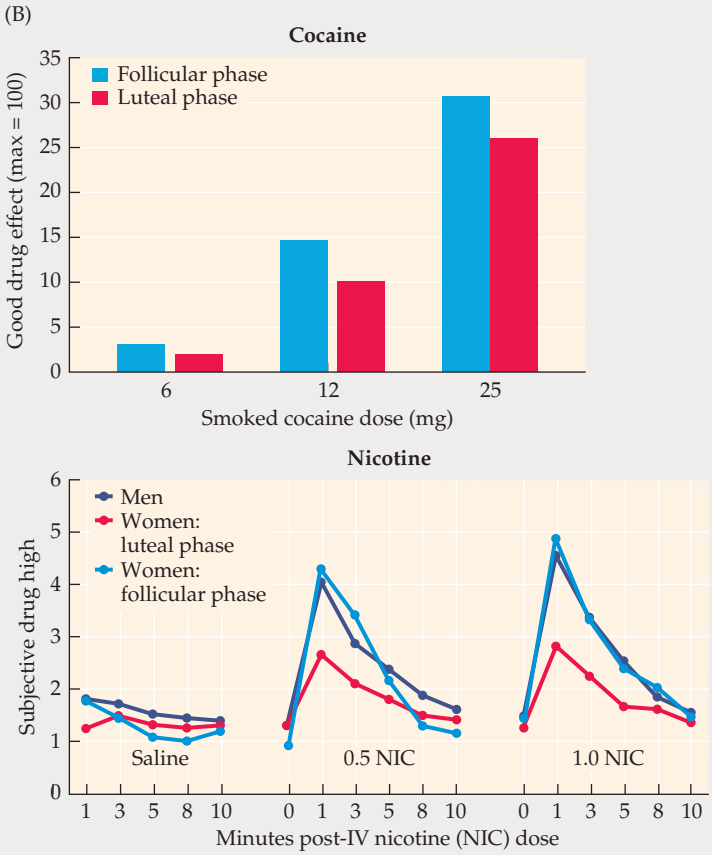
The human menstrual cycle has a period of approximately 28 days that consists of two main stages: follicular and luteal. During the follicular stage, a single follicle containing an immature egg (oocyte) in the woman's ovary begins to mature in preparation for ovulation. As shown in **Figure A**, circulating progesterone levels are low during this phase, whereas estradiol levels are beginning to rise. Because of positive feedback on the pituitary gland, the high estradiol levels eventually trigger a surge of LH that acts on the follicle to trigger ovulation. After releasing the egg (ovum), the follicle changes into a corpus luteum and begins to secrete large amounts of progesterone. If the egg is not fertilized, progesterone levels subsequently decline, menstruation occurs, and the cycle begins again.

Research on the effects of menstrual cyclicity, hormones, and drug sensitivity has focused on estradiol and progesterone rather than LH or FSH for two reasons. First, large changes in the levels of the two pituitary hormones LH and FSH are relatively short-lived, occurring primarily around the time of ovulation, whereas more sustained changes in estradiol and progesterone occur as a function of stage of the cycle. Second, LH and FSH have relatively little effect on the brain (in contrast to the gonads, of course),

BOX 3.2 Pharmacology in Action *(continued)*



(A) Changes in circulating hormone levels during the menstrual cycle



(B) Effects of the menstrual cycle on the subjective responses to smoked cocaine and IV nicotine (Top) The positive subjective response to smoking 6, 12, or 25 mg of cocaine (measured as "Good Drug Effect") was significantly greater when women were tested during the follicular phase of their menstrual cycle than during the luteal phase. (Bottom) In a separate study, the subjective "high" produced by two different intravenous (IV) doses of nicotine (NIC; 0.5 mg/70 kg or 1.0 mg/70 kg) was again significantly greater when women were tested during the follicular phase. Men's response was similar to that of women who were in the follicular phase of the cycle. (Top, after Evans and Foltin, 2010; bottom, after DeVito et al., 2014.)

whereas estrogen and progestin receptors are present in significant numbers of neurons, and activation of those receptors alters neuronal activity. When Evans and Foltin tested the positive subjective effects of different doses of smoked cocaine (6, 12, or 25 mg) on women during either the midfollicular phase (6–10 days after the beginning of menstruation) or the midluteal phase (7–12 days following the LH surge), the intensity of the drug effect at 12 mg and 25 mg doses was significantly lower during the luteal compared to the follicular phase (Evans and Foltin, 2010; **Figure B**, top). A more recent study examining the influence of intravenously administered nicotine similarly found that the subjective drug "high" in women was lower during the luteal compared to the follicular phase (DeVito et al., 2014; Figure B, bottom). Men tested under the same conditions showed a similar response to women in the follicular phase. One of the major hormonal differences between the luteal and follicular phases is the higher levels of circulating progesterone in the luteal phase. This difference may play a key role in the menstrual cycle–dependent changes in the effects of cocaine and nicotine, as researchers have demonstrated that progesterone administration reduces the subjective responses to both drugs (Sofuoglu et al., 2001; 2004; 2009).

The findings discussed here do not explain why men are more likely than women

(*Continued*)

BOX 3.2 Pharmacology in Action (*continued*)

to use psychoactive drugs, nor do they account for unexpected observations such as the more rapid progression to dependence in cocaine-using women. Nevertheless, the research clearly shows that hormones such as progesterone can influence the subjective responses to drugs of abuse. This information needs to be considered in assessing vulnerability to drug use and drug dependence, and it may also be helpful in devising new therapeutic approaches to these problems.

Section Summary

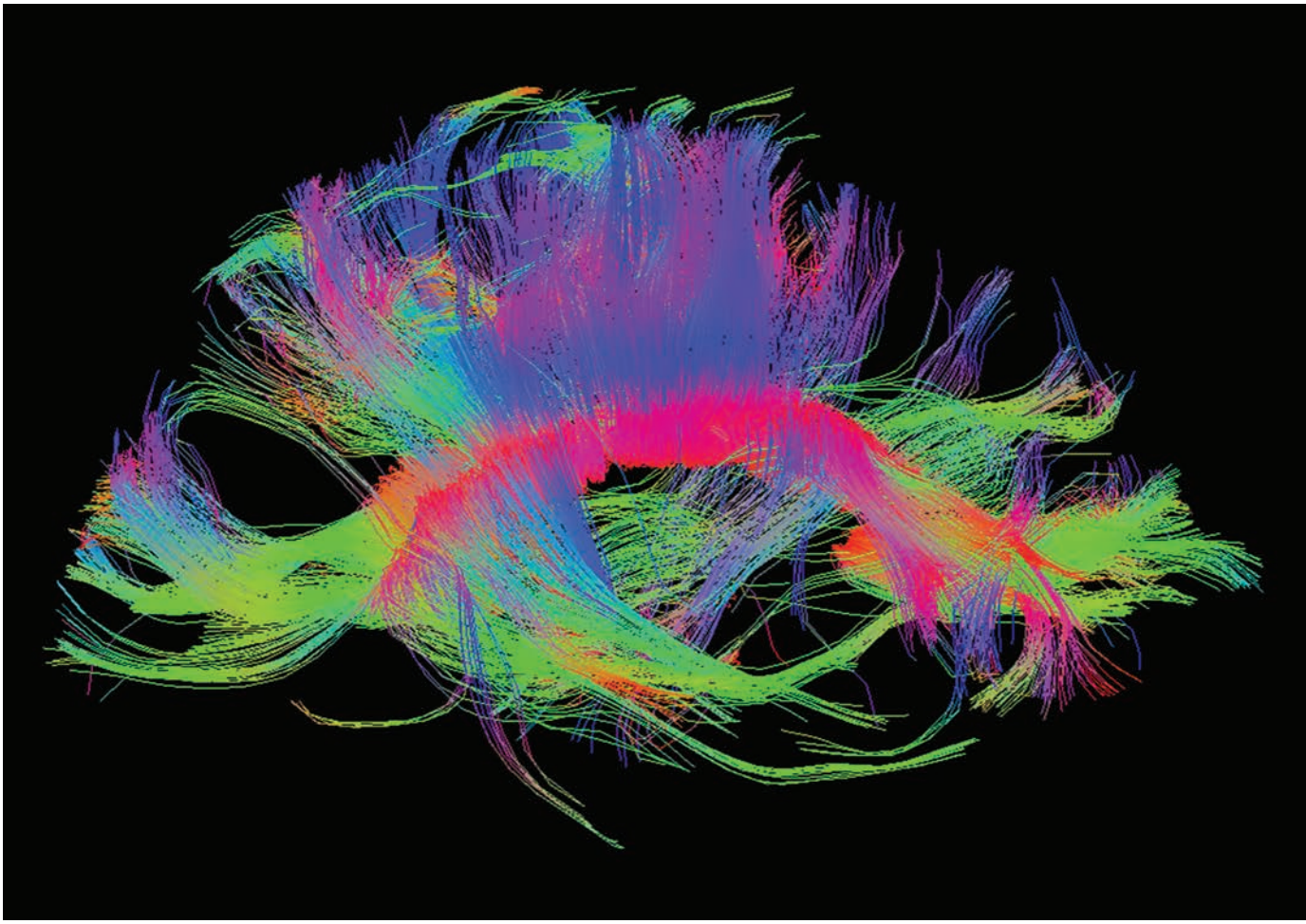
- Hormones are released into the bloodstream, where they may travel long distances before reaching target cells in the body. Despite important differences between synaptic and endocrine communications, the same substance is sometimes used as both a neurotransmitter and a hormone.
- The adrenal gland is composed of the inner medulla and the outer cortex, both of which are activated by stress. The chromaffin cells of the adrenal medulla secrete the hormones EPI and NE, whereas the adrenal cortex secretes glucocorticoid steroids such as cortisol and corticosterone.
- Other steroid hormones that are synthesized and released by the gonads include estrogens and progesterone from the ovaries in females, and androgens such as testosterone from the testes in males. These gonadal steroids are responsible for many of the secondary sex characteristics that appear after puberty; testosterone is additionally involved in sexual differentiation of the brain during early development, as well as in stimulation of sexual motivation later in life.
- The islets of Langerhans and the thyroid gland secrete hormones important in energy metabolism. Insulin and glucagon are released from separate populations of cells within the islets of Langerhans, and together these two peptide hormones regulate the disposition of glucose and other sources of metabolic energy. Lethargy and excessive energy are behavioral symptoms of hypothyroidism and hyperthyroidism, respectively, and are due to abnormally low or abnormally high levels of the two thyroid hormones, thyroxine and triiodothyronine.
- The pineal gland, which is situated just over the brainstem, synthesizes the hormone melatonin using 5-HT as a precursor. Melatonin has been implicated in the regulation of various types of rhythmic activity, including sleep.
- The pituitary gland is found just under the hypothalamus and is connected to it. The pituitary is divided into two separate glands, the anterior and posterior pituitary glands, which serve different functions.
- The anterior pituitary secretes TSH, ACTH, FSH, LH, GH, and PRL. TSH and ACTH stimulate the thyroid and adrenal glands (cortex), respectively, whereas FSH and LH together control the growth and functioning of the gonads. GH stimulates skeletal growth during development, and PRL plays an important role in promoting milk production during lactation.
- The hypothalamic releasing hormones TRH, CRH, and GnRH are neuropeptides synthesized within the hypothalamus that trigger the release of TSH, ACTH, and the gonadotropins FSH and LH. Because of this organizational structure, in which several glands must stimulate each other until the final hormone product is secreted, the endocrine system works much more slowly than chemical communication by neurotransmitters.
- The posterior pituitary secretes two small peptide hormones, vasopressin and oxytocin. Vasopressin enhances water retention by the kidneys, whereas oxytocin stimulates uterine contractions during childbirth and also triggers milk letdown from the breasts during lactation. These hormones may also promote affiliative behaviors in some species.
- The actions of hormones are mediated by several different kinds of receptors. Some are metabotropic receptors similar to those discussed for various neurotransmitters. Others are intracellular receptors that function as transcription factors that control gene expression, and still others are tyrosine kinase receptors.
- The endocrine system is important to pharmacologists for several reasons. These include the fact that (1) drugs can adversely alter endocrine function, (2) hormones may alter behavioral responses to drugs, (3) hormones themselves sometimes have psychoactive properties, and (4) the endocrine system can be used as a window to the brain to help us determine the functioning of a specific neurotransmitter system by measuring changes in hormone secretion under appropriate conditions.

STUDY QUESTIONS

1. Describe the structure of a typical axodendritic synapse, including both presynaptic and postsynaptic elements. How do axosomatic synapses, axoaxonic synapses, and neuromuscular junctions differ from axodendritic synapses?
2. List the criteria required for a substance to be verified as a neurotransmitter. Of these, which might be considered most important?
3. Neurotransmitters can be classified based on the chemical category to which they belong. Name these categories and give at least one example of a member of each category.
4. Describe how the synthesis of neuropeptides differs from that of other types of neurotransmitters.
5. What is the difference between a neurotransmitter and a neuromodulator? How are neuromodulators related to the concept of volume transmission?
6. What is exocytosis and what is its role in neurotransmitter release?
7. Describe the process of vesicle recycling and the various models that have been proposed to explain the recycling process. How do these models differ with respect to factors such as speed of recycling, area of the nerve terminal where vesicle membrane retrieval occurs, and the involvement of the protein clathrin and of endosomes?
8. Discuss the concept of a retrograde messenger and how this concept applies to lipid and gaseous transmitters.
9. What is the difference between somatodendritic and terminal autoreceptors? How do these receptors control the rate of neurotransmitter release?
10. Discuss the mechanisms by which neurotransmitters are inactivated.
11. Compare the functions of neurotransmitter transporters with those of neurotransmitter autoreceptors.
12. Why is a knowledge of receptor subtypes important when developing a new pharmaceutical medication?
13. Describe the structural and functional differences between ionotropic and metabotropic receptors.
14. Discuss the concept of allosteric receptor modulation. How is the influence of a positive or negative allosteric modulator different from the influence of a direct agonist or antagonist on a particular receptor's functioning?
15. What is a second messenger? List the main second messenger systems described in this chapter. What is the role of protein kinases in second messenger signaling?
16. What is meant by the term *synaptic plasticity*? Discuss the role of dendritic spines in mechanisms of synaptic plasticity.
17. List the major steroid hormones and the name of the endocrine gland responsible for synthesizing each hormone.
18. Discuss the relationships among the hypothalamic releasing hormones, the anterior pituitary gland, and the hormones secreted by the anterior pituitary. What is the anatomical relationship between the hypothalamus and the pituitary gland that contributes to the ability of hypothalamic releasing hormones to regulate anterior pituitary secretory activity?
19. List the hormones secreted by the posterior lobe of the pituitary gland and describe their functions.
20. Describe the difference in signaling mechanisms between steroid and peptide hormones.
21. Discuss the major reasons why the endocrine system is important to pharmacologists.

Go to the *Psychopharmacology* Companion Website at oup-arc.com/access/meyer-3e for animations, web boxes, flashcards, and other study aids.

CHAPTER 4



Images such as this diffusion tensor image (DTI) show some of the approximately 100,000 miles of axons connecting the many regions of the brain. (© Dr. Nicolas Menjot/Medical Images.)

Methods of Research in Psychopharmacology

THE BRAIN, THE MOST AMAZING ORGAN OF THE BODY, composed of tens of billions of neurons, perhaps 100 trillion synapses, and 100,000 miles of axons, is what allows us to think, comprehend speech, remember events, experience a wide variety of emotions, perceive complex sensory experiences, and guide our appropriate responses. Trying to decipher the functioning of this complex organ is an incredible undertaking. For much of history, philosophers and scientists believed our thoughts and emotions were due to "vapors" that swirled through our bodies and brains. By the seventeenth century, anatomical features of the brain began to be considered, and Luigi Galvani (an Italian physiologist) showed that if you electrically stimulated a nerve attached to a muscle, it could cause contraction of the muscle. But it was not until the Spaniards Golgi and Ramón y Cajal began to stain neurons to evaluate their structure microscopically (see Chapter 2) that they began to appreciate the complexity of the nervous system in its infinite detail. Now the sophisticated imaging techniques described in this chapter and other techniques yet to be developed have begun to piece together the intricate puzzle that is the human brain. Thanks to the National Institutes of Health (NIH) and public funding and the work of thousands of researchers, progress has been made toward mapping all the axonal pathways in the human brain. These researchers along with the entire scientific community have free access to the data. This initiative is called the Human Connectome Project (see www.humanconnectome.org), and in conjunction with the Human Genome Project that mapped the entire human gene sequence, neuroscience is leaping forward at an amazing pace, a true voyage of discovery that will explain what makes us human, yet different from one another. It may soon be able to change the lives of individuals with a variety of brain disorders. The opening figure provides an idea of the beauty and complexity of the neural networks that are being mapped. ■

Research Methods for Evaluating the Brain and Behavior

The discovery of chemical transmission of information between nerve cells paved the way for the birth of neuropsychopharmacology. Since then, an explosion of research has been directed toward understanding the nature of brain function and the biology of what makes us human. With the variety and power of new analytic tools and techniques, we can look inside the brain to find answers to questions that touch individual lives. Even nonscientists can appreciate the advances in neuroscience research that bring us ever closer to understanding the essence of human behavior, as well as some of the most troubling problems of mankind: dementia, depression, autism, and neurodegenerative disorders.

The new tools provide the means to explore the brain to answer our questions, but it takes disciplined and creative scientific minds and teamwork to pose the right questions and to optimally use available tools. The scientific method, utilizing rigorous hypothesis testing under controlled conditions, is the only real method that we have to investigate how molecules responsible for nerve cell activity relate to complex human behaviors and thinking. Analysis spans the entire range from molecular genetics to cell function to integrated systems of neuronal networks, and finally to observable behavior. To understand the brain requires a convergence of efforts from multiple disciplines, which together form the basis of neuroscience: psychology, biochemistry, neuropharmacology, neuroanatomy, endocrinology, computer science, neuropsychology, and molecular biology. Ultimately, we acquire knowledge by integrating information derived by a wide variety of research techniques from all of these fields.

As you might expect, the list of techniques is very long and gets longer every day. This chapter focuses on a few of the more common methods and helps you to understand the purpose of each method, as well as some of its potential weaknesses. Perhaps the most important goal of this chapter is to encourage you, when you read scientific papers, to critically evaluate the methods and controls used, because the conclusions we draw from experiments are only as good as the methodology used to collect the data.

In the first part of this chapter, we focus on behavioral pharmacology. Behavior, mood, and cognitive function represent the focus of neuropsychopharmacology, so it is of tremendous importance to understand and critically evaluate the techniques used to quantify behavioral changes. The second part of the chapter emphasizes techniques that look at the locations and functions of neurotransmitters and neurotransmitter receptors. The methods described are both **in vivo**, meaning observed in the living organism, and **in vitro**, which refers to measurements performed outside the living body (traditionally in a test tube). We also look at a variety of rather remarkable imaging techniques that permit us to visualize the activity of the living human brain. Because genetic engineering is an increasingly powerful tool, we will describe its use in psychopharmacology. Both the biochemical and behavioral techniques selected will be used in subsequent chapters. Although this chapter is artificially divided into sections, keep in mind that much of the most informative research in psychopharmacology utilizes techniques of neuroscience in combination with behavioral analysis. Feel free to return to this chapter to review a method when you encounter it later.

Techniques in Behavioral Pharmacology

Evaluating Animal Behavior

The techniques of behavioral pharmacology allow scientists to evaluate the relationship between an experimental manipulation, such as a lesion or drug administration, and changes in behavior. In a well-designed experiment, it is necessary to compare the behavior of the experimental treatment group with that of placebo control subjects. Neurobiological techniques such as selective lesioning and intracerebral drug administration, described in the second section of this chapter, tell us very little unless we have an objective measure of the behavioral consequences. Behavioral measures are crucial for (1) understanding the neurochemical basis of behavior, as well as drug-induced changes in that behavior; (2) developing animal models of psychiatric disorders; and (3) screening the large number of newly designed and synthesized drug molecules in preclinical pharmaceutical settings in an effort to determine therapeutic usefulness.

Animal testing needs to be valid and reliable to produce useful information

Animal studies clearly provide several advantages over studies using human participants. The most obvious advantage is the use of rigorous controls. The living conditions (e.g., diet, exercise, room temperature, exposure to stress, day–night cycle) of animal subjects can be regulated far more precisely than those of humans. In addition, the histories of animal subjects are well known, and the genetic backgrounds of a group of animals are very similar and well characterized. Finally, animals are the most appropriate subjects for the study of mechanisms of drug action, because an understanding of the electophysiological and neurochemical bases of drug effects often requires invasive techniques that are obviously unethical with human participants. Consider, for example, the valuable information gained

from transgenically manipulated animals. In addition, drugs can be administered to animal subjects in ways not generally appropriate for humans, for example, over long periods of time to determine toxic effects or the potential for addiction. Finally, the brains and behaviors of non-human mammals and humans are similar enough to allow generalization across species. For instance, lesions of the central nucleus of the amygdala of rats produce profound changes in the conditioned emotional response of these animals. Likewise, tumors, strokes, and surgical procedures that damage the human amygdaloid complex produce profound changes in fearfulness, anxiety, and emotional memory.

The impact of animal testing in biomedical research on the quality of human life (**FIGURE 4.1**) is discussed in a thought-provoking manner by Hollinger (2008), as are its alternatives. The need for animal experimentation is best seen under conditions when research using human participants is impossible, as when testing the effects of alcohol on fetal development. Ethical constraints prohibit researchers from administering varying doses of alcohol to groups of pregnant women to evaluate the effects on their newborns. Instead, data collected on alcohol consumption during pregnancy and the occurrence of fetal alcohol syndrome (FAS) suggest a relationship that tells us that the more alcohol a pregnant female consumes, the more likely it is that her infant will show signs of FAS. Although we know that infants of mothers who consume alcohol are more likely to show fetal abnormalities, the type of study described shows only a **correlational relationship**; we cannot assume that alcohol *causes* FAS, because other factors may be responsible for both. For example, poverty, poor diet, or other drug use may lead to increased alcohol consumption *and* cause developmental defects in the fetus. Therefore, to learn more about how alcohol affects fetal development, we need to perform animal studies. Since animal testing remains an important part of new drug development and evaluation, strict animal care guidelines have been developed to ensure proper treatment of subjects. The animal-testing stage provides an important step between basic science and the treatment of human conditions.

FIGURE 4.1 Poster used to counter the claims of animal rights activists Scientists hope to increase public awareness about the benefits of animal research. (Courtesy of the Foundation for Biomedical Research.)

The Health Research Extension Act of 1985 provided strict guidelines for the care of animals used in biomedical and behavioral research. The goals of that legislation include humane animal maintenance and experimentation that limits both the use of animals and animal distress. Each research institution must have an animal care committee that reviews each scientific protocol with three considerations in mind:

1. The research should be relevant to human or animal health, advancement of knowledge, or the good of society.
2. Alternative methods such as computer simulations that do not require animal subjects must be considered.
3. Procedures must avoid or minimize discomfort, distress, and pain.

Periodic inspection of living conditions ensures that they are appropriate for the species and contribute to health and comfort; size, temperature, lighting, cleanliness, access to food and water, sanitation, and medical care are ensured. Animal care and use committees have the authority to veto any studies that they feel do not meet all predetermined criteria. You can find a full copy of the guidelines prepared in 2011 on the Companion Website.

Some animal tests used to evaluate drug effects on physiological measures such as blood pressure or body temperature closely resemble tests used for humans. These tests have high **face validity**. However, for many psychiatric disorders, the symptoms are described in typically human terms, such as feelings of guilt, delusions, altered mood, or disordered thinking. In these cases, a correlated, quantifiable measure in an animal is substituted for a more cognitive human behavior for testing purposes. When the correlation is strong, a drug that modifies rat behavior in a specific way can be expected to predictably alter a particular human behavior, even though the two behaviors may seem unrelated. For instance, if a new drug were to reduce apomorphine-induced hyperactivity in rats, tests on humans might

show it to be effective in treating schizophrenia (see Chapter 19). Tests such as these have low face validity. However, if the drug effects observed in the laboratory test closely parallel or predict the clinical effect, the tests may be said to demonstrate **predictive validity**. **Construct validity** refers to the extent to which the animal measurement tool actually measures the characteristic being investigated. A test with high construct validity optimally would create the disease in the animal or would mimic the neural and behavioral features of the disorder. Unfortunately, the underlying neurobiology of most psychiatric disorders is not clear, and our understanding of the underlying causes change over time with enhancements in technology. To be optimal, an animal behavioral test, in addition to having predictive validity, should also do the following:

1. It should be *specific* for the class of drug being screened. For example, if antidepressants produce a consistent response in a behavioral test, we probably would not want to see analgesic drugs producing the same effect.
2. It should be *sensitive* so that the doses used are in a normal therapeutic range and show a dose–response relationship.
3. It should demonstrate the same *rank order of potency* (i.e., ranking drugs according to the dose that is effective) as the order of potency of the therapeutic action of the drugs.

In addition, good behavioral measures have high **reliability**, meaning that the same results will be recorded each time the test is used (Treit, 1985).

Valid and reliable animal tests are important for studying the neurobiological basis for complex disorders of the CNS and testing the potential of new therapeutic approaches. Although animal testing and disease modeling is crucial to the development of psychoactive drugs, many of the current approaches are very limited and are poor predictors of drug efficacy in humans (see the section on translational research). Unfortunately, many of the diagnostic symptoms, such as thoughts of suicide or feelings of guilt, cannot be easily translated to animal behavior. Also, since most psychiatric disorders are highly complex in symptomology, most animal testing is forced to evaluate only one or a few behavioral symptoms. Further, it is rare to have individuals with the same diagnosis demonstrate the same cluster of symptoms, so there are multiple animal models for the same disorder with little in common. In addition, there is insufficient information on the genetic or molecular basis of most disorders of the CNS. Nevertheless, neuroscientists are hopeful that better testing will be gradually achievable through genetic techniques, manipulations of environmental factors that have been associated with onset of the disorder, selective brain lesioning, or activation of specific brain cells using techniques such as optogenetics. The second part of this chapter will describe some of these neuropharmacological techniques. For an excellent review of animal models of CNS disorders as well as their utility and limitations, see McGonigle, 2014.

A wide variety of behaviors are evaluated by psychopharmacologists

There are many behavioral tests used by psychopharmacologists, and they vary considerably in complexity, time needed to be carried out, and cost, as well as validity and reliability. In this next section, we will describe just a few of the available procedures, many of which will be referred to in subsequent chapters.

SIMPLE BEHAVIORAL OBSERVATION Many simple observations of untrained behaviors require little or no instrumentation. Among the observations made are measures of tremors, ptosis (drooping eyelids), salivation, defecation, catalepsy, reflexes, response to tail pinch, and changes in eating or drinking. Animals demonstrating **catalepsy** are still and immobile and sometimes will remain in an unusual posture if positioned by the experimenter. The time it takes for the animal to return to normal posture gives an indication of the extent of catalepsy. The use of catalepsy as a test to identify antipsychotic drugs that produce motor side effects demonstrates the usefulness of screening tests that are not clearly related to human behavior.

MEASURES OF MOTOR ACTIVITY These measures identify drugs that produce sleep, sedation, or loss of coordination and, in contrast, drugs that stimulate activity. Spontaneous activity can be measured in a variety of ways. One popular method counts the number of times infrared light beams (invisible to rodents) directed across a designated space are broken. Automated video tracking with computerized analysis is a second method. A third, less automated technique (**open field test**) involves placing the animal in a prescribed area that is divided into squares so the investigator can record the number of squares traversed in a unit of time. It is also possible to count the number of fecal droppings and to observe the amount of time an animal spends along the walls of the chamber rather than venturing toward the open space. High fecal counts and low activity seen primarily at the perimeter of the cage are common indicators of anxiety.

OPERANT CONDITIONING Operant conditioning has also made contributions to the study of the effects of drugs on behavior. It is a highly sensitive method that can be used to evaluate a wide variety of behaviors including analgesia, anxiety, addiction potential, and drug discrimination, as well as learning and memory. The underlying principle of operant conditioning is that

FIGURE 4.2 Rat in an operant chamber The rat can be trained to press the lever (response) to activate a food delivery mechanism (reinforcement). An animal can also learn to press the lever to terminate or postpone shocks that can be delivered through the grid floor. (Courtesy of Med Associates, Inc.)

consequences control behavior. An animal performs because it is rewarded for doing so. Animals learn to respond to obtain rewards and avoid punishment.

Although it is possible to teach many types of operant responses, depending on the species of animal used, experiments are typically carried out in an operant chamber (Skinner box). An operant chamber is a soundproof box with a grid floor that can be electrified for shock delivery, a food or water dispenser for rewards, lights or a loudspeaker for stimulus cue presentation, and levers that the animal can press (**FIGURE 4.2**). Computerized stimulus presentation and data collection provide the opportunity to measure the total number of responses per unit time. In addition, the technique records response rates and interresponse times, which provide a stable and sensitive measure of continuous behavior.

In a brief training session, the animal learns to press a lever to receive a food reinforcer. Once the behavior is established, the requirements for reinforcement can be altered according to a predetermined schedule (**schedule of reinforcement**). The rate and pattern of the animal's behavior are controlled by the schedule; this allows us to examine the effect of a drug on the pattern of behavior. For instance, on a fixed-ratio (FR) schedule, reinforcement is delivered after a fixed number of responses. Thus, an FR-3 schedule means that the animal must press the lever three times to receive one food pellet. Changing the fixed ratio from 3 to 20 or 45 will tell us how hard the animal is willing to work for the reinforcement. Interval schedules also are commonly used and are characterized by the availability of reinforcement after a certain amount of time has elapsed (rather than a particular number of bar presses). Thus, on an FI-2 schedule (fixed interval of 2 minutes), reinforcement follows the first response an animal makes after 2 minutes have passed since the last reinforcement. Responses made during the 2-minute interval are "wasted," that is, elicit no reinforcement. This schedule produces a pattern of responding that includes a pause after each reinforcement and a gradual increase in the rate of responding as the interval ends. For a description of other variations in schedules and their use in drug testing, see Carlton (1983).

MEASURES OF ANALGESIA Analgesia is the reduction of perceived pain without loss of consciousness. Analgesia testing with human participants is difficult because the response to experimentally induced pain is quite different from that to chronic or pathological pain, in which anxiety and anticipation of more pain influence the individual's response. Of course, we cannot know whether an animal "feels pain" in the same way that a human does, but we can measure the animal's avoidance of a noxious stimulus. One simple test is the **tail-flick test**, in which heat produced by a beam of light (the intensity of which is controlled by a rheostat) is focused on a portion of a rat's tail (**FIGURE 4.3**). The latency between onset of the stimulus and the animal's removal of its tail from the beam of light is assumed to be correlated with pain intensity. Another method of measuring analgesic drug action using thermal stimuli is the **hot plate test**. The animal to be tested is placed in a cylinder on a metal plate maintained at a constant temperature, which can be varied between 55°C and 70°C. Drug evaluation is based on observation of the latency between an increase in temperature of the plate

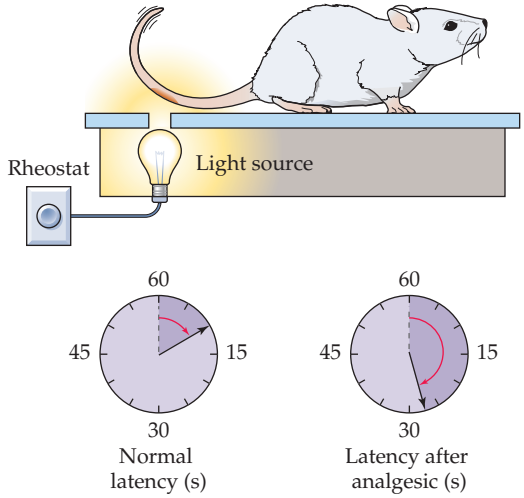


FIGURE 4.3 The tail-flick test of analgesia The response of the animal to a thermal stimulus is measured with this test. The quantitative measure made is the time between onset of the light beam, which provides heat, and movement of the tail. The clockfaces show that the response to the noxious stimulus is delayed after treatment with an analgesic that is known to reduce pain in humans. (After Hamilton and Timmons, 1990.)

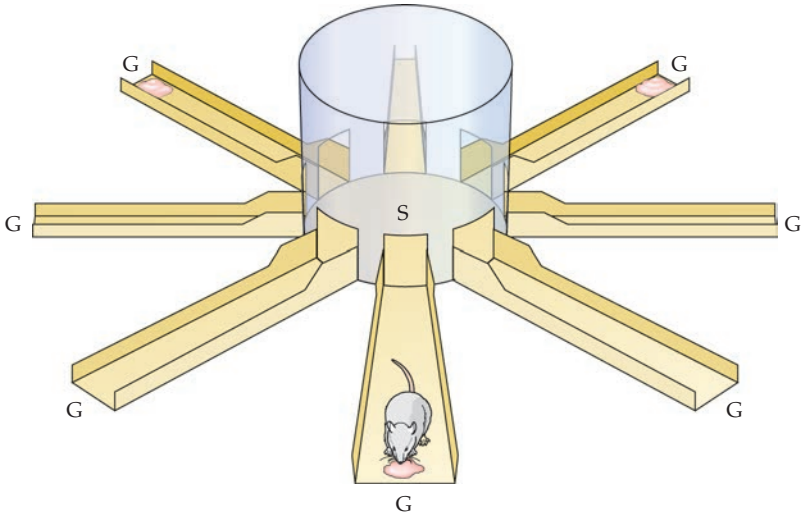


FIGURE 4.4 The radial arm maze
A central start box (S) has a number of arms or alleys radiating from it. Goal areas (G) containing food are at the ends of the arms.

and when the rat starts licking paws or kicking with the hind paws, vocalizing, or attempting to escape the cylinder. The sensitivity of this test is approximately equal to that of the tail-flick test. It seems to produce reliable and fairly stable pain thresholds with which to measure analgesic activity.

A variation of the operant FR schedule utilizes negative reinforcement, which increases the probability of a response that terminates an aversive condition. This technique can be easily applied to **operant analgesia testing**. First, the animal is trained to turn off an unpleasant foot shock by pressing the lever. In the test phase, the researcher administers increasing amounts of foot shock up to the point at which the animal responds by pressing the lever. The lowest shock intensity at which the animal first presses is considered the aversive threshold. Analgesic drugs would be expected to raise the threshold of electric shock. The method is very sensitive even to mild analgesics such as aspirin. However, an independent measure of sedation is necessary to distinguish between failure to respond due to analgesia and failure to respond due to behavioral sedation.

TESTS OF LEARNING AND MEMORY Objective measures of learning and memory, accompanied by careful interpretation of the results, are important whether you are using animal subjects or human participants. Keep in mind that these tests very often do not determine whether altered responses are due to drug-induced changes in attention or motivation, consolidation or retrieval of the memory, or other factors contributing to overall performance. Unless these other factors are considered, tests of learning are open to misinterpretation. Despite the challenges posed, finding new ways to manipulate the neurotransmitters involved in these functions will be central to developing drugs that are useful in treating memory deficits due to normal aging or neurological injuries or diseases such as Alzheimer's and other dementias. A wide variety of tests are available that depend on the presentation of information (training stage) followed by a delay and then the opportunity for performance (test stage). Higher cognitive processes can be evaluated by creating situations in which reorganization of the information presented is necessary before the appropriate response can be made.

MAZES Although the size and complexity of mazes can vary dramatically, what they have in common is a start box at the beginning of an alley with one (**T-maze**) or more (**multiple T-maze**) choice points that lead to the final goal box, which contains a small piece of food or another reward. A hungry rat is initially given an opportunity to explore the maze and find the food goal. On subsequent trials, learning is evaluated on the basis of the number of errors at choice points and/or the time taken to reach the goal box. Careful evaluation of results is needed because drug-induced changes in behavior may be due to a change in either learning or motivation (e.g., Does the drug make the animal more or less hungry? Sedated? Disoriented?). This task can also be used to evaluate working memory if the goal is alternated from side to side at each trial.

Spatial learning tasks help us investigate the role of specific brain areas and neurotransmitters, such as acetylcholine, in forming memories for the relative locations of objects in the environment. One special type of maze, the **radial arm maze**, is made up of multiple arms radiating away from a central choice point (**FIGURE 4.4**) with a small piece of food at the end of each arm. With very little experience, typical rats learn to forage efficiently by visiting each arm only once on a given day, indicating effective spatial memory for that particular episode. The task can be made more complex by blocking some arms on an initial trial. After the initial trial the animal is returned to the central choice point for a second trial. The animal is expected to remember which arms have already been entered and move down only those that still contain food. For a typical rat, the task is not complex because it mimics the foraging behavior of animals in the wild, where they must remember where food has been found. But animals with selective lesions in the hippocampus (and other areas), as well as those injected with a cholinergic-blocking drug, show significant impairment. Low doses of alcohol also interfere with spatial memory. Because the arms are identical, the animals must use cues in the environment to orient themselves in the maze, hence the need for spatial memory. The task is similar to our daily activity of driving home from work. Not only do we need to recognize each of the landmarks along our route, but we must learn the relative locations of these objects with respect to each other. As we move along our

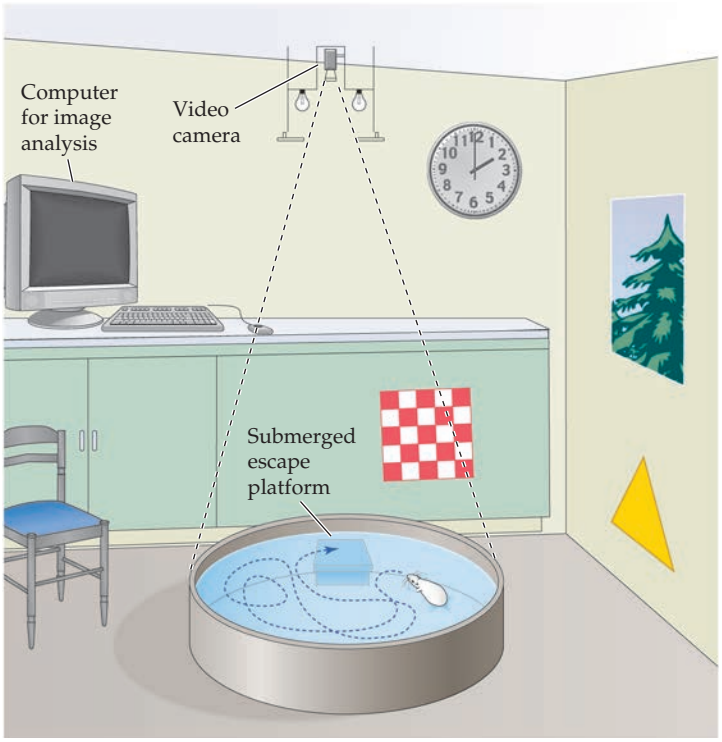


FIGURE 4.5 The Morris water maze A circular pool is filled with opaque water. The escape platform is approximately 1 cm below the water level. The rat's task is to locate the submerged, hidden platform by using visuospatial cues available in the room. A video camera is mounted above the pool and is connected to the video recorder and the computer link to trace the individual swim path. (After Kolb and Whishaw, 1989.)

route, our perceptions of the objects and their relative locations to us tell us where we are and where we should be going. Failure in this complex cognitive process is characteristic of patients with Alzheimer's disease who wander away and fail to find their way home.

A second test of spatial learning, the **Morris water maze**, uses a large, circular pool of water that has been made opaque by the addition of milk or a dye. Animals placed in the pool must swim until they find the escape platform that is hidden from view just below the surface of the water (**FIGURE 4.5**). The subject demonstrates that it has learned the spatial location of the submerged platform by navigating from different starting positions to the platform. Since there are no local cues to direct the escape behavior, successful escape requires that the subject learn the spatial position of the platform relative to landmarks outside the pool. When curtains surrounding the pool are drawn to block external visual cues, performance falls to chance levels, demonstrating the importance of visuospatial cues. As a laboratory technique, the water maze has several advantages. No extensive pretraining is required, and testing can be carried out over short periods of time. Escape from water motivates without the use of food or water deprivation or electric shock; this makes interpretation of drug studies easier, in that drug-induced changes in motivation are less likely. One disadvantage, however, is that water immersion may cause endocrine or other stress effects that can interact with the drugs administered. A video demonstration can be found on YouTube (Nunez, 2014) and on the Companion Website.

DELAYED-RESPONSE TEST This design assesses the type of memory often impaired by damage to the prefrontal cortex in humans. It is similar to tasks included in the Wechsler Memory Scale, which is used to evaluate working memory deficits in humans. In this task (**FIGURE 4.6**), an animal watches the experimenter put

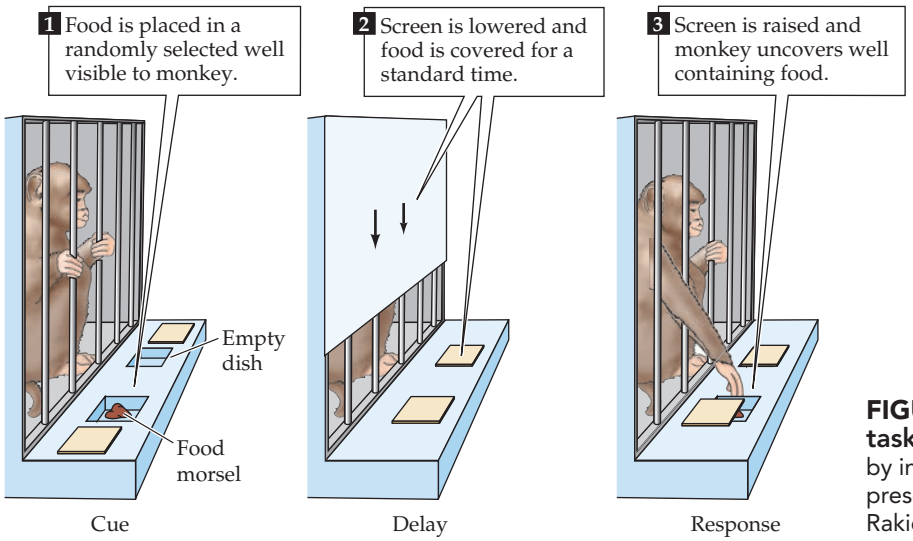


FIGURE 4.6 The delayed-response task This test evaluates working memory by imposing a delay between stimulus presentation and testing. (After Goldman-Rakic, 1987.)

a piece of food into one of the food boxes in front of it. The boxes are then closed, and a sliding screen is placed between the monkey and the boxes for a few seconds or minutes (the delay). At the end of the delay, the screen is removed and the animal has the opportunity to recall under which of the covers food is available. To receive its reward, the animal must hold in working memory the location of the food.

Visual short-term memory can be tested by slightly modifying the procedure. At the beginning of the trial, an object or other stimulus is presented as the sample. After a short delay, during which the sample stimulus is removed, the animal is given a choice between two or more visual stimuli, one of which is the same as the sample. If the animal chooses the pattern that matches the sample, it is given a food reward; an incorrect response yields no reward. This match-to-sample task can of course be modified to make a match-to-nonsample task, in which case the animal must choose the visual stimulus that is different from the original sample. This task is a good example of the use of operant conditioning. To make the correct choice after the interval, the animal must "remember" the initial stimulus.

MEASURES OF ANXIETY There are many biobehavioral measures available for identifying novel antianxiety compounds and for evaluating the neurochemical basis of anxiety. Cryan and Sweeney (2011) have reviewed the animal models and discuss their importance for drug discovery. Most anxiety models use induced fearfulness as an analogy to human anxiety. Some use unconditioned animal reactions such as a tendency to avoid brightly lit places or heights; others depend on traditional learning designs. The **light–dark crossing task** involves a two-compartment box with one side brightly lit (normally avoided by rodents) and the other side dark. Measures include the number of crossings between the bright and dark sections and the amount of time spent on each side, as well as total motor activity. Anxiety-reducing drugs produce a dose-dependent increase in the number of crossings and in overall activity while also increasing the amount of time spent in the light. Similar to the light–dark crossing task is the open field test. In this test, the animal is allowed to freely explore the test chamber, which is a novel and unprotected environment that is usually brightly lit. Both the number and pattern of the animal's movements are monitored either manually or with an automated system. Rodents typically explore the periphery of the chamber while staying in contact with the walls. Antianxiety drugs increase the amount of time spent in the unprotected center of the box. The **elevated plus-maze** is a cross-shaped maze raised 50 cm off the floor that has two open arms (normally avoided because of aversion to heights and open spaces) and two arms with enclosed sides (**FIGURE 4.7**). This quick and simple test shows a selective increase in

FIGURE 4.7 The elevated plus maze This maze is used to test anxiety and is based on rodents' natural aversion to heights and open space and their predisposition for enclosed places.

open-arm exploration following treatment with antianxiety drugs and a reduction after treatment with caffeine or amphetamine—drugs considered to increase anxiety. Very similar to the elevated plus-maze, the **zero maze** is a donut-shaped elevated platform that has two enclosed sections and two open sections. The circular construction lacks the ambiguous central square area in the plus-maze that sometimes makes scoring difficult. All of these naturalistic tests are easy to perform, relatively quick, and based on the assumption that the animals are in conflict between their natural desire to explore and the drive to avoid an aversive stimulus. Although the elevated mazes are among the most popular laboratory measures of anxiety, the construct validity of the tasks has been questioned (Ennaceur, 2014).

In another naturalistic task, the **one-chamber social interaction test** designed by File measures the amount of sniffing, following, walking over, crawling under, and mutual grooming behaviors that occur between two male rats when they are placed in an area where neither has established territory. In the original design it was found that these normal behaviors were reduced in an unfamiliar test chamber and when illumination was increased, conditions that may evoke anxiety in rodents. As the normal interactions decreased, traditional indicators of emotionality (defecation and freezing) increased, as did plasma levels of the rodent stress hormone, corticosterone. A review of 25 years of research using this test has shown that a variety of anxiolytics (drugs that reduce anxiety) increase the social interaction under the aversive conditions, while anxiogenic drugs, which increase anxiety, further reduce the social interaction (see File and Seth, 2003). A modification of this model has been created to test for sociability, social memory, and novelty (**BOX 4.1**).

In the **novelty suppressed feeding paradigm**, animals are presented their usual food in a new, potentially threatening environment, or conversely they are provided novel food in their usual environment. In either case, the novelty prolongs the latency to begin eating. In very young animals, anxiety can be evaluated by recording and quantifying the **ultrasonic vocalizations** of rat pups when they are separated from their mothers. These distress calls, which are inaudible to most predators and humans, reflect anxiety in the young and represent a call to their mothers to retrieve them. These vocalizations are very sensitive to a variety of antianxiety drugs from several drug classes.

The **water-lick suppression test** (**Vogel test**) is a **conflict procedure** that reliably screens anxiety-reducing drugs while requiring little training of the animals. Rats deprived of water quickly learn to lick the tip of a metal drinking spout for liquid. During testing, animals are given the opportunity to drink from the spout for a 3-minute session. However, after every 20 licks, the rats receive a mild tongue shock, which causes them to suppress responding. The conflict between the urge to lick the spout and the desire to avoid the shock is a classic paradigm for anxiety. Pretreatment with classic anxiety-reducing drugs such as the benzodiazepines increases water consumption; however, the test is insensitive to some newer anxiolytics.

MEASURES OF FEAR Several common measures of fear involve classical conditioning. The **conditioned emotional response** (often called emotional memory in humans) depends on presentation of a signal (a light or tone) followed by an unavoidable electric shock to form a classically conditioned association. When the warning signal is presented during ongoing behavior, the behavior is suppressed (i.e., "freezing" occurs). Although this method has not always produced consistent results when used to screen antianxiety drugs, it has become an important tool in understanding the role of the amygdala and its neurochemistry in the conditioned fear response.

A second method is **fear-potentiated startle**, which refers to enhancement of the basic startle response when the stimulus is preceded by the presentation of a conditioned fear stimulus. For example, if a light has been previously paired with a foot shock, the presentation of that light normally increases the magnitude of the startle response to a novel stimulus, such as a loud clap.

MEASURES OF DEPRESSIVE-LIKE BEHAVIOR Animal models are used to study the neurobiology of depression and to evaluate the mechanism of antidepressant drugs, as well as to screen new drugs for effectiveness. There is no available model that mimics all the

BOX 4.1 Pharmacology in Action

Using the Three-Chamber Social Interaction Test

The three-chamber social interaction test is a newer variation of the one-chamber social interaction test that evaluates anxiety. It was designed to measure social affiliation, social memory, and preference for social novelty in rodents. This task has relevance to human disorders that are characterized by poor social interaction, for example, autism spectrum disorders, schizophrenia, and bipolar disorder.

Jamain and colleagues (2008) used the three-chamber test to evaluate a genetically modified mouse model of autism. Autism is a neurodevelopmental disorder with high heritability, prompting major international efforts to identify candidate genes. Determining those genes would help to evaluate gene–environment interactions in the etiology of the disorder and provide a target for effective treatments. Since there are no identifiable biological markers for autism spectrum disorders, diagnosis is based solely on behavioral criteria. Since one core symptom cluster includes deficits in social interaction and reciprocal social communication, the social interaction test seemed to be an appropriate means to validate the animal model. Rodents are the natural choice of subjects because by nature they are sociable and will interact with and actively investigate a conspecific (an animal of the same species).

Numerous candidate genes have been implicated in autism, many of which regulate the formation and maturation of synapses. A good way to evaluate a gene's importance is to create mice with selective mutations of the genome (described later in this chapter) and evaluate their behaviors, which in this case should resemble the symptoms of autism. The gene that Jamain and colleagues eliminated is *Nlgn4*. Its elimination produces neuroligin 4–deficient mice, called *Nlgn4*-knockout mice. Mutation in this gene in humans, *NLGN4*, is associated with autistic symptoms. Expression of *NLGN4* is lowest prenatally and gradually increases until 3 weeks postnatally, a time that coincides with completed synapse formation. The *Nlgn4*-knockout mice and wild-type control mice were studied with a battery of behavioral

(*Continued*)

BOX 4.1 Pharmacology in Action (*continued*)

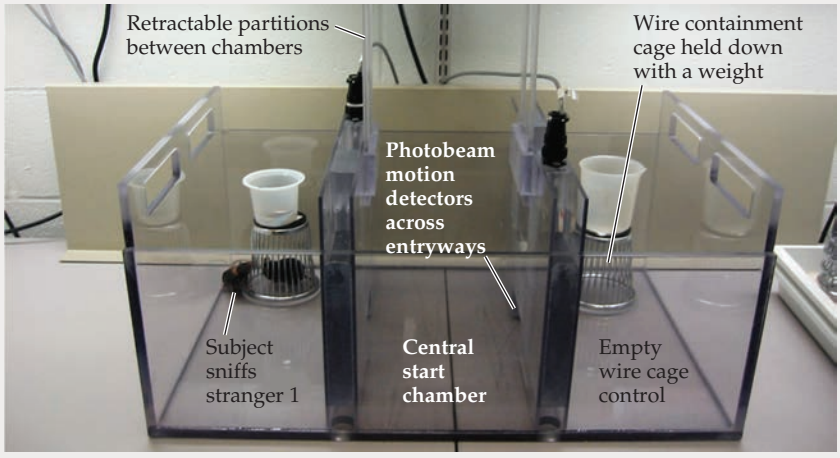
measures, including the three-chamber social interaction test.

The task required a three-chamber box with retractable doors. The test mouse was first acclimated to the test box that was empty except for a wire mesh containment cage in each of the side chambers (**Figure A**). For the social affiliation portion of the task, a novel mouse (stranger 1) was placed inside one of the wire containment cages, and the test mouse had access to the three chambers for 10 minutes. The spacing of the wire bars of the containment cages allowed olfactory, auditory, visual, and limited tactile interaction but prevented aggressive or sexual contact. The test mouse behavior was observed and scored for the duration and number of contacts, time spent in each chamber, and number of entries into each compartment, as well as other behaviors such as walking and grooming.

In **Figure B**, the wild-type mice spent more time in the chamber with stranger 1, whereas the *Nlgn4*-knockout mice spent equal time in both chambers. The wild-type mice had more interactions with stranger 1 than with the empty cage, as shown in **Figure C**. In contrast the *Nlgn4*-knockout mice showed no preference for spending time in the compartment with stranger 1 or interacting with it.

The next phase of the testing involved placing a new stranger mouse (stranger 2) in the previously empty cage, and the same behavioral evaluations were conducted. It is evident from **Figure D** that the wild-type mice remembered their previous experience with stranger 1 and interacted more with the novel mouse (stranger 2), demonstrating both memory of the previous social interaction and the preference for novel experiences. The *Nlgn4*-knockout mice did not show a similar preference for the new stranger. These results along with other test battery results led the researchers to conclude that *Nlgn4*-knockout mice were a viable model of autism and deserved further evaluation. For a detailed protocol of the procedure, including a video link, see Kaidanovich-Beilin et al., 2011.

(A)



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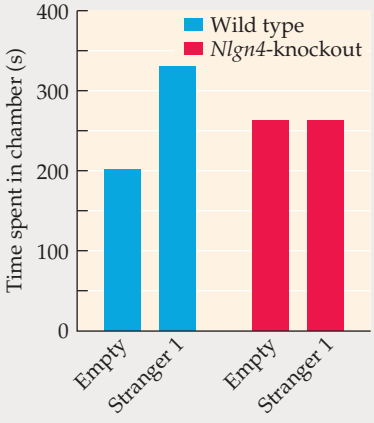

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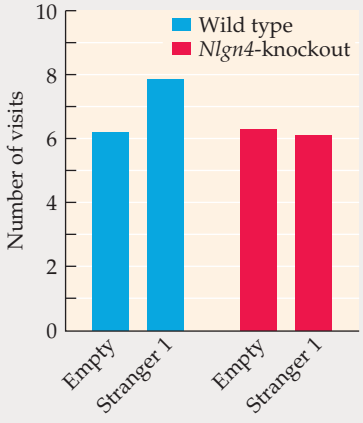
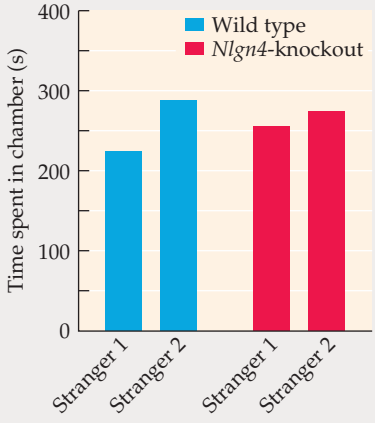


(D)



Social interaction test (A) Experimental setup to measure social affiliation, social memory, and preference for social novelty in rodents. Subjects included wild-type and *Nlgn4*-knockout mice. (B) Time spent in the compartment with stranger 1 versus the empty compartment. (C) Number of visits to the compartment of the stranger. (D) Time spent in the compartment with the familiar mouse (stranger 1) versus the novel stranger (stranger 2). (A from Crawley, 2004 and Nadler et al., 2000; B–D after Jamain et al., 2008.)

FIGURE 4.8 Forced swim test After its initial attempts to escape, the rat in the water-filled cylinder is shown in a posture reflecting a sense of futility. Antidepressant drugs reduce the amount of time spent in the immobile posture. (Courtesy of Porsolt and Partners Pharmacology.)

symptoms of depression; instead, each model is associated with a specific aspect of the disorder, such as reductions in psychomotor activity, neuroendocrine responses, cognitive changes, or such functions as eating, sleeping, and deriving pleasure from everyday activities. Therefore, the usefulness of any single model in evaluating the complex etiology of depression is limited, and the ability of models to predict which new drugs will be effective for the clinical population is challenging.

A commonly used measure that permits rapid behavioral screening of novel antidepressants, called the **behavioral despair** or **forced swim test**, requires rats or mice to swim in a cylinder from which there is no escape. After early attempts to escape are unsuccessful, the animals assume an immobile posture except for the minimal movements needed to keep their heads above water (**FIGURE 4.8**). In a closely related test, the **tail suspension test**, mice are suspended by the tail from a lever, and the duration of movements (a period of agitation followed by immobility) is recorded. These models are based on the anthropomorphic idea that the immobility reflects a lowered mood in which the animals are resigned to their fate and have given up hope. Acute administration of effective antidepressants reduces the time spent in this "freezing" phase of immobility relative to untreated control animals. This test has similarities to the **learned helplessness** test, in which subjects are initially exposed to aversive events such as *unescapable* foot shock for several hours or for periods over several days. When the subjects are placed in a new situation in which a response could alter an aversive event, they fail to make the appropriate response. The animal behavior resembles clinical depression in that depressed humans frequently fail to respond to environmental changes and express feelings of hopelessness and the belief that nothing they do has an effect. Although these three antidepressant screening tests are frequently used, all three have been criticized because the stress is only short-lived, the animals tested are not genetically vulnerable to depression, and the antidepressants reduce behavioral despair after acute drug treatment rather than after the chronic antidepressant administration that is needed to reverse symptoms in humans.

Several chronic stress procedures are more difficult to perform but are considered to have greater construct validity because stress is a risk factor for depression (Nestler and Hyman, 2010). The **chronic mild unpredictable stress** model exposes rodents to a series of physically stressful events such as cold temperatures, wet bedding, restraint, and sudden loud noises for several weeks. Following the stress, these animals show several behaviors reminiscent of depressed individuals, including multiple cognitive impairments, increased anxiety-like behaviors, social withdrawal, and a reduced preference for sucrose that models human anhedonia (i.e., failure to derive pleasure from normally pleasant events), all of which give the model face validity. Of significance is that chronic rather than acute antidepressant treatment is required to reverse the behaviors, so predictive validity is good. As is true for many of the most validated models, the downside of this model is that it is labor-intensive, requires significant laboratory space, and takes a long time. Also, because the procedure is complex, replication of studies across laboratories can be a challenge (Abelaira et al., 2013). **Chronic social defeat stress** uses the resident–intruder paradigm to create intense stress. The animal is placed in another conspecific's territorial cage, and this generates nonlethal aggression. Exposure to the conflict is repeated over several days, and between exposures the animal may be housed in a cage adjacent to the dominant animal to expose it further to visual and olfactory cues. Animals stressed in this manner show multiple depression-like behaviors including reductions in motor activity, exploration, sucrose preference, and copulatory behaviors, as well as social withdrawal and a metabolic syndrome (e.g., weight gain and insulin resistance) characteristic of depressed humans. Chronic, but not acute, antidepressant administration reverses these behaviors. Hence this model also has construct, face, and predictive validity, as does the model using maternal separation, discussed next.

To evaluate the importance of early life stress as a vulnerability factor in the development of depression, **maternal separation** can be used. Stress is induced

by separating young animals (usually rats) from their mothers for brief periods daily during the first few weeks of life. Early stress provides the opportunity to evaluate long-term behavioral and neuroendocrine abnormalities in the animals as adults. Nemeroff (1998) originally used this model to evaluate the hypothesis that abuse or neglect early in life activates the stress response at the time but also produces long-term changes in CRF function that may predispose the individual to clinical depression later in life. The relationship of early life stress to clinical depression is discussed further in Chapter 18.

The **sucrose preference test** is a measure of anhedonia. In the test, the animals are provided with two drinking tubes, one of which contains water, the second a solution sweetened with sucrose or saccharin. The animals have free access to the tubes over several days. Each day the volume of water and sucrose is measured, and the positioning of the bottles is reversed to prevent a bias toward one side of the enclosure. The typical animal will show a strong preference for the sweetened solution. Animals failing to show that preference demonstrate anhedonia, a common symptom of clinical depression. A variety of stressors can reduce sucrose preference. As described earlier, those animals exposed to chronic mild unpredictable stress tend to experience anhedonia in this test, and their behavior can be returned to normal with chronic, but not acute, antidepressant treatment. Other reinforcers, such as male preference for a sexually receptive female, can be used in a similar manner. Evidence for stress-induced anhedonia provides incentive to examine the neurobiology of the reward circuitry as a component of major clinical depression.

METHODS OF ASSESSING DRUG REWARD AND REINFORCEMENT The simple operant FR schedule has been used very effectively in identifying drugs that have abuse potential, that is, drugs that are capable of inducing dependence. We assume that if an animal will press a lever to receive an injection of drug into the blood or into the brain, the drug must have reinforcing properties. The drug **self-administration method** (**FIGURE 4.9**) used with rodents is a very accurate indicator of abuse potential in humans. For instance, animals will readily self-administer morphine, cocaine, and amphetamine—drugs that we know are readily abused by humans. In contrast, drugs like aspirin, antidepressants, and antipsychotic drugs are neither self-administered by animals nor abused by humans. **TABLE 4.1** lists some of the drugs that are reinforcing in the rhesus monkey. Compare this list with what you know about substances abused by humans. Drug self-administration is considered the most valid model of drug seeking in humans.

Furthermore, we can ask the animal which of several drugs it prefers by placing two levers in the operant chamber and training the animal to press lever A for one drug and lever B for the alternative. If given free access to the levers, the animal's choice will be readily apparent. An additional question we can pose involves how much the animal really "likes" a particular drug. By varying the schedule of reinforcement from FR-10 to FR-40 or FR-65, we can tell how reinforcing the drug is by how hard the animal works for the injection. The point at which the effort required exceeds the reinforcing value is called the **breaking point**. The higher the breaking point, the greater is the reinforcement of the drug and, presumably, the greater is the abuse potential in humans. Drugs like cocaine sustain incredibly high rates of responding; animals will lever press for drug reinforcement until exhaustion.

In addition to modeling the acquisition and maintenance of the drug-taking habit, self-administration

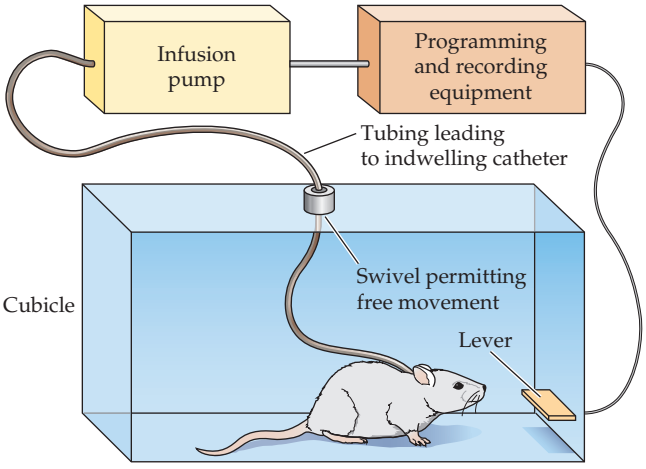


FIGURE 4.9 The drug self-administration method
Pressing the lever according to a predetermined schedule of reinforcement triggers drug delivery into a vein or into discrete brain areas. This method predicts abuse liability of psychoactive drugs.

TABLE **4.1** Drugs That Act as Reinforcers in the Rhesus Monkey

Category	Specific drug
Central stimulants	Cocaine
	Amphetamine
	Methylphenidate (Ritalin)
	Nicotine
	Caffeine
Opioids	Morphine
	Methadone
	Codeine
CNS depressants	Pentobarbital
	Amobarbital
	Chlordiazepoxide (Librium)
	Ethyl alcohol

studies can be used to examine the neurochemical and neuroanatomical correlates of the withdrawal syndrome, which is the central component of physical dependence. By substituting saline for the active drug, the abstinence syndrome occurs in physically dependent animals. Although there may be an initial increase in lever pressing, ultimately the operant responses will decline and eventually cease. At that point, we say extinction of the behavior has occurred. Animals that have undergone extinction of the drug-taking behavior can then be used to study those factors that lead to relapse, that is, the reinstatement of drug-taking behavior. Much more detail on substance abuse can be found in Chapter 9.

A modification of the drug self-administration method allows the animal to self-administer a weak electrical current to discrete brain areas via an indwelling electrode. The underlying assumption of **electrical self-stimulation** is that certain brain areas constitute "reward" pathways. It is assumed that when the animal works to stimulate a particular cluster of neurons, the electrical activation causes the release of neurotransmitters from nerve terminals in the region, which, in turn, mediate a rewarding effect. The fact that pretreatment with certain drugs, such as morphine or heroin, increases responding for even low levels of electrical stimulation indicates that the drugs enhance the brain reward mechanism (Esposito et al., 1989). In combination with mapping techniques, this method provides an excellent understanding of the neural mechanisms of reward and the effects of psychoactive drugs on those pathways.

Although several popular measures used to evaluate the rewarding and reinforcing effects of drugs are operant techniques, a method called **conditioned place preference** relies on a classically conditioned association between drug effect and environment (**FIGURE 4.10**). During conditioning trials over several days, the animal is injected with either drug or saline and is consistently placed in one compartment or the other so that it associates the environment with the drug state. The rewarding or aversive effect of the drug is determined in a test session in which the animal is given access to both compartments and the amount of time spent in each is monitored. If the drug is rewarding, the animal spends much more time in the compartment associated with the drug. If the drug is aversive, the animal prefers the compartment associated with saline injection. Additionally, researchers may study the biological basis for the rewarding effects by pretreating animals with selected receptor antagonists or neurotoxins to modify the place preference. O'Brien and Gardner (2005) have reviewed many behavioral principles and methods related to drug reward and reinforcement.

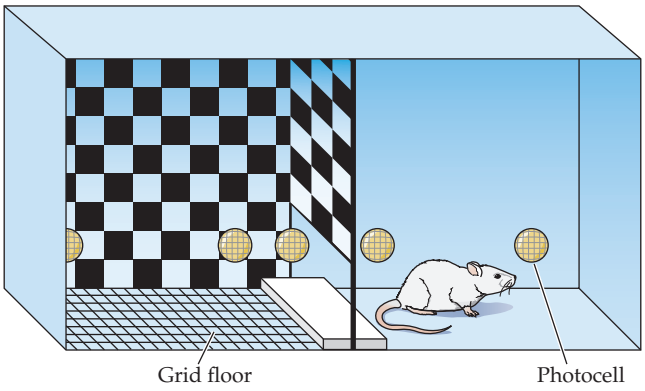


FIGURE 4.10 Place-conditioning apparatus
The apparatus consists of two distinctly different compartments that vary in the pattern and texture of the floor and walls. Photocells monitor the animal's movement. Each compartment is repeatedly paired with either drug or saline injection. On the test day, the animal is allowed free access to both compartments, and the amount of time spent in each tells us whether the drug effect was rewarding or aversive. (After Stolerman, 1992.)

DRUGS AS DISCRIMINATIVE STIMULI A discriminative stimulus is any stimulus that signals reinforcement for a subject in an operant task. For example, "light on" in the chamber may signal that reinforcement is available following pressing one of two available levers, while "light out" may signal that reinforcement is available only after pressing the other lever. An animal that learns to press one lever when the light is on and the opposite lever when the light is out can discriminate between the two conditions. Although discriminative stimuli are usually changes in the physical environment, internal cues after drug administration can also be discriminative (**FIGURE 4.11**). Thus, an animal can learn that on days when it receives an injection of a particular drug and experiences the internal cues associated with that drug state (like the "light on"), it can press one lever for reinforcement, and on days when it receives a saline injection and experiences different internal cues, it can press the alternative lever for reinforcement, as in the "light off" condition. The animal's response depends on its discriminating among internal cues produced by the drug.

By using this technique, we can find out how the animal experiences a drug cue. For example, if an animal has been trained to lever press on the left after receiving morphine, and on the right on days when it has been pretreated with saline, other opioids can be substituted for the internal cue and signal to the animal that reinforcement can be obtained by pressing the "morphine" lever. In this way, it has been shown that the opioid drugs heroin and methadone are experienced in a way that is similar to that for morphine (i.e., they show generalization to morphine). In contrast, drugs like amphetamine or marijuana, which apparently produce subjective effects very different from those of morphine, do not lead to pressing the "morphine"

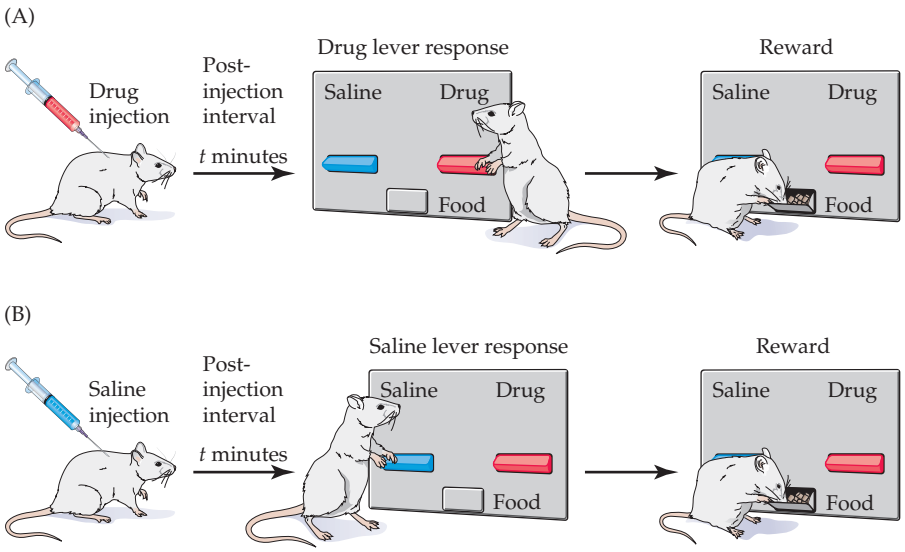


FIGURE 4.11 Drug discrimination testing
(A) A rat is injected with a drug before being placed in an operant chamber. If it presses the right lever ("drug"), it receives a food reward. If it presses the left (saline) lever, no reinforcement occurs. (B) On days that the rat receives a saline injection before it is placed in the operant chamber, it receives food reinforcement if it presses the saline lever, and no reinforcement if it presses the drug lever. Discrimination of internal drug cues determines the animal's response.

lever. In this way, novel drugs can be characterized according to how similar their internal cues are to those of the known drug. This can be useful in screening new therapeutic drugs by determining whether animals respond as they did when trained on a known effective agent. The same technique can be used to identify the neurochemical basis for a given drug cue. The drug cue can be challenged with increasing doses of a suspected neurotransmitter antagonist until the cue has lost its effect. Likewise, neurotransmitter agonists can be substituted to find which more closely resembles the trained drug cue. Drug discrimination testing is relatively easy to do and requires no surgical procedures. However, it is time-consuming because of the extensive training time and the limitation of generalization testing to only once or twice a week (with continued retraining on the remaining days). The technique has high predictive validity, meaning that results from animal tests are usually quite similar to those from human testing in analogous experimental designs. Solinas and colleagues (2006) provide an excellent description of the basic methodology of drug discrimination, as well as an assessment of advantages and disadvantages. For an example of the utility of drug discrimination testing, read **Web Box 4.1**.

TRANSLATIONAL RESEARCH Despite the difficulties associated with developing animal models of human psychiatric conditions, it is an extremely important step in translational research. Translational research represents an effort to transform discoveries from basic neuroscience research into clinical applications for treating mental and neurological disorders—an important goal of psychopharmacology. Translational research is not a new concept, and a good deal of early research in psychopharmacology was directed toward the ultimate goal of developing more effective medications. However, more recently, there has been greater emphasis on improving the predictability of therapeutic effects based on animal research, because running lengthy and expensive human clinical trials (**BOX 4.2**) on drugs that prove ineffective makes new drug development both slow and costly. This, in turn, makes medication more expensive and delays the opportunity for effective treatment. It also takes valuable research time and money away from the pursuit of potentially more successful drug development. Because there have been so many failures in translation of animal models to clinical effectiveness, many pharmaceutical companies are no longer eager to pursue therapeutics for disorders such as depression and anxiety (see McGonigle and Ruggeri, 2014). Since pharmaceutical companies have been criticized for emphasizing research and development of highly profitable drugs rather than those that are most innovative and/or those that address unmet medical needs, the research contribution of biotechnology companies and universities has become increasingly important. In response to the need for additional cutting-edge therapeutics for significant psychiatric disorders, the NIH has begun a consortium of academic health centers to nurture *interdisciplinary* research teams, whose goal is to develop inventive research tools to stimulate the development of drugs for clinical use. Yu (2011) describes some of the advances that have been made in the development of translational tools with the collaboration of preclinical and clinical scientists. It is estimated that up to 60 such research teams comprising molecular and psychopharmacological researchers, clinical investigators, and representatives of the pharmaceutical industry will be established in the very near future. Open-access websites and journals will further

BOX 4.2 Clinical Applications

Drug Testing for FDA Approval

All new drugs produced and sold in the United States by pharmaceutical companies must be approved by the Food and Drug Administration (FDA). For approval, they must be demonstrated to be both effective and safe. Design and testing of new drugs is a long, complex, and expensive procedure involving extensive evaluation in both laboratory and clinical settings. The approval process utilizes many of the methods we have discussed so far, in addition to extensive testing in humans (Zivin, 2000).

The **Figure** shows a timeline of typical drug development beginning with preclinical trials, which include in vitro neuropharmacological methods, such as receptor binding, autoradiography, and so forth. In vivo animal studies provide important information about pharmacokinetics (absorption, distribution, and metabolism), effective dose range, and toxic and lethal doses. In addition, animal behavioral models and animal models of neurological and psychiatric disorders provide a means to screen and evaluate potentially useful drugs. After preclinical testing, a drug that is considered safe is tested with humans in three distinct phases. In phase 1, the drug is evaluated for toxicity and pharmacokinetic data in a small group of healthy human volunteers. Although serious injuries during phase 1 testing are rare, they are particularly disturbing because the subjects are typically young and healthy individuals who may join the study for the monetary compensation because they are poor and unemployed. One recent example involves the testing of a new drug to relieve mood, anxiety, and motor problems that often accompany neurodegenerative diseases. Of the 90 study participants, 6 were hospitalized. One of these was brain dead, and 3 were likely to have irreversible brain damage (Chan, 2016). The examples of harm, though limited, point to the inherent dangers in clinical drug trials. In phase 2, limited clinical testing is conducted to evaluate the effectiveness of the drug in treating a particular disease. It has been argued that genomic screening should be a part of the FDA approval process (Urban and Goldstein, 2014; see Chapter 1). Such routine screening would provide a database of genetic information that predicts effective clinical outcomes and potential side effects, which is a necessary step in personalized medicine (i.e., drug treatment assigned to patients based on their genetic profile). An advantage for pharmaceutical developers would be that the screening and treatment outcome would give them an indication of the potential market for the new drug and might reduce their costs by including patients in phase 3 that have genetic indications of potential efficacy. Finally, the drug is tested again in large clinical trials (phase 3) involving thousands of patients at multiple testing sites around the country. After the third phase has been completed, the FDA can evaluate the data collected on both effectiveness and safety. If the drug receives FDA approval, it can be marketed and sold. Once in general use, the drug may still be evaluated periodically and new warnings may be issued to maximize safety by monitoring adverse reactions, dangerous drug interactions, and product defects.

Although arguments that new drugs are excessively expensive for the consumer are valid, it is important to understand that as few as 20% of new drugs that are tested reach final approval. This means that the

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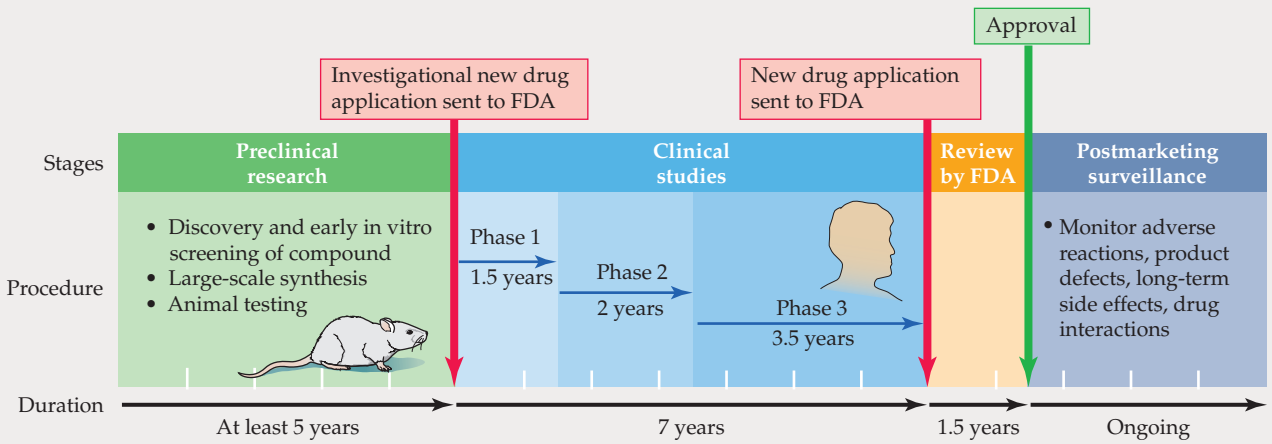


BOX 4.2 Clinical Applications (*continued*)

remaining 80% are eliminated only after testing that is both time-consuming and expensive. If we look at the total cost in today's dollars for research and development of the average drug approved by the FDA, we see a startling increase over time. In 1975, the average cost was $100 million; 12 years later costs increased to $300 million. By 2005 the cost of the average drug was over four times greater at $1.3 billion, and in 2011 the figure rose further to an amazing $5.8 billion (Roy, 2012). This enormous increase in the cost of developing new drugs has been primarily driven by the expenses associated with phase 3 trials, which have gotten larger, more complex, and longer in duration. For these reasons, improvements in translational research that transfers results from animal testing and basic neuroscience to predictable clinical outcomes for patients are critical (see the text). If more drugs moved successfully through the three phases, the average development costs for drugs ultimately approved by the FDA would go down.

To protect its investment, a pharmaceutical company patents its drug so that no other company can sell it for a period of 20 years. The company develops a trade name (also called a proprietary name) and has exclusive rights to market that product. After 20 years, the drug becomes "generic," and other companies can manufacture and sell their own formulations of the drug after proving to the FDA that they are equivalent to the original, with similar bioavailability. Once the patent has expired, the drug may acquire a variety of trade names, each developed by the individual manufacturer. For example, the newly patented antidepressant drug vilazodone is called Viibryd by its manufacturer. In contrast, because the patent on chlordiazepoxide has expired, the original manufacturer is still marketing Librium, but other pharmaceutical companies now produce chlordiazepoxide under the trade names Reposans, Sereen, and Mitran.

enhance communication among researchers and will encourage information sharing.

As is true for methods in neuropharmacology and molecular biology, animal testing and behavior analysis must continue to undergo improvement and refinement. Animal modeling will improve with advances in understanding disease causality and development of objective measures for clinical diagnosis. For a review of some of the challenges in developing translational animal models, see McGonigle and Ruggeri (2014).

It has long been wondered whether the translation of animal data into useful human drug treatments was hindered because drug effects were frequently tested on an adaptive animal response to a specific challenge, which may produce effects quite different from the response in humans with existing pathology. For instance, is it likely that drugs that suppress the normal aggression of a resident rodent on an intruder in its territory will also be effective in reducing pathological aggression in humans? Such questions have led researchers to suggest that the best models for translation to human treatment are those that depend on creating abnormal behaviors or neurobiological changes that closely resemble some of the focal symptoms of the disorder (Miczek and de Wit, 2008). Certainly, as molecular research continues to identify genetic differences associated with psychiatric disorders, additional animal models utilizing genetic mutations will be developed. For example, in one mouse model of obsessive-compulsive disorder (OCD), selective deletion of genes produces animals that as adults show OCD-like behaviors such as excessive grooming with severe hair loss analogous to the compulsive washing seen in some individuals with OCD, self-injurious behavior, and increased anxiety. Furthermore, these behaviors respond to treatment with the same serotonergic agonists that are effective for individuals treated for OCD. Additionally, the mice show abnormal corticostriatal connectivity—again, similar to human neuropathology (OCD will be described further in Chapter 17). These similarities suggest the value of this genetic model in studying the etiology of OCD and in screening potential therapeutic agents (Yang and Lu, 2011). Be sure to refer to the section on genetic engineering for a discussion of the limitations of gene mutations.

Selective brain lesioning can also be used to induce a pattern of behavior and neurochemical change that mimics significant components of psychiatric syndromes. In one model of schizophrenia developed in the early 1990s, neonatal mice are subjected to ventral hippocampal lesioning, which alters the developmental trajectory of the animals. Although their behaviors are normal early in life, at rodent pubescence, behaviors and physiological changes emerge that resemble multiple symptoms of schizophrenia. This model is appealing because schizophrenia is considered a neurodevelopmental disorder whose diagnostic symptoms most often begin in adolescence and early adulthood. Similar

to patients with schizophrenia, these lesioned rats show limbic dopamine dysregulation, hyperresponsiveness to stress and amphetamine injection, impaired functional development of the prefrontal cortex, and other abnormalities. This model is providing a template to study the impact of genetic, environmental, and neurobiological factors contributing to the pathophysiology of schizophrenia. The model was reviewed in detail and evaluated by Tseng and colleagues (2009) and is discussed in **Web Box 19.4**.

Another approach to improving translational research is to make the results from animal studies better predictors of therapeutic efficacy by developing parallel methodologies. To this end, the National Institute of Mental Health (NIMH) has funded a series of conferences for interdisciplinary researchers focused on cognitive processes, because cognitive dysfunction, including deficits in attention, memory, and behavioral regulation, is central to many psychiatric disorders. Researchers first worked to identify the most important cognitive processes that should be tested, and then they developed testing methods for human participants in parallel with animal models (Barch et al., 2009). Among the tasks that evaluate cognitive function that have been adapted for use with humans, non-human primates, and rodents is the **stop-signal task**. This is a commonly employed procedure used to measure impulsivity. Impulsivity or failure of behavioral inhibition is a significant component of disorders such as attention deficit hyperactivity disorder (ADHD), OCD, and substance abuse, among others. Impulsivity represents failure of an important cortical control mechanism that suppresses the execution of responses that are inappropriate in a changing environment, hence decreasing flexible behaviors. The stop-signal task requires the subject (human or otherwise) to rapidly press one button or lever when a square is displayed, and the other button or lever when any other shape appears. Periodically, a tone, which is the "stop" signal, is sounded following the visual presentation. The tone indicates that the subject should withhold responding. This paradigm can be used to integrate neurochemical contributions to the task performance derived from rodent pharmacological studies with imaging results in humans performing the same tasks. It is anticipated that the synthesis of discoveries will translate into more effective treatments for enhancing behavioral inhibition. For a more complete discussion, refer to the paper by Eagle et al. (2008). The translational technique called prepulse inhibition of startle is another example of a task that is performed in the same manner across species and has been used to examine the sensory processing of schizophrenic individuals (see Chapter 19 and **Web Box 19.2**).

Section Summary

- Techniques in behavioral pharmacology provide a means of quantifying animal behavior for drug testing, developing models for psychiatric disorders, and evaluating the neurochemical basis of behavior. Creating animal models of complex psychiatric disorders with uniquely human symptoms poses numerous hurtles.
- Advantages of animal testing include having a subject population with similar genetic background and history, maintaining highly controlled living environments, and being able to use invasive neurobiological techniques.
- Animal testing includes a wide range of measures that vary not only in validity and reliability, but also in complexity, time needed for completion, and cost.
- Some measures use simple quantitative observation of behaviors such as motor activity, response to noxious stimuli, and time spent in social interaction.
- Operant behaviors controlled by various schedules of reinforcement provide a sensitive measure of drug effects on patterns of behavior. Operant behaviors are also used in tests of addiction potential, anxiety, and analgesia.
- Other methods assess more complex behaviors such as learning and memory, using a variety of techniques such as the classic T-maze, as well as mazes modified to target spatial learning: the radial arm maze and Morris water maze. The delayed-response task assesses working memory.
- Many measures of anxiety use unconditioned animal reactions such as the tendency to avoid brightly lit places (light–dark crossing task, open field test), heights (elevated plus-maze, zero maze), electric shock (Vogel test), and novelty (novelty suppressed feeding).
- To assess fear a classically conditioned response is established by presenting a light or tone followed by an unavoidable foot shock. The light or tone becomes a cue associated with shock, and when presented alone, it produces physiological and behavioral responses of fearfulness. When the conditioned fear stimulus precedes a startle-producing stimulus, the startle is much greater.
- Common measures of depressive behaviors such as the forced swim test, tail suspension test, and learned helplessness utilize acute stress to create a sense of helplessness but have been criticized for responding to acute rather than chronic antidepressant treatment. Other models

of depression use more prolonged stress (chronic mild unpredictable stress, chronic social defeat stress), which more closely resembles the human experience. These stress-induced depressive behaviors respond to chronic but not acute drug treatment. Early maternal separation models the impact of stress early in life on later biobehavioral outcomes.

- The sucrose preference test is a measure of anhedonia (i.e., loss of interest in normally reinforcing stimuli).
- The operant self-administration technique, in which animals lever press for drugs rather than food reward, is an accurate predictor of abuse potential in humans. Varying the schedule of reinforcement indicates how reinforcing a given drug is, because when the effort of lever pressing exceeds the reinforcement value, the animals fail to press further (the "breaking point"). Drug self-administration can also be used to study factors leading to relapse following extinction of the drug-taking response.
- In conditioned place preference, animals learn to associate a drug injection with one of two distinct compartments, and saline with the other. On test day, if the drug is rewarding, the animal spends more time in the environment associated with the drug. If aversive, the animal stays in the saline-associated environment.
- Drug effects act as discriminative stimuli in operant tasks, which means that the lever-pressing response of an animal depends on its recognizing internal cues produced by the drug. Novel drugs can be characterized by how similar their internal cues are to those of the known drug.
- Translational research is the interdisciplinary approach to improving the transfer of discoveries from molecular neuroscience, animal behavioral analysis, and clinical trials, with the goal of more quickly and inexpensively developing useful therapeutic drugs.
- To make animal research more predictive of therapeutic benefits in humans, animal models that create abnormal behaviors or neurobiological changes that closely resemble the pathophysiology and focal symptoms of the disorder are needed. Genetic manipulations and brain lesions are two approaches for achieving this goal.
- Developing parallel tasks for humans, non-human primates, and rodents will allow integration of neurochemical data from rodent studies with findings of imaging studies in humans performing the same task.

Techniques in Neuropharmacology

Multiple Neurobiological Techniques for Assessing the CNS

Answering questions in psychopharmacology requires expertise not only in the techniques of behavioral analysis, but also in methods that allow us to examine the anatomy, physiology, and neurochemistry of the brain. The most productive research will utilize multiple approaches to the same problem.

Stereotaxic surgery is needed for accurate in vivo measures of brain function

The classic techniques of physiological psychology (lesioning, microinjection, and electrical recording) are equally important in understanding the actions of psychoactive drugs. Stereotaxic surgery is an essential technique in neuroscience that permits a researcher to implant one of several devices into the brain of an anesthetized animal with significant precision. The stereotaxic device itself (**FIGURE 4.12A**) essentially serves as a means of stabilizing the animal's head in a fixed orientation so that the carrier portion can be moved precisely in three dimensions for placement of the tip of an electrode or a drug delivery tube in a predetermined brain site. Brain site coordinates are calculated using a brain atlas, which is a collection of frontal sections of brain of appropriate species, in which distances are measured from skull surface features (**FIGURE 4.12B**). Accuracy of placement is determined histologically after the experiment is complete. The halo bracket (**FIGURE 4.12C**) is the equivalent apparatus used in human neurosurgery; the target site is identified with the use of a computerized imaging technique such as magnetic resonance imaging (MRI) or computerized tomography (CT) (see the section on imaging techniques later in this chapter).

LESIONING AND MICROINJECTION Experimental ablation, or **lesioning**, uses a stereotaxic device to position a delicate electrode, insulated along its length except for the exposed tip, deep within the brain. The tissue at the tip is destroyed when a very-high-frequency radio current is passed through the electrode to heat the cells. The rationale of the experiment is that a comparison of the animal's behavior before and after the lesion will tell us something about the function of that brain area. Electrolytic lesion studies in animals can be used by cognitive neuroscientists to model human functions altered by injury to specific brain areas.

Electrolytic lesions destroy all tissue at the tip of the electrode, including cell bodies, dendrites, and axons. Alternatively, a **neurotoxin** (a chemical that is

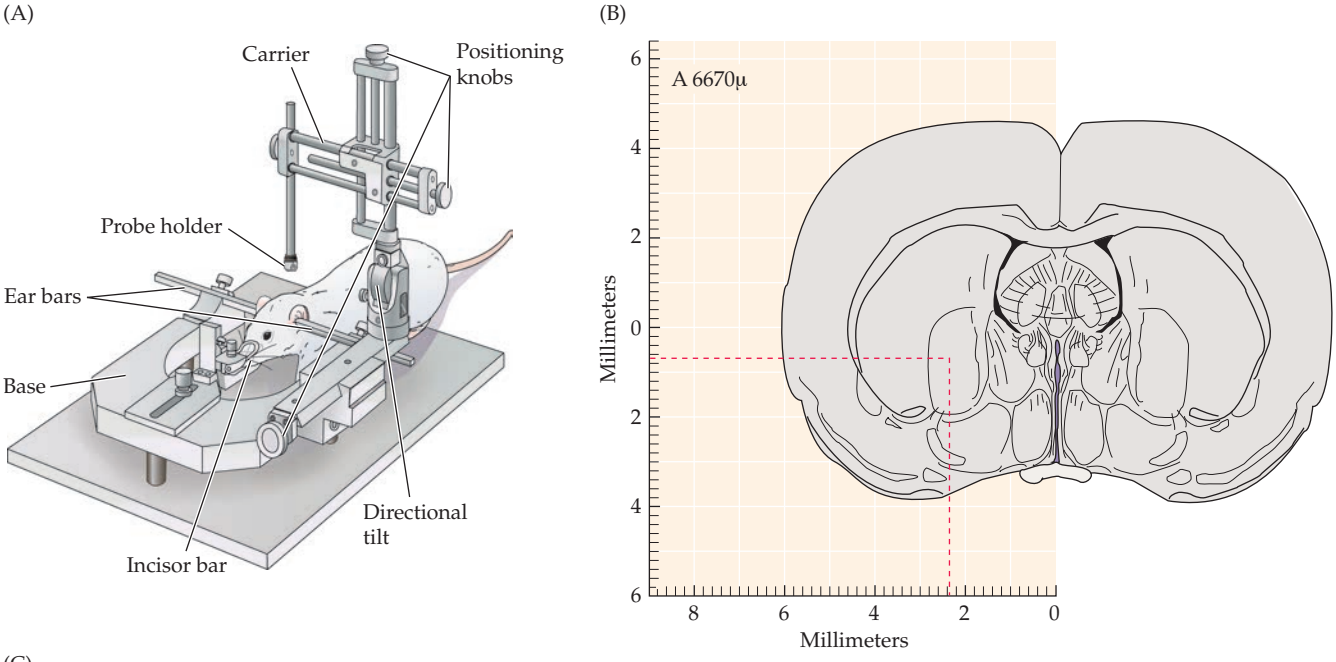


FIGURE 4.12 Stereotaxic surgery (A) A stereotaxic device used for precise placement of electrodes during brain surgery on animals. The base holds the anesthetized animal's head and neck in a stationary position. The carrier portion places the electrode or the cannula in a precise location based on the coordinates of the target area identified with the brain atlas (B). The precise target within the brain is defined by the intersection of three planes. The measurement (A 6670μ) in the upper left corner indicates the anterior–posterior position of the brain slice. The lateral and dorsal–ventral dimensions can be read directly from the axes provided. (C) A similar apparatus is used for human brain surgery. The location of the procedure is determined by CT or MRI.

damaging to nerve cells) can be injected via a cannula (a hollow tube inserted like an electrode) to destroy cells. Of course, the same type of cannula can be used to administer drugs or neurotransmitters that stimulate cells in the CNS before behavior is evaluated (see the discussion of intracerebroventricular administration in Chapter 1). Chemical lesions have the advantage of being significantly more specific because neurotoxic chemicals, such as kainic acid or ibotenic acid, kill cell bodies in the vicinity of the cannula tip but spare the axons passing through the same area. In either case, this procedure can be used to identify the brain area responsible for a drug-induced change in behavior.

Because neuropharmacologists are interested in neurochemical regulation of behavior, the lesioning techniques used are often specific for different neural pathways that use particular neurotransmitters. These **specific neurotoxins** most often are injected directly into the brain, where they are taken up by the normal reuptake mechanism of neurons. Once inside the cell, the toxin destroys the cell terminal. In this way, behavioral measures obtained before and after a neurotoxic lesion tell us about the role of the neurotransmitter in a particular behavior. For example, intracerebroventricular administration of 6-hydroxydopamine (6-OHDA) produces nerve terminal degeneration in both noradrenergic and dopaminergic cells and profound neurotransmitter depletion. More selective effects are achieved when the neurotoxin is injected directly into a target area. For example, we might test our understanding of the role of the nucleus accumbens in reinforcement by selectively destroying the large number of dopamine cell terminals in that area, using the neurotoxin

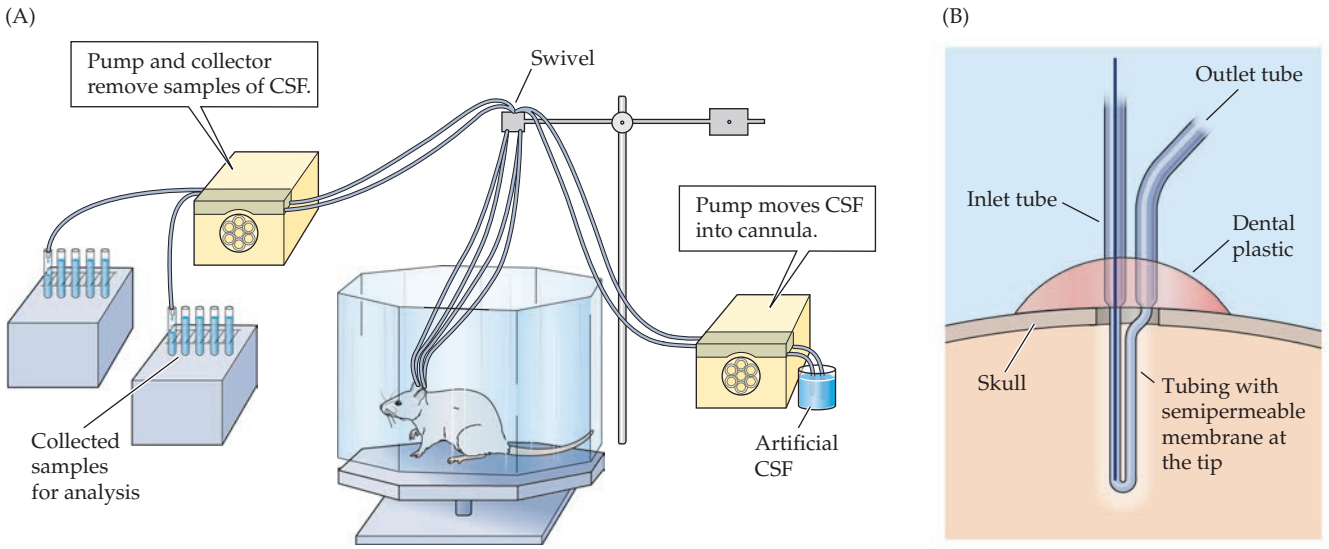


FIGURE 4.13 Collection of extracellular fluid with microdialysis (A) Microdialysis allows the collection of samples from deep within the brain in unanesthetized and freely moving animals under relatively normal conditions. The collected samples are identified and are measured by one of several analytic techniques, such as high-performance liquid chromatography (HPLC). (B) Typical microdialysis probe, which uses flexible tubing that is sealed except at the tip, where it is semipermeable. It is held in place by dental plastic on the animal's skull. CSF, cerebrospinal fluid. (A after Philippu, 1984; B after Ungerstedt, 1984.)

6-OHDA, before evaluating the drug-taking behavior in the self-administration paradigm.

MICRODIALYSIS A different technique that uses stereotaxic surgery is **microdialysis**. Although researchers have been able to measure neurotransmitters released from brain slices in vitro for many years, microdialysis lets us measure neurotransmitters released in a specific brain region while the subject is actively engaged in behavior (**FIGURE 4.13A**).

The technique requires a specialized cannula made of fine, flexible tubing that is implanted stereotaxically (**FIGURE 4.13B**). The cannula is sealed along its length except at the tip, allowing investigators to collect material in extracellular fluid at nerve terminals at precise sites even deep within the brain. Artificial cerebrospinal fluid (CSF) is gently moved into the microdialysis cannula by a pump. The CSF in the cannula and in the extracellular fluid are identical except for the material to be collected. On the basis of the difference in concentration, the chemicals of interest move across the membrane from the synaptic space into the cannula. A second pump removes the CSF from the cannula into a series of tubes to be analyzed by high-performance liquid chromatography (HPLC) or another method.

A major improvement over older collection methods is that only tiny amounts of material need to be collected for accurate measurement. This improved accuracy is due to the development of highly sensitive analytic techniques (such as HPLC), which can be combined with microdialysis collection. HPLC, like other types of chromatography, serves two purposes. First, chromatography separates the sample into component parts depending on characteristics of the sample, such as molecular size or ionic charge. Second, the concentration of the molecules of interest can be determined (**FIGURE 4.14**).

Microdialysis is important to neuropsychopharmacology because it can be used in several types of experiments that combine biochemical and behavioral analyses. For example, we might evaluate the released neurochemicals during ongoing behaviors, such as sleep and waking, feeding, or operant tasks, to obtain a window into the functioning CNS. We might also investigate the effects of drugs on extracellular concentrations of neurotransmitters in selected brain areas. Since the sample can be collected from freely moving animals, correlated changes in behavior can be monitored simultaneously. Finally, we might collect extracellular materials at nerve terminals following discrete electrical or chemical stimulation of neural pathways.

A second method used to measure neurotransmitter release is **in vivo voltammetry**. Whereas microdialysis collects samples of extracellular fluid for subsequent analysis, in vivo voltammetry uses stereotaxically implanted microelectrodes to measure neurochemicals in the extracellular fluid of freely moving animals. In voltammetry, a very fine electrode is implanted, and a small electrical potential is applied. Changes in the flow of current at the electrode tip reflect changes in the concentration of electroactive substances such as neurotransmitters or their metabolites. A major advantage of this method is that because measurements are made continuously and require as little as 15 ms to complete,

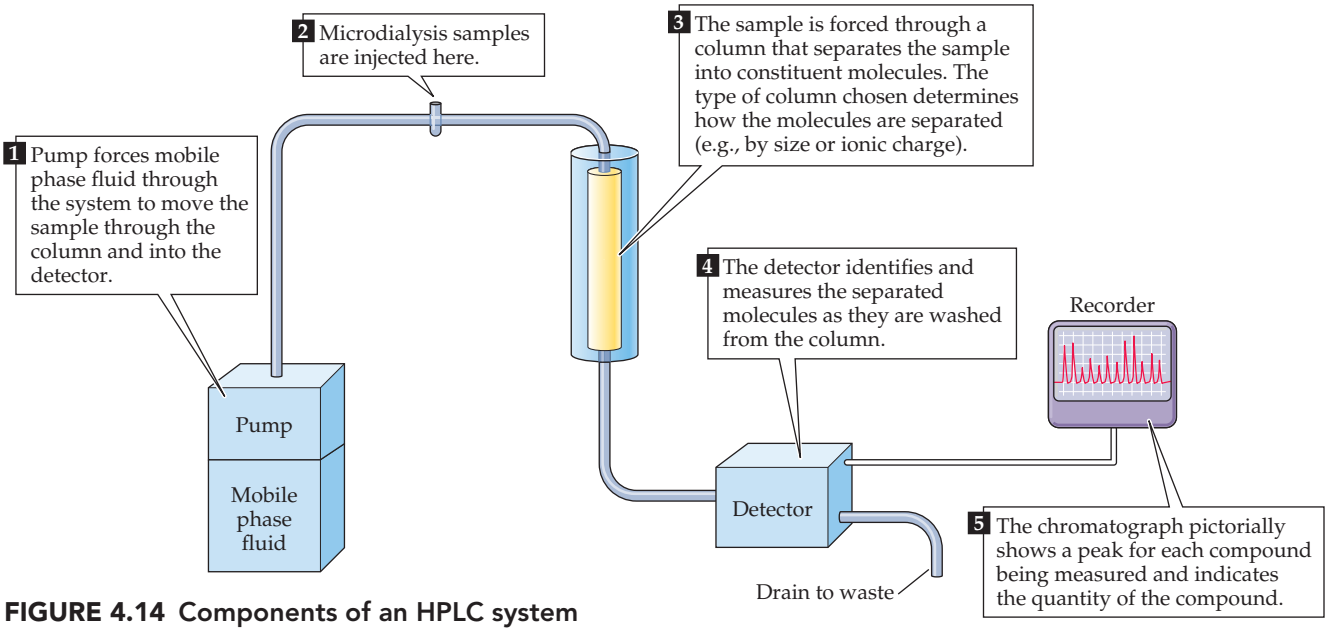


FIGURE 4.14 Components of an HPLC system

researchers can evaluate neurotransmitter release as it is occurring in real time.

ELECTROPHYSIOLOGICAL STIMULATION AND RECORDING In a similar fashion, implanted **macroelectrodes** (**FIGURE 4.15A**) can be used to activate cells at the tip while the change in animal behavior during stimulation is evaluated. The minute amount of electrical current applied changes the membrane potential of those cells and generates action potentials. The action potentials in turn cause the release of neurotransmitter at the cell terminals to mimic normal synaptic transmission. Hence the electrical stimulation should produce biobehavioral effects that are similar to those seen upon injection of the natural neurotransmitter or neurotransmitter agonists into the brain. In addition, one would expect that stimulation of a given cell group would produce effects opposite those caused by a lesion at the same site. Macroelectrodes can also be used to record the summated electrical response of thousands of neurons in a specific brain region following drug treatment or

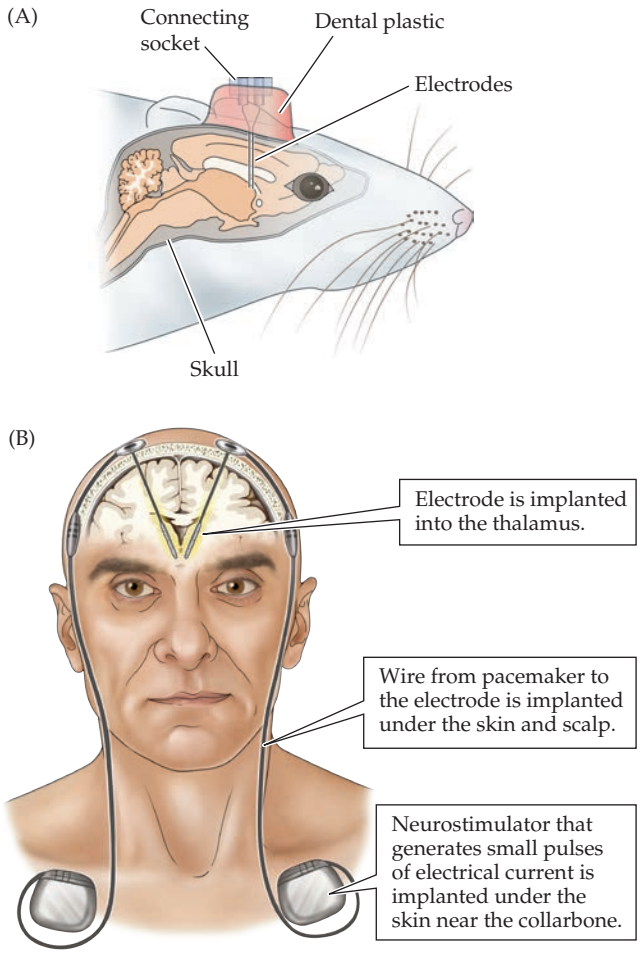


FIGURE 4.15 Electrical brain stimulation and recording (A) Stereotaxically implanted electrodes are held in place on the skull with dental plastic. After recovery, the animal can be plugged into a device that can electrically stimulate cells at the tip or monitor and record changes in electrical activity. (B) Diagram of a system used for tremor control, consisting of an insulated wire electrode surgically implanted deep in the brain. The electrode is connected to a pulse generator implanted under the skin near the collarbone. The generator is programmed to deliver the amount of electrical current needed to reduce the tremor on the opposite side of the body. Patients also can have individual control by passing a handheld magnet over the skin above the pulse generator.

other experimental manipulation in a freely moving animal. If we had found, for example, that lesioning the periaqueductal gray (PAG) in the midbrain prevented the pain-reducing effects of morphine, we might want to find out what effect activating those PAG neurons has. What we would find is that if electrodes implanted in the PAG are activated, the animal fails to respond to painful stimuli. Likewise, if pain-killing opioids like morphine or codeine are microinjected into that brain area via an indwelling cannula, the animal also demonstrates profound analgesia.

Many years of animal research into stereotaxic implantation of electrodes into the brain have translated into clinical benefits. **FIGURE 4.15B** shows the adaptation of the technique to humans being treated for Parkinson's disease. Small pulses of electrical current applied to the thalamus cause the cells to fire and release neurotransmitter, which reduces the tremor on the opposite side of the body.

An alternative to macroelectrode recording, which is a summation of electrical activity in a brain region, is single-unit recording, which uses **microelectrodes**. Stereotaxically implanting a fine-tipped electrode either into a single cell (**intracellular recording**) or into the extracellular fluid near a single cell (**extracellular recording**) allows the response of individual cells under various conditions to be monitored. Intracellular recording must involve an anesthetized animal, because the electrode must remain in a precise position to record the membrane potential of the cell. An advantage of extracellular single-cell recording is that it can be done in a mobile animal (**FIGURE 4.16**). The downside to extracellular recording is that the electrode records only the occurrence of action potentials in the nearby neuron and cannot monitor the change in the cell's membrane potential. Returning to our earlier example of morphine action, we find that the drug produces strong selective inhibition of neurons in the spinal cord, and this prevents the projection of pain information to higher brain centers, thereby contributing to the analgesic effect.

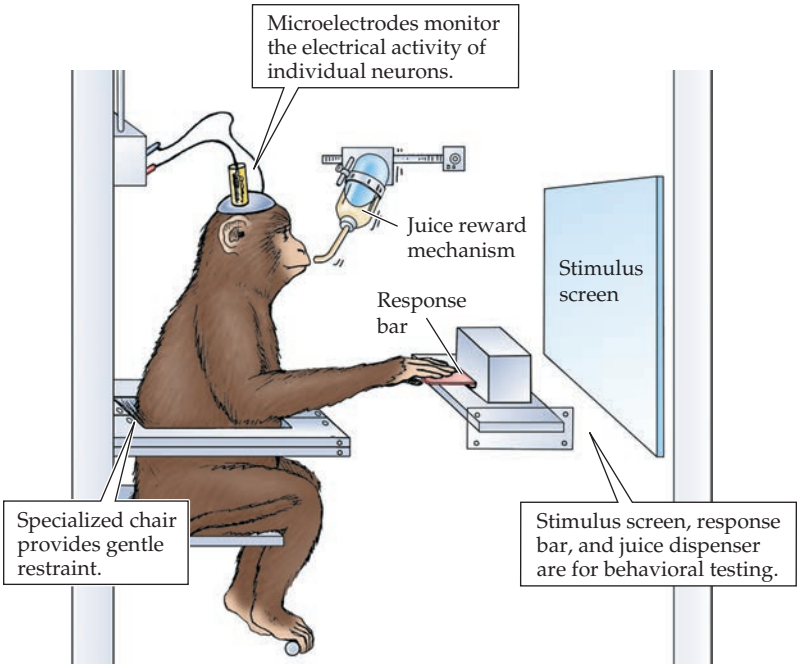


FIGURE 4.16 Extracellular microelectrode recording This electrophysiological technique records the electrical activity from single neurons in the brain of an awake, responding animal. This experimental setup might be used to evaluate the effects of a previously administered drug on the animal's response to visual stimuli and on the electrical activity of a single cell.

In addition to measuring membrane potentials of groups of cells and single cells, thanks to the Nobel Prize–winning research of Neher and Sakmann conducted in the 1970s, neuroscientists can study the function of *individual* ion channels, which collectively are responsible for the membrane potential. This technique, known as **patch clamp electrophysiology**, works best with individual cells in culture but can also be used on exposed cells in slices of brain. The method involves attaching a recording micropipette to a piece of cell membrane by suction. When the pipette is pulled away, a small membrane patch containing one or more ion channels remains attached. The subsequent electrical recording through the pipette represents in real time the channel opening, the flow of ions (electrical current) during the brief period when it is open, and the channel closing.

Neurotransmitters, receptors, and other proteins can be quantified and visually located in the CNS

To both quantify and locate neurotransmitters and receptors in the CNS, several methods are required. To count or measure a particular molecule, a "soup" method is often used, in which a tissue sample is precisely dissected out and ground up, creating a homogenate before it is evaluated. Homogenates are used in many possible neurochemical analyses, which are referred to as *assays*. In contrast, for localization, the landmarks of the tissue and the relationships of structures must be preserved, so the visualization method is done on an intact piece or slice of tissue. Hence, when we want to measure the number of receptors in a particular brain area, we are likely to use a radioligand binding assay in a tissue homogenate, but if we want to see where in the brain particular receptors are located (and measure them), we are more likely to use a slice preparation with

TABLE **4.2** Methods Used to Quantify and Visualize Target Molecules in the Nervous System

Target molecule	Tissue extract assay to quantify	Brain slice preparation to visualize
Receptor site	Radioligand binding	Receptor autoradiography
Receptors and other proteins	Radioimmunoassay (RIA); Western blot; ELISA	Immunocytochemistry (ICC)
mRNA	Dot blot or Northern blot	In situ hybridization (ISH)

autoradiography. **TABLE 4.2** summarizes the "soup" and "slice" techniques described in the following section of the chapter.

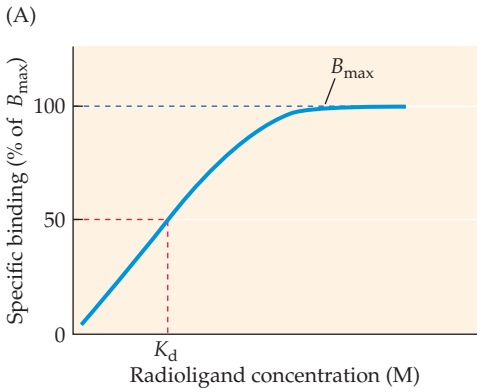
RADIOLIGAND BINDING To study the number of receptors in a given brain region and their affinity for drugs, the **radioligand binding** method was developed. Once the brain region we are interested in is dissected out, it is ground up to make a homogenate. A ligand (usually a drug or chemical) that is radioactively labeled (now called the *radioligand*) is incubated with the tissue under conditions that optimize its binding. After a brief time, any radioligand that has not bound is removed, often by washing and filtering. The amount of radioligand bound to the tissue is then measured with a scintillation (or gamma) counter and reflects the number of receptors in the tissue.

Although the binding procedure is quite simple, interpretation of the results is more complex. How can we be sure that the radioligand is actually binding to the specific biological receptors of interest, rather than to other sites that represent artifacts of the procedure? Several criteria that must be met include (1) specificity, (2) saturability, (3) reversibility and high affinity, and (4) biological relevance. Specificity means that the ligand is binding only to the receptor we are concerned with in this tissue, and to nothing else. Of course, drugs often bind to several receptor subtypes, but they may also attach to other cell components that produce no biological effects. To measure the amount of a ligand that binds to the site that we are concerned with, we add very high concentrations of a nonradioactive competing ligand to some tubes to show that most of the radioactive binding is displaced. That which remains is likely to be nonspecifically bound to sites such as assay additives (e.g., albumin) or cellular sites (e.g., enzymes), which we are less interested in at the moment. Nonspecific binding is subtracted when the data are calculated for specific binding. When binding to specific subtypes of receptors is necessary, ligands must be designed to distinguish between receptor proteins.

Saturability means that there are a finite number of receptors in a given amount of tissue. By adding increasing amounts of radioligand to a fixed amount of tissue, one would expect to see gradual increases in binding until all sites are filled (**FIGURE 4.17A**). The point at which the binding curve plateaus represents maximum binding, or B_{max}. Binding within the assay must also be reversible because a neurotransmitter in vivo will bind and release many times to initiate repeated activation of the cellular action. This reversibility is demonstrated in binding assays because the radioactive ligand can be displaced by the same drug that is not radiolabeled (**FIGURE 4.17B**). If you compare the rate of dissociation with the rate of binding, you get an estimate of receptor affinity called the dissociation constant or K_d. Clearly those ligands that bind readily and dissociate slowly have the highest affinity for the receptor. The unbinding (dissociation) of the ligand from the receptor must also be consistent with the reversal of physiological effects of the ligand.

Ideally, binding of chemically similar drugs should correlate with some measurable biochemical or behavioral effect to show the biological relevance of the receptor. For example, the classic antipsychotic drugs all bind to a particular subtype of receptor (D_2) for the neurotransmitter dopamine. Not only do the drugs in this class bind to the D_2 receptor, but their affinity for the receptor correlates with the effectiveness of the drugs in reducing the symptoms of schizophrenia (see Chapter 19). Unfortunately, in vitro binding data does not always transfer to in vivo results, because in vitro receptors are not in their natural environment. Also, drug effects in the intact organism are dependent on many factors in addition to drug–receptor interaction, for example, absorption and distribution. The use of in vivo receptor binding (see the section below) can help to account for absorption, distribution, and metabolism of the drug.

RECEPTOR AUTORADIOGRAPHY Receptor binding is a classic tool in neuropharmacology that tells us about receptor number and affinity for a particular drug in a specific piece of brain tissue. When we want to visualize the distribution of receptors within the brain, we may use receptor autoradiography. The process begins with standard radioligand binding as described previously, except that slide-mounted tissue slices rather than ground-up tissue are used. After the unbound radioactively labeled drug is washed away, the slices are processed by **autoradiography**. The slides are put into cassettes, a specialized autoradiographic film is placed on top of the slides so that it is in physical contact with the tissue sections, and the cassettes are stored in the



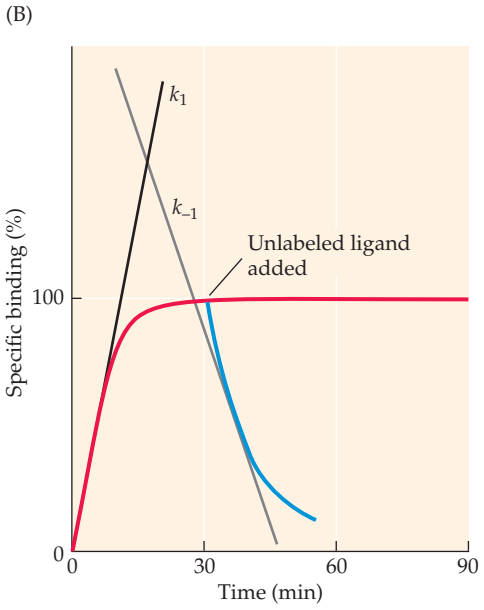


FIGURE 4.17 Radioligand binding to receptors (A) A hypothetical saturation curve shows that as radioligand concentration increases, specific binding to the receptor also increases until all sites are filled (B_{max}). The K_d is defined as the ligand concentration at which 50% of the receptors are occupied; it is an indication of receptor affinity and is called the dissociation constant. (B) Hypothetical association and dissociation curves. The red line represents the association of a radioligand with its receptors over time. The rate of association (k_1) is estimated by calculating the slope of the straight line that best fits the curvilinear data. After maximum binding has occurred (association and dissociation are in equilibrium), excess unlabeled ligand is added. The blue line represents the dissociation of the radioligand from its receptors in the presence of large amounts of unlabeled ligand. The slope of the straight line that best fits that portion of the curve provides an estimate of the rate of dissociation (k_{-1}).

dark to allow the radioactive material that is bound to receptors to act on the film. The particles that are constantly emitted from the radioactive material in the tissue expose the film and show not only the amount of radioligand bound but also its location. This method is especially good for studying the effects of brain lesions on receptor binding because each lesioned animal can be evaluated independently by comparing the lesioned and nonlesioned sides of the brain. This method might give us clues about how various psychoactive drugs produce their behavioral effects. For instance, mapping of the binding of cocaine in monkey brain shows a distinct pattern of localization and density in selected brain areas (**FIGURE 4.18**). With a clear understanding of anatomical distribution, we can begin to test specific hypotheses regarding the behavioral consequences of activating these receptors, using microinjections of receptor-selective agonists and antagonists.

IN VIVO RECEPTOR BINDING The same autoradiographic processing can be done on brain slices of an animal that had previously been injected in vivo with a radiolabeled drug. The drug enters the general circulation, diffuses into the brain, and binds to receptors. Then the animal is killed, and the brain is sliced and processed by autoradiography. This technique shows the researcher where a particular drug or neurotransmitter binds in an intact animal.

ASSAYS OF ENZYME ACTIVITY Enzymes are proteins that act as biological catalysts to speed up reaction rates but are not used up in the process. We find many different enzymes in every cell, and each has a role in a relatively specific reaction. The enzymes that are particularly interesting to neuropharmacologists are those involved in the synthesis or metabolism of neurotransmitters, neuromodulators, and second messengers. In addition, neuropharmacologists are interested in identifying the conditions that regulate the rate of activity of the enzyme. For example, acute morphine treatment inhibits adenylyl cyclase activity. Adenylyl cyclase is the enzyme that synthesizes the second messenger cyclic

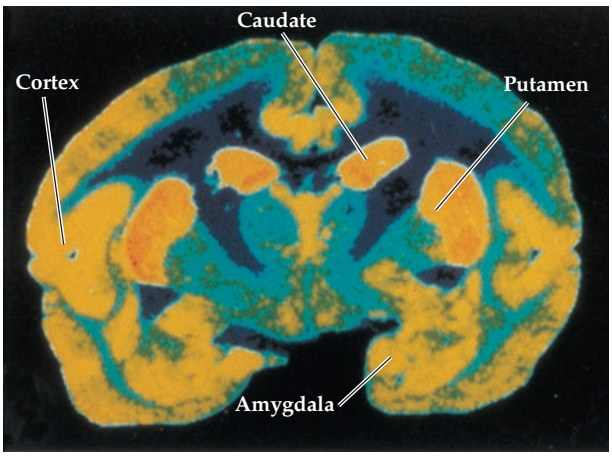
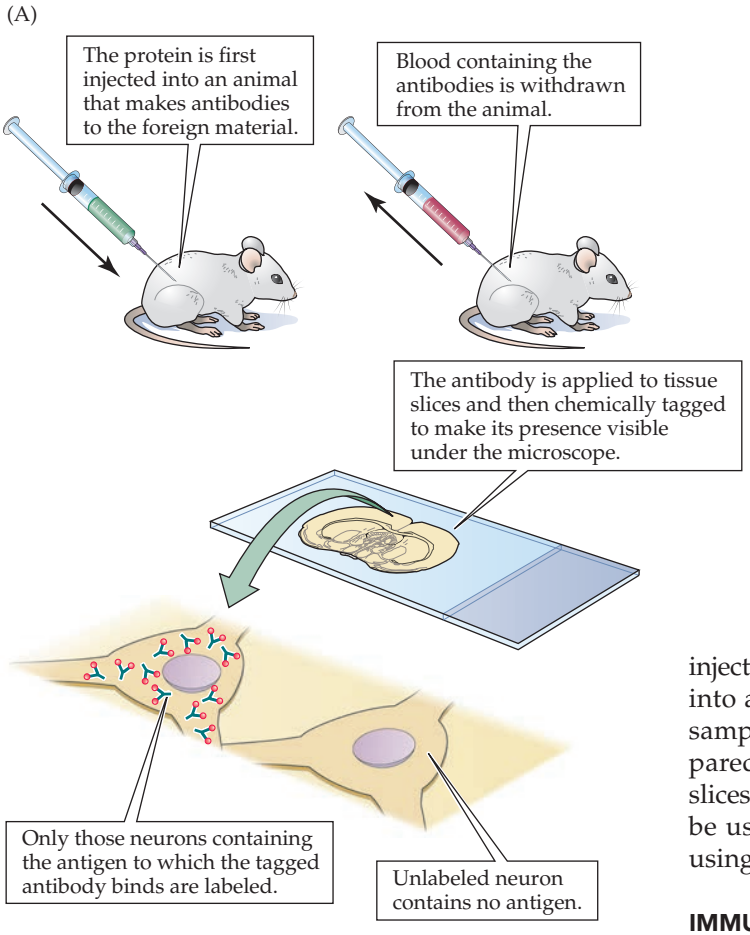


FIGURE 4.18 Autoradiogram of the distribution of cocaine binding in monkey brain The highest levels of cocaine binding are in areas colored yellow and orange. (Courtesy of Bertha Madras and Marc J. Kaufman.)



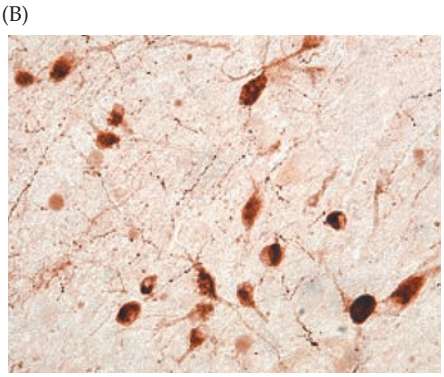


FIGURE 4.19 Immunocytochemistry (ICC) uses tagged antibodies to locate molecules within cells (A) Steps in ICC localization. (B) Immunocytochemical identification of cells containing the neuropeptide hypocretin in the human lateral hypothalamus. (A after Bear et al., 2001; B courtesy of Jerome Siegel.)

adenosine monophosphate (cAMP). However, long-term exposure to morphine produces gradual but dramatic up-regulation of the cAMP system, suggesting that the second-messenger system acts to compensate for the acute effect of opioid inhibition. It is perhaps one of the best-studied biochemical models of opioid tolerance and is discussed further in Chapter 11.

Sometimes the mere presence of an enzyme within a cell cluster is important because it can be used to identify those cells that manufacture a specific neurotransmitter. The next section describes the use of antibodies and immunocytochemistry to locate enzymes and other proteins in the brain.

ANTIBODY PRODUCTION Some of the newest methods for identifying and measuring receptors and other proteins are far more specific and sensitive than ever before because they use an antibody. An **antibody** is a protein produced by the white blood cells of the immune system to recognize, attack, and destroy a specific foreign substance (the antigen). Researchers use this immune response to create supplies of antibodies that bind to specific proteins (receptors, neuropeptides, or enzymes) that they want to locate in the brain (**FIGURE 4.19A**). The first step is to create an antibody by injecting the antigen (e.g., the neuropeptide hypocretin) into a host animal and at various times taking blood samples to collect antibodies. With the antibody prepared, we are ready to look for the peptide in tissue slices using immunocytochemistry. Antibodies can also be used to quantify very small amounts of material using radioimmunoassays (see later in this chapter).

IMMUNOCYTOCHEMISTRY For **immunocytochemistry** (**ICC**), the brain is first fixed (hardened) using a preservative such as formaldehyde. Tissue slices are then cut and incubated with the antibody in solution. The antibody attaches to the antigen wherever cells that contain that antigen are present. In the final step, the antibody is tagged so that the antigen-containing cells can be visualized (see Figure 4.19A). This is usually accomplished by means of a chemical reaction that creates a colored precipitate within the cells or with the use of a fluorescent dye that glows when exposed to light of a particular wavelength. The researcher can then examine the tissue slices under a microscope to see which brain areas or neurons contain the antigen. This technique is limited only by the ability to raise antibodies, so it is suitable for a wider range of protein than autoradiography. **FIGURE 4.19B** shows the visualization of cells that contain the neuropeptide hypocretin in the lateral hypothalamus of a healthy human. In patients with the sleep disorder narcolepsy, the number of hypocretin neurons is reduced by about 90% (Thannicakal et al., 2000). These results, along with animal experiments using neurotoxin lesioning and genetic modification, suggest that hypocretin in the hypothalamus may regulate the onset of sleep stages. ICC is similar to autoradiography in principle, but it is far more selective because the antibody (which recognizes

only a very specific protein) is used, and it is much quicker because it does not require the development time of autoradiographic film. A related technique called **Western blot** uses antibodies to detect specific proteins in a tissue rather than a slice. The proteins in the homogenate are separated out based on size, using a method called gel electrophoresis, and placed onto a blotting membrane. As is true for ICC, the membrane is incubated with the antibody of the protein of interest. Because the antibody–antigen interaction is so specific, the method can identify the target protein even in a complex mixture of proteins. After brief rinsing to remove unbound antibody, a second antibody is applied that binds to the first antibody and provides a chemical reaction to create a detectable product, either an insoluble colored dye that stains the membrane or a luminescence. These reaction products are formed in proportion to the amount of bound antibody and provide an accurate quantification of the protein of interest in the sample.

RADIOIMMUNOASSAY Antibodies are useful in quantifying physiologically important molecules in body fluids such as blood, saliva, or CSF, as well as in tissue extracts. **Radioimmunoassay** (**RIA**) is based on competitive binding of an antibody to its antigen (the molecule being measured). The use of antibodies makes the procedure highly specific for the molecule of interest and very sensitive (**FIGURE 4.20**).

RIA involves preparing a standard curve of known antigen concentrations against which unknown samples can be compared. The standard curve is created by first combining a preset amount of antibody with a known concentration of radioactively labeled antigen in all the assay tubes. At this point, all the tubes are identical, that is, all of the antibody would be reversibly attached to radioactive antigen. However, the experimenter then adds different, known concentrations of unlabeled antigen, which compete with the radioactively labeled antigen. The higher the concentration of unlabeled competitor antigen added, the lower the amount of bound radioactive antigen there will be after the mixture has been incubated. Values are plotted as a standard curve and analyzed using appropriate computer software.

To determine how much of the antigen is present in any experimental sample, other test tubes are prepared in just the same way, except that samples containing unknown amounts of antigen are added, instead of the known antigen used to create the standard curve. By measuring the amount of radioactive antigen bound in the sample tubes compared with the standard curve, the amount of antigen in the sample can be calculated.

Although the RIA was an extremely popular analytic technique following its development in 1960, there were several problems that made it costly and hazardous. Many laboratories felt compelled to build special facilities to protect investigators from the hazardous radioactivity (powerful beta and gamma radiation) needed to label the antigen. A second significant expense was the purchase of costly radioactivity-counting equipment. Finally, consideration of how to dispose of the radioactive waste became an increasing problem. Even though the radioisotopes typically used now in

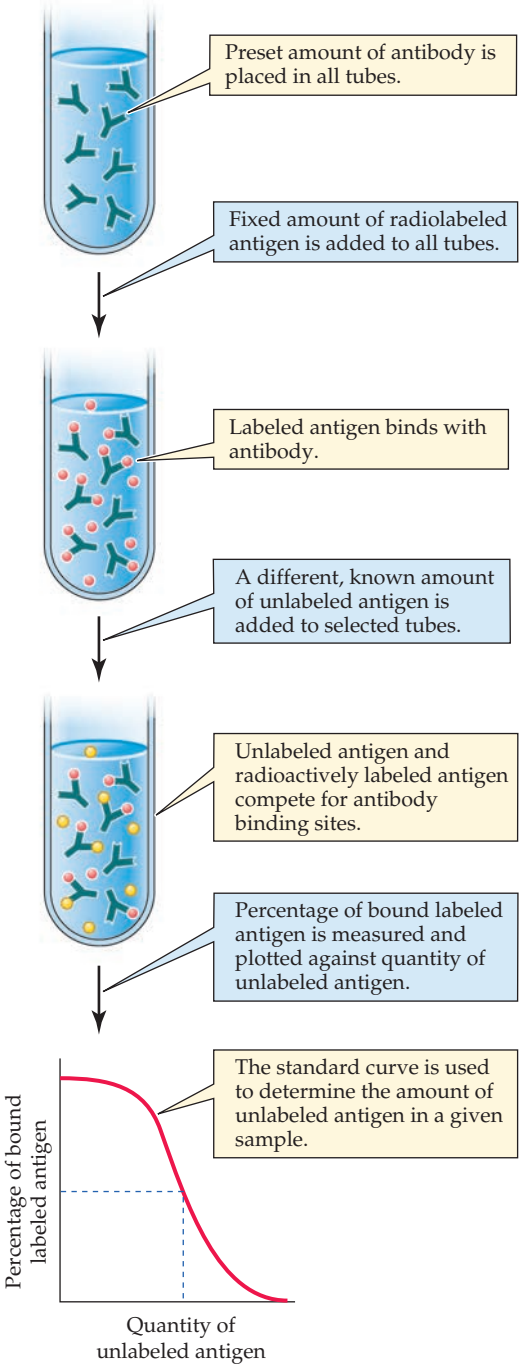


FIGURE 4.20 Radioimmunoassay (RIA) The steps in the RIA procedure that produce a typical standard curve. The curve in turn is used to calculate the amount of unknown antigen in a given sample.

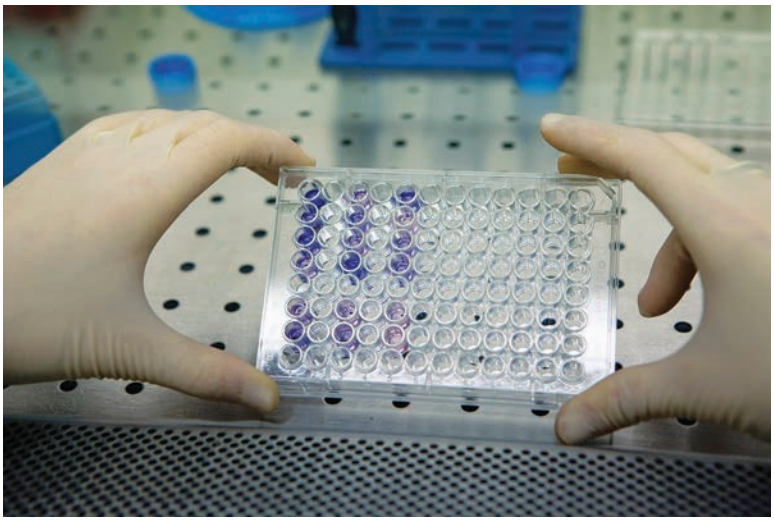


FIGURE 4.21 A sample 96-well ELISA plate A colored reaction product can be seen in some of the wells of the plate. Commercially available ELISA or EIA kits are available in a few different configurations. The most common type of ELISA is the competitive ELISA in which the greater the amount antigen present in a given well, the less colored product is produced. However, other formats exist in which there is a positive instead of an inverse relationship between antigen concentration and colored product formation. In either case, after the enzymatic reaction has been terminated, the optical density in each well (an index of light absorbance by the colored reaction product in the wells) is measured by a microplate reader, and the concentrations of antigen in the experimental samples are determined by means of a standard curve using known amounts of antigen provided by the manufacturer. (BSIP SA/Alamy Stock Photo.)

RIAs are much less dangerous than those used before, some of the disadvantages of this technique are still present. Because of the need to develop safer and more convenient alternatives to RIA, the **enzyme-linked immunosorbent assay** (**ELISA**) was developed in Sweden and the enzyme immunoassay (EIA) was developed in the Netherlands by two independent groups of researchers. Like the RIA both are immunoassays, but instead of using radioactivity for detection, they rely on an enzyme that acts on a substrate (i.e., starting material) to form a colored product. There are many modified protocols, but one of the most commonly found in commercial kits is the competition ELISA. Rather than using individual tubes or cuvettes as are used in the RIA, ELISA uses a plate with typically 96 wells (**FIGURE 4.21**) that immobilizes the reactants. For example, to measure the amount of a given substance (called the antigen, as in RIA) in a biological sample, the wells are first coated with a thin film of the antibody against the antigen. Standards (known quantities) of antigen (protein of interest) or test samples containing unknown amounts of antigen are then added to various wells, followed by antigen that has been linked to a reporting enzyme. The unlabeled antigen from samples or the known concentrations constituting the standard curve compete with the enzyme-linked antigen molecules for the limited number of antibodies in each well. After the unbound antigen is rinsed away, a colorless chemical substrate is added. The enzyme on any labeled antigen will turn the colorless substrate into a colored product. The product is measured in a microplate reader, and samples are compared with the color change in the known standards. In this assay, the amount of colored product at the end of the assay reaction is inversely related to the amount of the substance of interest (standard or unknown). Hence, the more substance is in the sample, the less color change will occur. Enzyme-linked immunoassays are quick and simple to perform, because they are designed to process a large number of samples in parallel. They are very popular methods to measure a wide variety of proteins, peptides, antibodies, and hormones.

IN SITU HYBRIDIZATION **In situ hybridization** (**ISH**) makes it possible to locate cells in tissue slices that are *manufacturing* a particular protein or peptide, in much the same manner that ICC identifies cells *containing* a particular protein. ISH is particularly useful in neuropharmacology for detecting the specific messenger RNA (mRNA) molecules responsible for directing the manufacture of the wide variety of proteins essential to neuron function, such as enzymes, structural proteins, receptors, ion channels, and peptides. For example, **FIGURE 4.22A** shows the location of the mRNA for enkephalin, one of several opioid peptides in the adult rat brain (see Chapter 11). Because the method detects cells with a precise RNA sequence, it is exceptionally specific and extremely sensitive. Besides locating cells that contain specific mRNA, ISH is used to study changes in regional mRNA levels after experimental manipulations. The amount of mRNA provides an estimate of the rate of synthesis of the particular protein. This means that if chronic drug treatment caused a decrease in enkephalin mRNA, we could conclude that the protein the mRNA codes for had been down-regulated, that is, less of that protein was being synthesized.

As you may recall from Chapter 2, the double strands of DNA and the corresponding mRNA (**FIGURE 4.22B,C**) have a unique base-pair sequence responsible for directing the synthesis of each particular protein with its unique amino acid sequence. ISH depends on the ability to create probes by labeling single-stranded fragments of RNA made up of base-pair sequences complementary to those of the mRNA of interest (**FIGURE 4.22D**). After the single strands have been

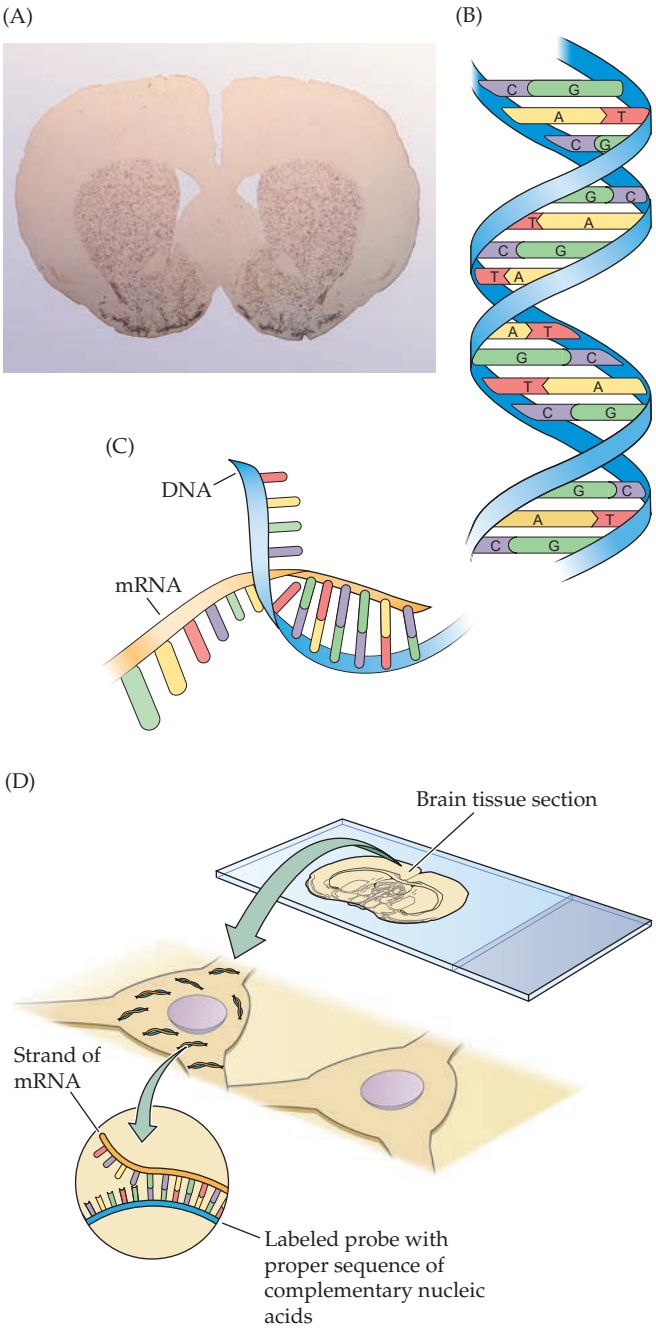


FIGURE 4.22 In situ hybridization (ISH)
(A) Localization of enkephalin mRNA in a slice from rat brain. (B) Structure of a DNA molecule. The nucleotide bases always bind in a complementary fashion: thymine to adenine, and guanine to cytosine. (C) A strand of mRNA has copied the code from a partially unraveled DNA molecule in the nucleus and will carry the genetic code to ribosomes in the cytoplasm, where the protein will be created. (D) In ISH, a labeled probe has been created with the correct sequence of complementary bases. When the strand of mRNA in the cell and the labeled probe hybridize, or bond to one another, the product will label the cell that contains the genetic code for the protein of interest. (A courtesy of Brian Sauer and Suzanne Pham.)

prepared, they are labeled radioactively or with dyes. When the tissue slices or cells are exposed to the labeled probe, the probe attaches (binds, or hybridizes) to the complementary base-pair sequences. After incubation, the tissue is washed and dehydrated before it is placed in contact with X-ray film or processed in other ways for visualization of cells containing the specific mRNA. This technique is extremely sensitive and can detect a very small number of cells that express a particular gene. If the researcher is interested only in measuring the amount of mRNA rather than visualizing its location, hybridization can be done using a tissue homogenate rather than a tissue slice. Two available methods of ISH that use homogenates are called Northern blot and dot blot.

DNA MICROARRAYS Microarrays, also called DNA chips or gene chips, provide the newest and most dramatic improvement in gene technology. Because the nervous system exhibits the greatest complexity of gene expression of all tissues, being able to examine all of the genes simultaneously can tell researchers which genes switch on and off together in response to a disease state, drug treatment, or environmental condition. One would assume that genes that increase or decrease their expression under the same condition probably work together to induce a cellular response. In addition, measuring the number of various RNAs in a sample tells us both the types and quantities of proteins present. A study by Mirnics and colleagues (2000) demonstrated the technical elegance of microarray by identifying multiple presynaptic proteins that are underexpressed in the frontal lobes of individuals with schizophrenia. Their results provide a predictive and testable model of the disorder.

The method is similar to that described for ISH, but rather than measuring a single mRNA, microarrays consist of between 1000 and 20,000 distinct complementary DNA sequences (spots) on a single chip (a structural support) of approximately thumbnail size. Each spot is only about 50 to 150 μm in diameter. This makes it possible to screen the expression of the entire genome of an organism in a single experiment on just a few chips. The tissue to be evaluated (e.g., the frontal lobe from an individual with schizophrenia compared with a frontal lobe from a healthy individual) is dissected, and the mRNAs are isolated and labeled, then hybridized to the large number of immobilized DNA molecules on the chip. A scanner automatically evaluates the extent of hybridization of each of the thousands of spots on the chip, and computer analysis is used to identify the patterns of gene activity. Several excellent reviews of the microarray procedure

and its application in areas such as aging, neuropharmacology, and psychiatric disorders are available (Luo and Geschwind, 2001; Marcotte et al. 2001).

A modification of the standard microarray technology spurred the development of **genome-wide association studies** (**GWAS**). This technique utilizes gene chips consisting of large sets of single-nucleotide polymorphisms (SNPs) (i.e., gene alleles that differ in only one nucleotide). DNA samples from people with a disorder and matched controls are assayed on the chips. If a particular allele is found more often in people with the disease, it is "associated" with the disorder. The method is data driven rather than hypothesis driven, meaning that researchers do not need to know anything about the function of the gene, although sometimes this makes the results difficult to interpret. Although originally limited to detecting common SNPs (i.e., those found in more than 5% of the population), it is now possible to identify rare SNPs, as well as more of the copy number variants (CNVs). CNVs are chromosomal abnormalities in which portions of a chromosome are duplicated, deleted, inverted, or translocated. The technology is highly accurate, relatively inexpensive, and very fast, and it uses powerful statistical methods that reduce the risk of finding false-positive associations between genotype and phenotype. For details on the history, rationale, and application of genome-wide association studies, see a paper by the Psychiatric GWAS Consortium Coordinating Committee (2009).

New tools are used for imaging the structure and function of the brain

Most conventional neurobiological techniques are designed to quantify or to localize significant substances in the nervous system. One of the greatest challenges in psychopharmacology has been to evaluate the functioning of the brain under various conditions, particularly in the living human being. Advances in technology not only make visualization of the CNS far more precise, but also provide the opportunity to visualize the functioning brain.

AUTORADIOGRAPHY OF DYNAMIC CELL PROCESSES

You are already familiar with the technique of autoradiography for mapping cell components such as neurotransmitter receptors that have been radioactively labeled. A second application of autoradiography is the tracing of active processes in the brain such as cerebral blood flow, oxygen consumption, local glucose utilization, or local rates of cerebral protein synthesis that indicate neural activity.

The technique called **2-deoxyglucose autoradiography** is based on the assumption that when nerve cell firing increases, the metabolic rate, that is, the utilization of glucose and oxygen, also increases. By identifying cells that take up more glucose, we can tell which brain regions are most active. A modified form of the glucose molecule, 2-deoxyglucose (2-DG) is taken up by active nerve cells but is not processed in the same manner as glucose and remains trapped in the cell. If the 2-DG has been labeled in some way, the most active cells can be identified. A similar (but nonlethal) technique can be performed with human participants using PET, as described in the next section.

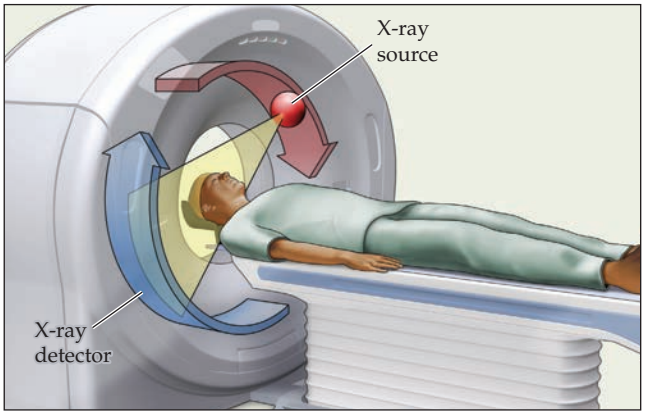
A second way of identifying which brain cells are active is to locate cells that show increases in nuclear proteins involved in protein synthesis. The assumption is that when cells are activated, selected proteins called transcription factors (such as **c-fos**) dramatically increase in concentration over 30 to 60 minutes. The c-fos protein subsequently activates the expression of other genes that regulate protein synthesis. In order to visualize which neurons are active, ICC is used to stain the cells that have increased levels of the fos protein.

IMAGING TECHNIQUES

Since our ultimate goal is to understand how drugs affect the human brain and behavior, the most exciting advance in recent years has been the ability to visualize the living human brain. Although we can learn a lot by studying individuals with brain damage, until recently we could only guess at where the damage was located, because the brain was not accessible until the individual died, often many years later. It was virtually impossible to know which specific brain area was responsible for the lost function. The human brain remained a bit of a "black box," and our understanding of the neural processes responsible for human thinking and behavior was advanced primarily through animal experiments. Because of recent advances in X-ray and computer technology, neuroscience now can not only safely visualize the detailed anatomy of the human brain, but also identify the neural processes responsible for a particular mental activity. CT and MRI are techniques that create pictures of the human nervous system that show far greater detail than was previously possible with standard X-ray. Other techniques are designed to see functional activity in the human brain. These include PET, functional MRI, and computer-assisted electrical recording (see the electroencephalography section below).

When standard X-rays are passed through the body, they are differentially absorbed depending on the density of the various tissues. Rays that are not absorbed strike a photographic plate, forming light and dark images. Unfortunately, the brain is made up of many overlapping parts that do not differ dramatically in their ability to absorb X-rays, so it is very difficult to distinguish the individual shapes of brain structures. **Computerized tomography** (**CT**) not only increases the resolution (sharpness of detail) of the image but also provides an image in three dimensions.

(A)



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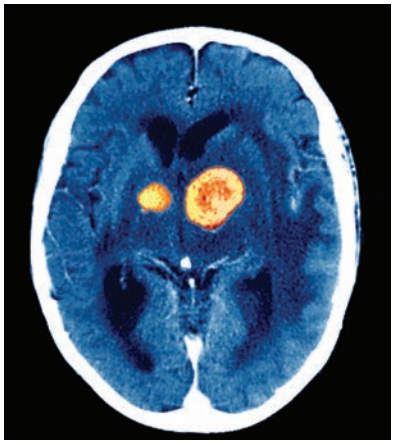


FIGURE 4.23 Computerized tomography (CT) (A) The cylindrical CT scanner rotates around the head, sending parallel X-ray beams through the tissue to be detected on the opposite side. A computerized image in the form of a brain slice is constructed from the data. (B) Horizontal CT scan showing a tumor (orange) at the level of the basal ganglia. Anterior is toward the top of the scan. (B © Zephyr/SPL/Science Source.)

The individual undergoing a CT scan (sometimes called CAT scan, for computerized axial tomography) lies with his head placed in a cylindrical X-ray tube (**FIGURE 4.23A**). A series of narrow, parallel beams of radiation are aimed through the tissue and toward the X-ray detectors. The X-ray source is rotated around the head while the detectors move on the opposite side in parallel. At each point of rotation, the source and detectors also move linearly. In this manner, they make a series of radiation transmission readings, which is calculated by a computer and visually displayed as a "slice" through the brain (**FIGURE 4.23B**). The slices can be reconstructed by the computer into three-dimensional images for a better understanding of brain structure.

Magnetic resonance imaging (**MRI**) further refines the ability to view the living brain by using computerized measurements of the distinct waves that different atoms emit when placed in a strong magnetic field and activated by radio-frequency waves. This method distinguishes different body tissues on the basis of their individual chemical composition. Because tissues contain different amounts of water, they can be distinguished by scanning the magnetically induced resonance of hydrogen. The image provides exquisite detail and, as is true for CT, sequential slices can be reconstructed to provide three-dimensional images (**FIGURE 4.24**).

Magnetic resonance spectroscopy (**MRS**) compliments MRI because it can use the MRI-generated data to calculate the concentration of brain chemicals as well as evaluating metabolic changes in individuals with Alzheimer's disease, Parkinson's disease, depression, epilepsy, and other conditions. Although MRI shows only structure, MRS is an important new tool for researchers and physicians to measure levels of specific molecules such as glutamate, choline, and neurotransmitter metabolites in discrete brain regions in living individuals. Although only a few molecules can be measured presently, the future expansion of the field is likely.

A further modification of MRI technology called **diffusion tensor imaging** (**DTI**) depends on the ability to scan the microscopic three-dimensional movement of water in neural tissue. While water molecules normally disperse in every direction, in axon bundles that are generally myelinated with fatty sheaths, movement is restricted and the water can diffuse only along the

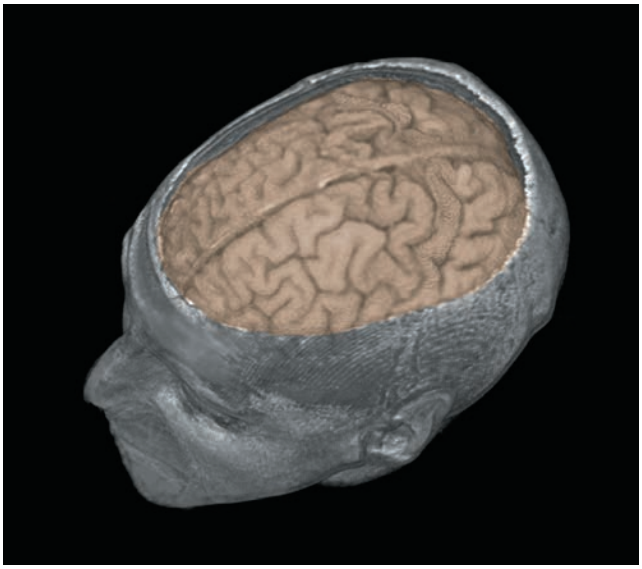
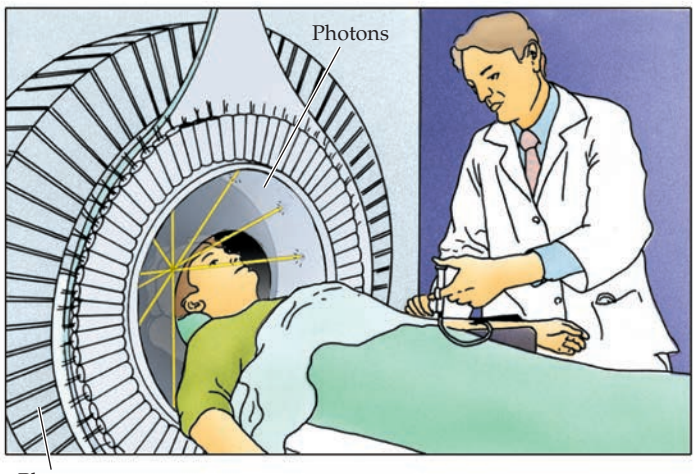


FIGURE 4.24 A three-dimensional image formed with MRI Computer technology provides the opportunity to create three-dimensional representations of the brain from sequential slices. (© S. Mark Williams, Pyramis Studios, and Leonard E. White and James Voyvodic, Duke University).

(A)



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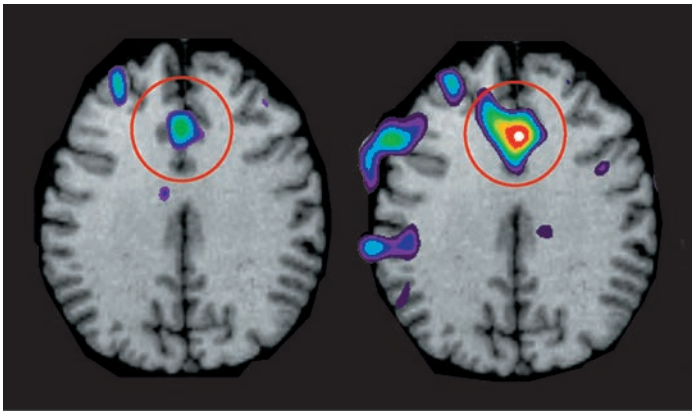


FIGURE 4.25 Positron emission tomography (PET) (A) A typical scanning device for PET. Notice the photodetectors that surround the head to track the gamma photons produced when a positron expelled from the nucleus collides with an electron. (B) PET scan image showing active brain areas by measuring regional cerebral blood flow under two conditions. The person on the left, who was told to expect only mild discomfort from putting a hand into 47°C (116.6°F) water, showed less neuronal activity (correlated with less blood flow) in the anterior cingulate cortex than the person on the right, who expected more pain. Highly active areas are colored orange, red, and white. Additional experiments might assess how certain drugs change the pattern of activation. (B from Rainville, 2002; courtesy of Pierre Rainville.)

length of the myelinated axons. Scanning the water movement provides the data needed to visualize the axonal pathways and provides a structural view of connectivity among brain structures. The data collected tell not only the total amount of water molecule diffusion, but also the directionality of diffusion, called anisotropy. The higher the anisotropy, the more tightly bundled the axons; higher diffusion levels indicate poorly myelinated or damaged axon connectivity. This technology is the basis for the Human Connectome Project, introduced in the chapter opener, that aims to map all the neural connections in the human brain. The chapter opening photo is an image from a DTI scan. The significance of connectivity to mental function will be described in subsequent chapters (see Chapters 18, 19, and 20). What is becoming clear is that any single brain structure may not be functionally important to a brain disorder, but the *connections* between regions may be the critical consideration.

It did not take long for scientists to realize the power of their new tools, and they proceeded to use the computerized scanning techniques to view the localization of radioactively labeled materials injected into a living human. **Positron emission tomography** (**PET**) does not create images of the brain but maps the distribution of a radioactively labeled substance that has been injected into an individual. To do this safely, we must use radioisotopes that decay quickly rather than accumulate. Although radioactive isotopes used in many laboratory experiments have relatively long half-lives, on the order of 1200 years for ^{3}H or 5700 years for ^{14}C, those used for PET have half-lives of 2 minutes (^{15}O), 20 minutes (^{11}C), or 110 minutes (^{18}F). Isotopes that decay and lose their radioactivity quickly (i.e., have short half-lives) emit positrons, which are like electrons except that they have a positive charge. When a positron expelled from the nucleus collides with an electron, both particles are annihilated and emit two gamma photons traveling in opposite directions. In a PET scanning device (**FIGURE 4.25A**), detectors surround the head to track these gamma photons and locate their origin. The information is analyzed by computer and visualized as an image on the monitor.

PET is useful in neuropharmacology in several ways (Farde, 1996). First, a radioactively labeled drug or ligand can be administered, and the location of binding in brain tissue can be seen. The technique has been used successfully to localize neurotransmitter receptors and identify where drugs bind. Perhaps even more exciting is the use of PET to determine which parts of the brain are active during the performance of particular tasks or cognitive problem solving (**FIGURE 4.25B**). PET allows us to visualize brain activity, which is reflected in increases in glucose utilization, oxygen use, and blood flow, depending on which reagent has been labeled. Very much like autoradiography in living humans, PET can be used along with 2-DG to map brain areas that utilize increased glucose or demonstrate increased blood flow—both indicative of heightened neural activity.

Single-photon emission computerized tomography (**SPECT**) is very similar to PET imaging, but it is much simpler and less expensive because the radiolabeled probes do not have to be synthesized but are commercially available. When scanned, the radioactive compounds, either inhaled or injected, show the changes in regional blood flow. Although resolution is less accurate than with PET, SPECT data can be combined with CT or MRI scans to localize active areas more precisely than with SPECT alone.

While MRI visualizes brain structure, **functional MRI** (**fMRI**) has become the newest and perhaps the most powerful tool in the neuroscientist's arsenal for visualizing brain activity. To meet the increased metabolic demand of active neurons, the flow of blood carrying oxygen to these cells increases. Functional MRI can detect the increases in blood oxygenation caused by cell firing (called BOLD, for blood-oxygen-level-dependent, brain activity) because oxygenated hemoglobin (the molecule that carries oxygen in the blood and provides the red color) has a different magnetic resonance signal than oxygen-depleted hemoglobin. Functional MRI offers several advantages over PET. First, fMRI provides both anatomical and functional information for each individual, and the detail of the image is far superior. Second, because the individual does not have to be injected with radioactive material, the measures can be made repeatedly to show changes over time. For the same reason, the procedure is essentially risk-free, except for the occasional case of claustrophobia caused by the scanner. Third, the process is so rapid that brain activity can be monitored in real time (i.e., as it is occurring). In combination with recording of electrical activity with electroencephalography (see the next section), fMRI can produce three-dimensional images that show neural activity in interconnecting networks of brain centers. Temporal sequencing of information processing becomes possible, so one can see the changing locations of brain activity during tasks and cognitive processes.

Resting-state fMRI (**rs-fMRI**) is a relatively new modification and powerful tool to investigate connectivity among brain regions when the individual is awake but not actively engaged in a task that requires attention. Examining BOLD brain activity at rest allows researchers to find highly reliable patterns of correlated spontaneous neural activity. Such data can be used to compare connectivity in healthy individuals with that in individuals with neurological or psychiatric diseases. For example, a large number of studies have compared healthy individuals and those with major depressive disorder (reviewed by Dutta et al., 2014). There have been multiple distinct networks identified in the resting state that are associated with particular symptoms of depression and that are different in depressed individuals than in controls. One such network, called the default mode network (DMN), includes the posterior cingulate cortex, medial prefrontal cortex, ventral anterior cingulate cortex, and portions of parietal cortex. The DMN is more active in depressed individuals than in controls, and this hyperactivity is greatest when the individual is not actively involved in any activity that requires attention or performance of an explicit task. That fact has led to the idea that DMN hyperactivity is the basis for self-referential processing, which refers to the negative inwardly directed ruminations of depressed individuals. Perhaps of greatest interest to psychopharmacologists and clinicians is that resting-state connectivity is differentially altered by various antidepressant treatments. Additionally, there is indication that the extent of connectivity may help identify which individuals will respond or not respond to a given treatment. Such ability to predict responders and nonresponders has the potential to greatly improve the treatment of major depressive disorder (reviewed by Dichter et al., 2015). For an older but excellent introduction to brain imaging and its relationship to cognitive processes, refer to Posner and Raichle (1994).

Pharmacological MRI (**phMRI**), a spin-off of fMRI, has developed into an important technique in drug development. It images the mechanism of drug action by analyzing changes in brain function following drug administration and identifies the location of drug action within the CNS. It aids early clinical studies that evaluate relationships between drug dose, drug plasma levels, brain receptor occupancy (using PET), and changes in brain function. Further, visualizing the time course of drug action can provide pharmacokinetic data. Most important, it has the potential to predict treatment response, avoiding weeks of treatment that often is needed to ultimately see a measurable clinical response. It could do this by screening patients' brain function profiles in response to the drug to determine whether they will ultimately respond to that particular treatment. This use of phMRI would be an important tool in personalized medicine (see Chapter 1) by matching a given treatment to those individuals most likely to show beneficial results, and avoiding expensive and time-consuming treatment of a patient who will not have a good treatment outcome. This would be particularly important for high-risk, high-cost treatments or those taking months to show clinical effectiveness.

ELECTROENCEPHALOGRAPHY (EEG) In addition to improved visualization techniques and methods of mapping metabolic function in the human brain, a third noninvasive method of investigating human brain activity is now used in neuropharmacology: electrical recording with **electroencephalography** (**EEG**). As is true for other imaging techniques, the EEG has become more sophisticated with the assistance of computer analysis. Electrodes are taped to the scalp in multiple locations

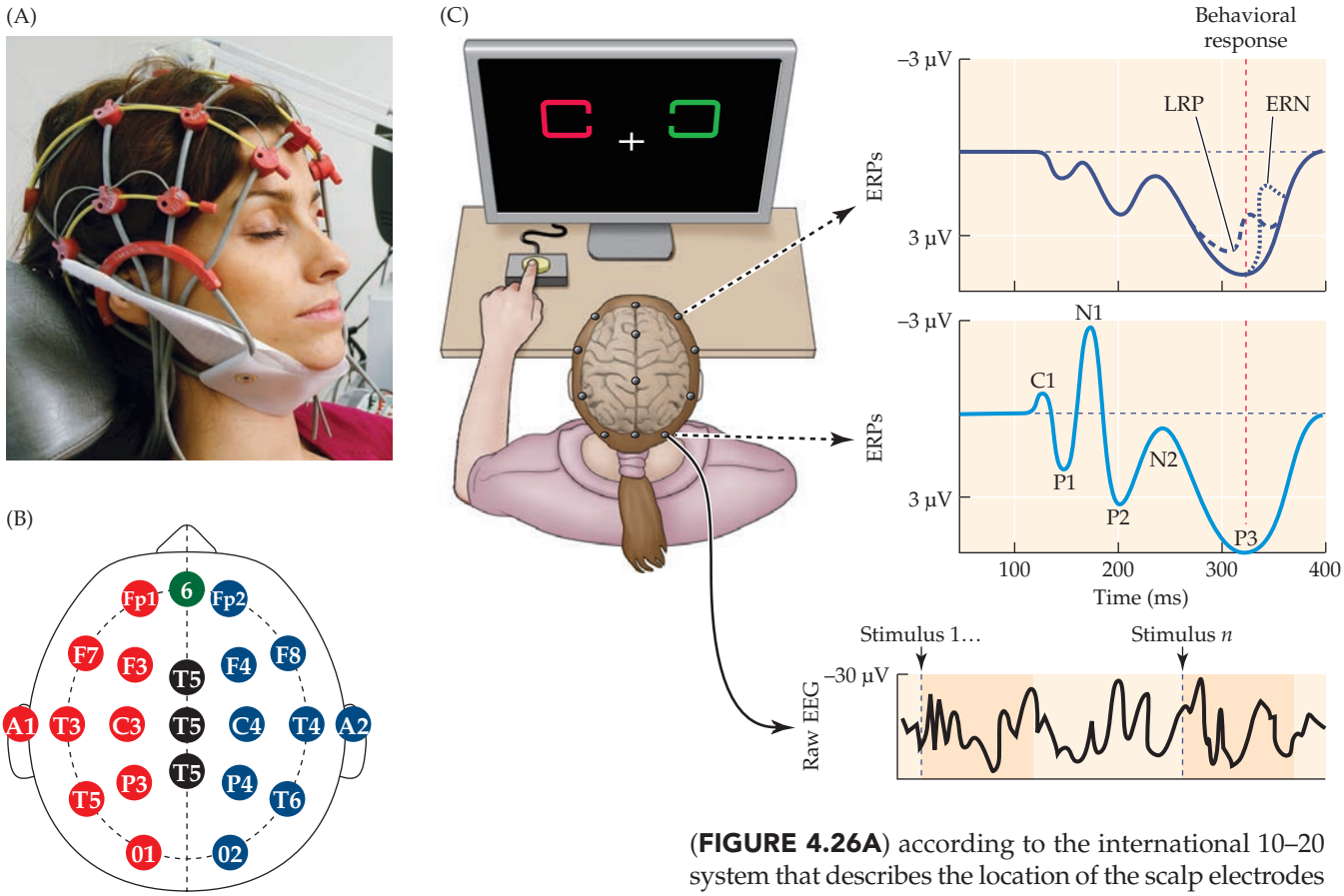


FIGURE 4.26 Electroencephalography (EEG)
(A) In humans, changes in electrical activity of the brain are detected by recording electrodes that are attached to the surface of the individual's scalp. The electrodes record the activity of thousands of cells simultaneously. (B) The standardized international 10–20 system for electrode placement, with the view of the skull from above, nose indicating frontal, ears to each side. The electrodes are marked as the ground (G), frontal pole (Fp), frontal (F), central (C), parietal (P), occipital (O), and temporal (T). The midline electrodes are marked as FZ, CZ, and PZ, with the Z standing for zero. Odd numbers are used for points over the left hemisphere, and even numbers over the right. The ear lobes are indicated with (A). (C) Event-related potential (ERP) patterns of voltage change in response to a visual stimulus in a discrimination task. On the left is a model of a human head with the placement of a subset of electrodes from the 10–20 system. The right side of the figure shows how the ERPs are extracted from the raw EEG record while the subject performs the visual discrimination task. The lower ERP (blue) shows the wave forms at occipitotemporal lobe electrode sites during the visual task. The upper ERP (purple) shows the wave forms while the finger response occurs. Processes associated with voltage fluctuations of the ERP are labeled: C1, primary visual cortex; P1 and N1, information moves through the visual system; P2, not well understood; N2, categorizing the stimulus; P3 (often called P300 because it occurs 300 ms after the stimulus onset), working memory encoding; LRP, preparation for the motor response; ERN, evaluation of performance after the response. (A, Phanie/LARIBOISIERE-APHP-GARO/Medical Images; C After Woodman, 2010).

(**FIGURE 4.26A**) according to the international 10–20 system that describes the location of the scalp electrodes (**FIGURE 4.26B**) based on the underlying area of the cerebral cortex. This type of mapping is important for standardization of placement and permits comparison and reproducibility from person to person and in the same individual over time. The electrical activity that is recorded reflects the sum of electrical events of populations of neurons. Multiple electrodes are used because a comparison of the signals from various locations can identify the origin of some waves. **Quantitative EEG** (**qEEG**) uses computerized analysis to evaluate the large amount of complex data collected with an EEG, which is frequently converted into colored maps of the changes taking place in the brain during cognitive processing tasks. While useful to clinicians to evaluate brain functions associated with things such as traumatic brain injury or epilepsy, application of qEEG in psychopharmacology includes evaluating the effects of medications on neural function. Additionally, it can be used to predict individual response to particular treatments to enhance personalized medicine. Hence qEEG can be used to determine whether a given individual will be helped by medication and which of the classes of medications will be most beneficial (see Arns and Olbrich, 2014).

Because EEG can detect electrical events in real time, one of the most useful applications of EEG data is recording electrical changes that occur in response to momentary sensory stimulation; these changes are called **event-related potentials** (**ERPs**). ERPs allow

researchers to visualize the processing of the cognitive response to a given stimulus as it occurs. Because ERPs are quite small, the raw EEG response must be computer averaged over many (perhaps several hundred) stimulus presentations to reduce the appearance of random brain activity (**FIGURE 4.26C**) while the significant electrical wave form is retained (the ERP). Evaluation of electrical responses in various clinical populations has led to improved understanding of attention deficits and processing differences in individuals with schizophrenia, Huntington's disease, attention deficit hyperactivity disorder, and many other disorders. It has been suggested that these differences in processing may help to identify subtypes of clinical disorders that might be used for selecting appropriate treatment. Other brain scans may be used in combination with qEEG because while PET and fMRI can show which areas of the brain are active during a task, ERPs help to understand the time course of the activation.

Genetic engineering helps neuroscientists to ask and answer new questions

The excitement surrounding completion of the Human Genome Project, in which all human genetic material has been mapped, has permeated both the scientific and the popular press. Although the term *genetic engineering* evokes both excitement and some trepidation in most people, the technology has at the very least provided amazing opportunities for neuroscience. Genetic engineering involves procedures in which the DNA of the organism is altered (knockouts or knockins) or a foreign gene is added to the genetic material of the organism (transgenic). These procedures provide the opportunity to produce highly specific mutations in the mouse genome to study subsequent changes in brain function and behavior. **FIGURE 4.27** shows the essential steps in genetic engineering. Genes of interest are extracted or extracted and modified and then are injected into fertilized mouse eggs taken from a pregnant mouse. After the injection procedure, the eggs are implanted in the uterus of a surrogate mother. Offspring are assessed for evidence of the mutation. Chimeric mice, that is, those in which the transgene is expressed in some cells and not in others, are subsequently mated with wild-type mice. The offspring from these matings produce some heterozygous mice (i.e., those having one transgene and one wild-type gene) that in turn will be mated to other heterozygous mice to ultimately produce some mice homozygous for the transgene. Because the gestation time of mice is approximately 3 months, the entire procedure takes about 9 to 12 months.

Creating **knockout mice** may represent the most sophisticated of all lesioning techniques yet described. With the ability to identify which piece of chromosomal DNA (i.e., the gene) is responsible for directing the synthesis of a particular protein, neuroscience has the opportunity to alter that gene, causing a change in expression of the protein. In essence, we are producing an animal model that lacks a particular protein (e.g., enzyme, ion channel, receptor) so that we can evaluate postlesioning behavior. Comparing the behavior and the drug response of altered mice with those of unaltered animals will tell us about the function of the protein that has been deleted. We can also use these animals to identify the importance of that protein in specific drug effects. The knockout technology has led to exciting new discoveries in neuroscience and the development of animal models of human diseases, leading to more effective therapeutics. (For a video animation on creating knockout mice, see the Companion Website.)

What is needed is a faster and less expensive method to genetically engineer mice. One of the newest techniques is **CRISPR** (clustered regularly interspaced short palindromic repeat), which uses "guide RNA" to identify a specific genomic sequence to be modified. Along with the "guide," a nuclease (Cas9) is also introduced, and it cuts out the specified section of DNA. A new section of DNA is then inserted. In the future, CRISPR may make it possible to correct genetic mutations at specific locations for the treatment of human diseases. The new technique is highly specific, avoids the prolonged process of traditional gene-targeting methods used in animal research (see Figure 4.27), and is therefore less expensive. Additionally, modifications of the method can damage the Cas9 enzyme, which still

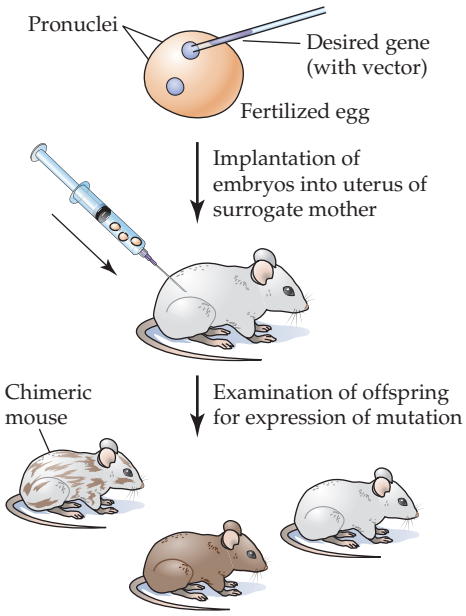


FIGURE 4.27 Illustration of genetic engineering
Genetic material is extracted from cells, injected into fertilized eggs, and implanted in the uterus of a surrogate mother. Offspring that are chimeric are mated with wild-type mice. Any of their offspring that are heterozygous for the genetic trait of interest are subsequently mated to produce some homozygous mice of interest.

allows it to identify the specified DNA sequence, but rather than cutting it out, it prevents gene expression. Gene expression can also be stimulated by attaching an activating protein to the damaged Cas9 enzyme, which means that genes can be turned on or off using this technique. Other alternatives in the procedure can examine epigenetic modifications of gene expression and chemical- or light-induced control of Cas9 (see Ledford, 2016, for more detail). Many variations of the technique have been developed by multiple research labs.

In contrast to the knockout technique, to create **knockin mice**, the inserted gene is modified so that it produces a slightly different protein from that found in wild-type mice. Often the protein manufactured is different from that of wild-type mice by only a single amino acid residue, allowing investigation of the relationship between protein structure and function. For neuropharmacologists, the protein of interest is often a receptor subtype or an enzyme that controls an important synthesizing or metabolizing process.

A second strategy involves the substitution of one gene for another, producing **transgenic mice**. As we learn more about the pathological genes responsible for neuropsychiatric diseases such as Huntington's and Alzheimer's diseases, it is possible to remove the human genes and insert them into mice to produce true animal models of the disorders. For an example, see the work by Carter and coworkers (1999), which measures motor deficits in mice transgenic for Huntington's disease (**BOX 4.3**). With authentic animal models, neuroscience will be able to identify the cellular processes responsible for a disorder and develop appropriate treatments.

As is true for any revolutionary new technique, caution in interpreting the results is warranted. First, because behaviors are regulated not by single genes but by multiple interacting genes, changing or eliminating only one alters only a small part of the overall behavioral trait. Second, compensation by other genes for the missing or overexpressed gene may mask the functional effect of the mutation. Third, since the altered gene function occurs in all tissues at all stages of development, it is possible that changes in other organs or in other brain areas are responsible for the behavioral changes. Finally, because these animals are developing organisms, environmental factors also have a significant effect on the ultimate gene expression. Several articles provide greater detail on the potential pitfalls of gene-targeting studies (Crawley, 1996; Gerlai, 1996; Lathe, 1996).

In addition to its use in creating "mutant" animals, genetic material can be inserted into cells (maintained in cell culture) that do not normally have a particular protein (e.g., receptor). The normal cell division process produces large numbers of identically altered cells, which we call **cloning**. These cells can then be used to screen new drugs, using conventional pharmacological techniques for identifying agonists and antagonists.

Optogenetics is an exciting field of biology that uses light (hence *opto*) to exert temporally and spatially precise control over the functioning of genetically specified cells (hence *genetics*). This approach has particular utility in neurobiology by enabling researchers either to excite or to inhibit selected populations of nerve cells almost instantaneously (reviewed by Deisseroth, 2015; Guru et al., 2015). Optogenetic control of neuronal firing is accomplished using microbial opsins, light-sensitive proteins synthesized by several different types of microorganisms.[1] **FIGURE 4.28A** illustrates three kinds

[1]Of course, the prototypical opsin is rhodopsin, the light-sensitive retinal pigment found in rod photoreceptors.

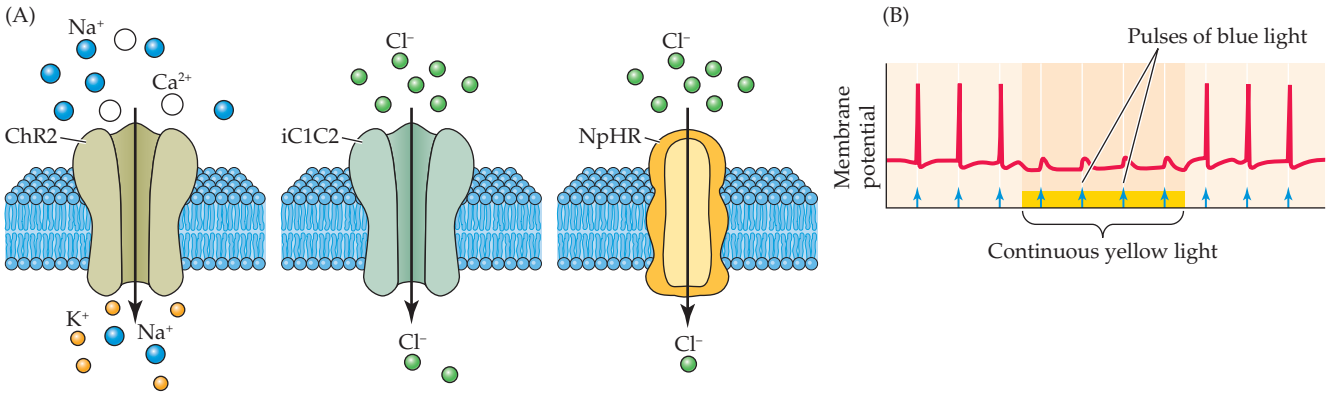


FIGURE 4.28 Light-sensitive proteins used in optogenetic studies (A) The figure depicts three of the major opsins presently used in optogenetic research, each embedded in a neuronal membrane along with the ions it causes to pass through the membrane. ChR2 and iC1C2 are ion channels, whereas NpHR is an ion pump. (B) This electrical tracing was taken from a neuron in vitro that was engineered to express both ChR2 and NpHR. Vertical blue arrows represent brief flashes of blue laser light; the horizontal yellow line represents continued application of yellow light from a different laser. Large upward deflections of the red line are action potentials elicited by ChR2 stimulation. (A after Guru et al., 2015, CC-BY 4.0; B after Zhang et al., 2007.)

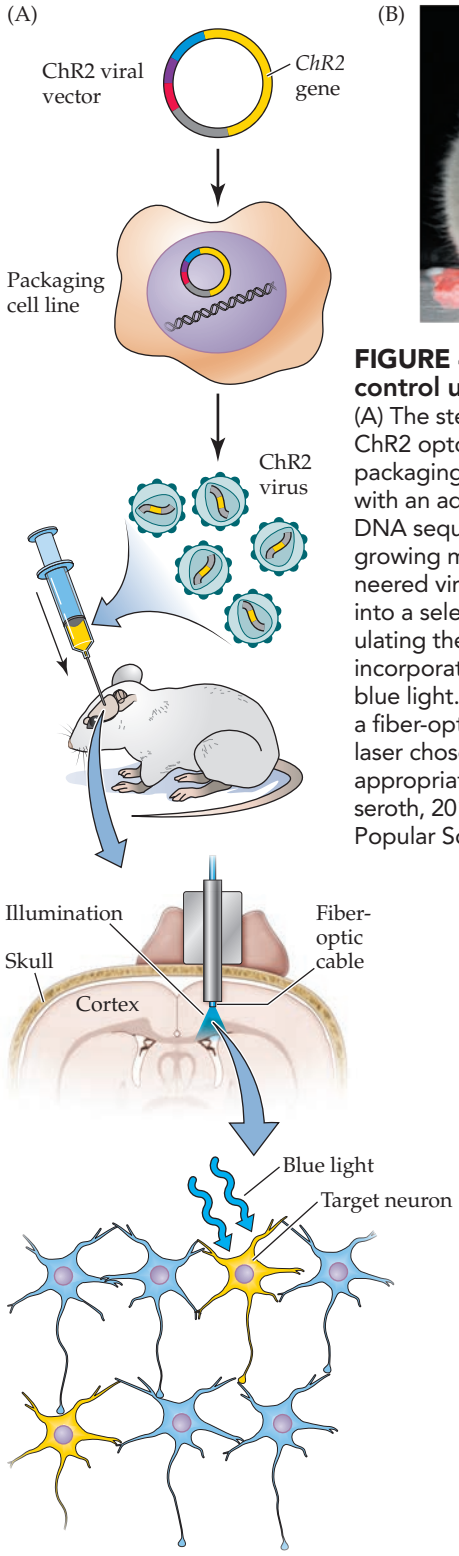


FIGURE 4.29 Behavioral control using optogenetics
(A) The steps required for an in vivo ChR2 optogenetic study include packaging the ChR2 gene along with an additional cell-targeting DNA sequence into a viral vector, growing many copies of the engineered virus, implanting the virus into a selected brain area, and stimulating the target neurons that have incorporated the ChR2 gene with a blue light. (B) Mouse implanted with a fiber-optic probe connected to a laser chosen to deliver light of the appropriate wavelength. (From Deisseroth, 2015. Photo: John B. Carnett/Popular Science/Getty Images.)

of opsins currently being used in optogenetic research. ChR2 (channelrhodopsin-2) forms a nonspecific cation channel permeable to sodium (Na^+), potassium (K^+), and calcium (Ca^{2+}) ions, so light-activated channel opening depolarizes the cell membrane and rapidly stimulates cell firing primarily due to Na^+ influx. A genetically modified form of channelrhodopsin called iC1C2 forms an inhibitory chloride (Cl^-) channel. NpHR (halorhodopsin) is a Cl^- pump instead of a channel, thus enabling it to inhibit neuronal firing as iC1C2 does but with a slower onset of action. Each of these opsin proteins is sensitive to a particular wavelength of light, which is used to selectively activate the ion channel or pump. This can be seen in **FIGURE 4.28B**, which shows electrophysiological recording from a single neuron in vitro that was made to express both ChR2 and NpHR. Note that each short pulse of blue light, which activates ChR2, led to the firing of an action potential (left and right sides of the trace); however, this ChR2-mediated effect was completely blocked by NpHR activation using simultaneous application of a yellow light (middle of the trace).

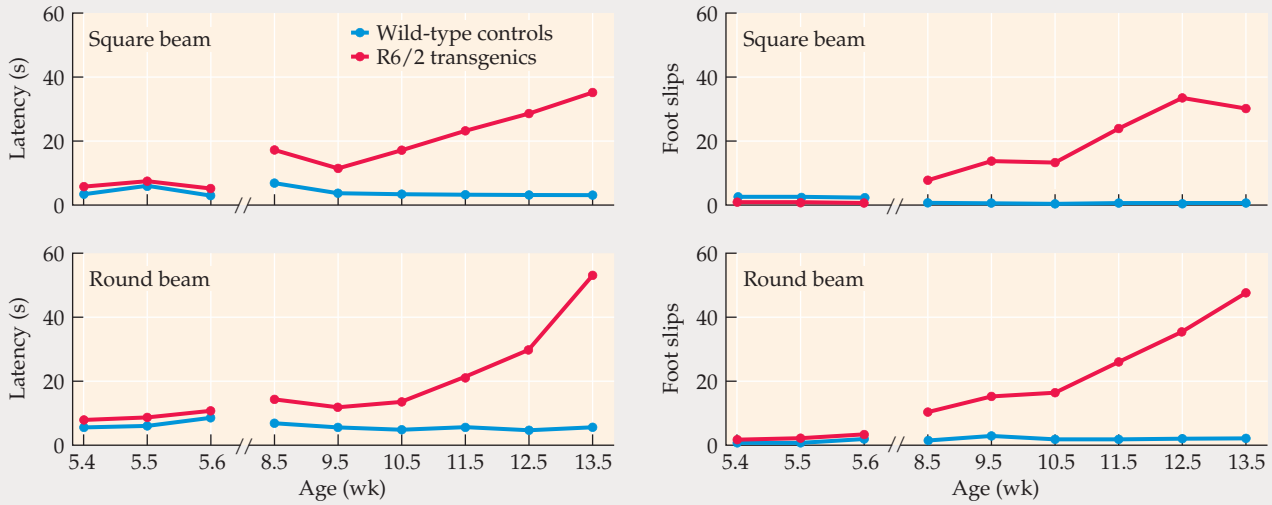
The power of optogenetics stems from the ability of researchers to express the gene for ChR2, iC1C2, NpHR, or any other opsin in a specific population of neurons (e.g., dopamine or serotonin neurons) using viral vectors to target the cells of interest in a particular brain area (**FIGURE 4.29A**). The viruses used in optogenetics and related methods deliver genetic material to neurons but are otherwise harmless to the cells. Once the gene-bearing virus has infected the target cells and the opsin transgene has been expressed by those cells, the animal is surgically implanted with a fiber-optic probe aimed at the area containing the target. After the animal has recovered from the surgery, a laser connected to the probe by a fiber-optic cable can deliver light of the appropriate wavelength to the target area while the animal is freely behaving in an apparatus such as an open field or an operant chamber (**FIGURE 4.29B**). Using this setup, researchers can determine the behavioral effects of either activating or silencing the specific neurons engineered to express the light-sensitive protein.

Since the introduction of optogenetics to neuroscience less than 15 years ago, there has been an enormous upsurge in research using this technology. It's easy to see why. For most of the history of neuroscience, one of the few ways to determine the effects of stimulating a group of nerve cells was to use the electrophysiological stimulation approach described earlier in the chapter. Unfortunately, electrical stimulation affects all neurons within range of the electrode, not just the ones of interest to the experimenter. Therefore, you could never be sure that the behavioral effects of the stimulation were due to the specific neurons you were studying and not to adjacent cells with a different neurochemistry

BOX 4.3 Pharmacology in Action

Transgenic Model of Huntington's Disease

(A)



(A) Beam walking Mice were taught to walk across square or round elevated beams to reach an enclosed safety platform. The time to cross and the number of foot slips were recorded. Each data point represents the mean time to cross. (After Carter et al., 1999.)

Huntington's disease (HD) is characterized by progressive impairment in movement, such as slow, uncoordinated actions; involuntary jerking; and impaired gait, posture, and balance. Additionally, individuals develop cognitive dysfunction, which includes difficulty in planning, lack of flexibility in thinking, poor impulse control, inability to focus attention, learning deficits, and others. Furthermore, there are marked mood and personality changes accompanied by a variety of psychiatric disorders. Further details of the symptoms of this disease, its progression, and approaches to treatment can be found in Chapter 20. This genetically transmitted disease strikes individuals when they are in their 30s and 40s and progresses over time. Individuals typically live 15 to 20 years after onset of symptoms, which means that their life span is significantly shortened. At present, although some symptoms can be treated initially, no treatment is available to modify the course of the disease. What is needed is a translational animal model of the disease that would lead to improved drug development.

Using a host of behavioral measures, Carter and colleagues (1999) characterized the progressive neurological symptoms in a model created by inserting the human Huntington's disease gene into mice (R6/2 mice). Because patients with HD show a variety of gradually worsening motor deficits and progressive hypoactivity, a battery of tests measuring the motor function of the transgenic mice was started at 5 to 6 weeks of age and administered weekly for 10 weeks. Functioning of the transgenic mice was compared with that of wild-type control mice. To evaluate fine motor coordination and balance, the investigators used **beam walking**, in which the raised beam resembles a human gymnastic balance beam. They found no difference in performance between R6/2 transgenic mice and wild-type control mice at 5 to 6 weeks of age on either the square or the round beam, although R6/2 mice were somewhat slower in performing the task (**Figure A**). However, by 8 to 9 weeks of age, the R6/2 mice took 12 times as long and had many more foot slips. At 13 to 14 weeks, many of the transgenic mice fell off the beam repeatedly. Motor coordination and balance were further evaluated on a **rotarod**—a horizontally oriented cylinder that is mechanically rotated at set speeds (**Figure B**). The researchers timed how long the mice remained on the rod (i.e., latency to fall). As you can see in **Figure C**, control mice readily maintained their balance and coordination for the maximum trial length at all speeds tested. In contrast, transgenic mice had difficulty at the highest speeds at 5 to 6 weeks, and by 13 to 14 weeks, they failed to maintain their coordination at any speed. When their **swimming performance** was examined at 5 to 6 weeks, R6/2 mice showed overall slowness, a tendency to float temporarily without making efforts to swim, and

(*Continued*)

BOX 4.3 Pharmacology in Action *(continued)*

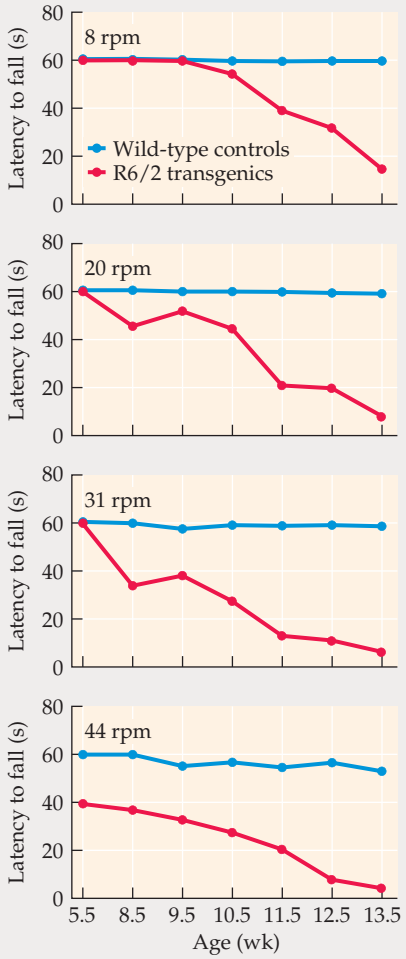
significant incoordination in swim movements that progressively worsened over time. Additionally, rather than the fluid, coordinated **gait** shown by healthy mice, transgenic mice showed staggering and weaving with uneven short strides, reminiscent of the typical patient with HD. In a final task, researchers examined the startle reflex to an intensely loud sound. Transgenic mice showed a decline in response only at the final time point. More important, the acoustic startle response is normally reduced when the intense stimulus is preceded by a small prepulse stimulus. Patients with HD characteristically show a deficit in that **prepulse inhibition of startle**. Likewise, by 8 weeks, the transgenic mice showed significant deficits.

A variety of behavioral measures that reflect the symptoms of HD show clearly that R6/2 mice represent a valid model of the motor deficits that gradually appear in patients with HD. Subsequent studies have shown other analogous characteristics, including the development of diabetes, deficits in spatial learning, shortened life span, and a host of pathological changes in the brain. You might be interested in reading more about these animals and some of the newer drugs tested on them that target progressive brain pathology and gene transcription (Li et al., 2005). It is hoped that these drugs may become effective agents in preventing the progression of this devastating disease.

(B)

(B) Rotarod (Courtesy of Susan Urmy, Vanderbilt University.)

(C)



(C) Rotarod performance Data points represent the mean latency to fall from the rotarod (maximum trial length = 60 seconds) at various speeds of rotation. rpm, revolutions per minute. (After Carter et al., 1999.)

(e.g., GABA neurons intermingled with dopamine neurons). Furthermore, the only techniques that were previously available for temporarily silencing neurons were very crude and also nonspecific. These limitations have all been overcome by the advent of optogenetics. As with other areas of neuroscience research, the long-term goal of optogenetic studies is not just to identify the basic behavioral and physiological functions of specific neuronal populations, but also to learn more about the causes of neuropsychiatric disorders and to help develop more effective treatments (Huang et al., 2013).

An alternative to optogenetics is the technique called **chemogenetics**, sometimes referred to as **DREADD** (designer receptor exclusively activated by a designer drug). As its name suggests, a genetically engineered receptor is stereotaxically targeted to specific brain cells, usually with viral vectors, and then is either activated or suppressed by administering a ligand that has been created to very specifically bind to the inserted receptor and nothing else. The ligand can be injected or given orally in the animals' drinking water, so the technique is easier to implement and is less invasive than optogenetics. Because these designer drugs bind to altered G protein–coupled receptors, their actions take longer to be initiated and are more prolonged (over minutes to hours) compared with the neuronal excitation and inhibition produced optogenetically. Hence while optogenetics is optimum for controlling changes over milliseconds, providing the finest level of temporal control over the behavior investigated, chemogenetics may be chosen for studying behavioral effects of longer duration, such as anxiety or feeding, because the designer ligands last

longer than pulses of light. The longer duration and the fact that designer ligands can be administered orally suggest the potential for translation to therapeutic applications such as treatment of obesity, epilepsy, and addiction disorders. For further details of the method and discussion of therapeutic potential, refer to Urban and Roth (2015).

Because both optogenetics and chemogenetics have drawbacks, multiple efforts have been made to find new ways to use noninvasive technology to rapidly and reversibly activate neurons with high spatial specificity even deep within the brain. Such a technology is **magnetogenetics** (Long et al., 2015; Wheeler et al., 2016). In essence, this technique involves genetic manipulations that make cells sensitive to magnets. When the animal expressing these magnetoreceptors is exposed to remote magnetic stimulation, the cells depolarize and a behavioral response is triggered. Magnetogenetics is one more highly advanced tool for neuroscientists to study how the brain interprets sensory information, processes it, and generates behavior and cognition. In addition, the fact that the magnetic activation, in the millitesla[2] range, can be maintained continuously without causing side effects means it may replace deep brain stimulation (Figure 4.15B), which is used to treat Parkinson's disease, epilepsy, obsessive-compulsive disorder, chronic pain, and other neurological conditions. Although deep brain stimulation is effective, it requires invasive surgical implantation of metal electrodes with associated risk of infection and other complications.

DART The goal of a very new technique called DART (drugs acutely restricted by tethering) is to attach a drug to the surface of a biochemically defined population of nerve cells, thereby greatly increasing the effective concentration of the drug at those cells while minimizing exposure of other kinds of cells to the drug. This is accomplished by using genetically engineered animals to express a bacterial enzyme (HaloTag) only in the selected group of neurons. The drug to be studied, for example a neurotransmitter receptor antagonist, is attached to a small molecule, called the HaloTag ligand, by a long flexible linker. When the drug–ligand complex is administered to the genetically engineered animal or to a brain slice obtained from such an animal, the ligand part binds to the HaloTag on the selected neurons while the drug is able to bind to its receptor on the cell membrane (**FIGURE 4.30**). For example, researchers used DART to test the behavioral effects of blocking AMPA receptors (a particular type of receptor for the neurotransmitter glutamate) either in neurons that possess D_1 dopamine receptors or in neurons with D_2 receptors. That is, possessing a particular dopamine receptor subtype was the defining property of the nerve cells being targeted. The power of this technique is that without using DART, the antagonist would block AMPA receptors everywhere they are present, not just in cells with one or the other dopamine receptor subtype. Ultimately, this method will be of great value in helping researchers determine cell-specific drug mechanisms of action in animal models of clinical disorders (Shields et al., 2017).

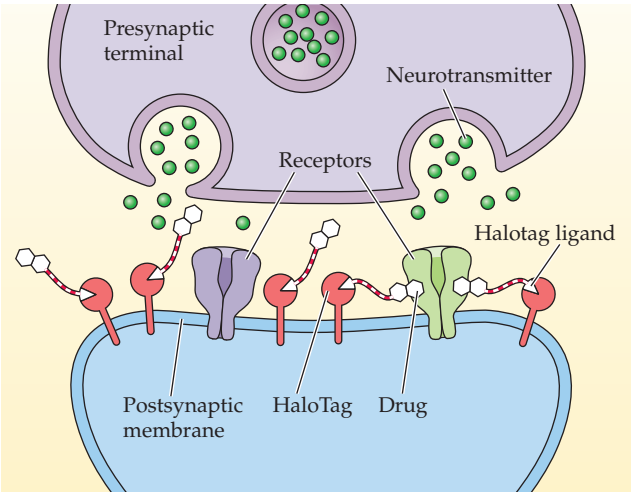


FIGURE 4.30 Drugs acutely restricted by tethering (DART) HaloTag (red) is shown attaching a drug to the postsynaptic membrane of a biochemically defined cell type. Drug molecules, depicted as white ring structures, are linked via tethers (red-and-white dashed curved lines) to small molecule ligands that bind to the HaloTag. The drug is specific for the green-colored, but not the purple-colored, receptors on the postsynaptic membrane. Hence any drug-induced behavioral effects can be attributed to the drug action on those receptors in the defined population of cells. The green spheres represent neurotransmitter molecules released from the presynaptic nerve terminals. (After Shields et al., 2017.)

Behavioral and neuropharmacological methods complement one another

Bear in mind that under normal circumstances, several of these techniques are used in tandem to approach a problem in neuroscience from several directions (see Web Box 4.2). The power of these experimental tools is that when they are used together, a more reasonable picture emerges, and conflicting results can be incorporated into the larger picture. Only in this way can we uncover the neurobiological substrates of cognitive function and dysfunction. In every case, interpretation of these sophisticated approaches is subject to the same scrutiny required by the earliest lesion experiments. Remember, healthy skepticism is central to the scientific method.

[2]Tesla is the standard unit of magnetic flux intensity.

Section Summary

- Using a stereotaxic device, lesioning destroys brain cells in selected areas with high-frequency radio current. More selective lesions are made by injecting an excitatory neurotoxin that destroys cell bodies in the region without damaging axons passing through, or by injecting a neurotoxin selective for a given neurotransmitter.
- Using a specialized cannula, microdialysis allows researchers to collect material from extracellular fluid from deep within the brain in a freely moving animal, so corresponding changes in behavior can be monitored simultaneously. The material is analyzed and quantified by high-pressure liquid chromatography. In vivo voltammetry is a second way to measure the neurotransmitter released into the synapse.
- Macroelectrodes that are stereotaxically implanted are used to electrically stimulate deep brain regions while monitoring behavioral changes. They can also record electrical response following drug treatment or other experimental manipulation. Microelectrodes can record electrical activity from either inside a cell (intracellular) or near a single cell (extracellular).
- The function of individual ion channels is monitored with patch clamp electrophysiology.
- The radioligand binding method evaluates the number and affinity of specific receptor molecules in tissue homogenates. Verification of binding requires proof of selectivity, saturability, reversibility, high affinity, and biological relevance.
- To visualize the location of receptors in the brain, receptor autoradiography, both in vitro and in vivo, is used.
- The ability to make antibodies to proteins allows more precise cellular localization of receptors or other protein cell components such as enzymes with ICC. Antibodies are also used to very sensitively measure proteins in tissue homogenates with Western blot. Radioimmunoassays and ELISA measure important molecules in body fluids or tissue extracts with competitive binding of an antibody to its antigen.
- ICC identifies cells that *contain* a given protein; the complementary technique ISH is used to locate cells in a tissue slice that are *manufacturing* a particular protein by detecting the corresponding mRNA. It can also determine changes in mRNA levels, which provide an estimate of the rate of synthesis of that protein. Although ISH measures a single mRNA, microarrays (or gene chips) screen up to 20,000 genes on a single support (chip). The entire genome can be screened in one experiment with the use of just a few chips.
- Although they use two distinct scanning technologies, CT and MRI both create extremely detailed "slices" through the brains of living individuals that can also be displayed as three-dimensional images. Their excellent detail of brain structure. depends on computer analysis. MRS uses MRI-generated data to calculate brain chemical concentration. Diffusion tensor imaging is one of several methods available to show structural connectivity among brain structures.
- PET maps the distribution of a radioactively labeled substance that has been injected into an individual. A labeled drug or ligand is administered to identify where drugs bind and to localize neurotransmitter receptors. It can also allow visualization of regional brain activity during task performance, which is reflected in increased glucose utilization, oxygen use, and blood flow, depending on the reagent labeled. SPECT depends on similar technology at a lower cost but with less resolution.
- Functional MRI (fMRI) provides both anatomical and functional imaging of the human brain by allowing visualization of changes in regional blood oxygenation caused by cell firing. It monitors brain activity as it is occurring and, unlike PET, requires no radioactivity. Resting-state fMRI shows correlated patterns of brain activity when the individual is not engaged in a task. Pharmacological MRI utilizes fMRI to image brain function following drug administration, provides information on the location of drug action and pharmacokinetic data, and potentially can predict patient treatment response.
- EEG utilizes electrodes placed on the scalp of humans to measure the electrical activity of populations of neurons. Quantitative EEG requires computer analysis of the data to produce color-coded maps of brain activity. EEG recording of event related potentials (ERPs) shows the processing of the cognitive response to momentary sensory stimulation in real time.
- Deleting a specific gene in mice produces an animal model that lacks a particular protein (knockout) in order to evaluate postlesion behavior and drug effects. Knockin mice have a gene inserted, so they produce a slightly different protein than is produced by wild-type mice. Transgenic mice are mice in which one gene is replaced by another.
- Optogenetics provides the opportunity to temporally and spatially excite or inhibit genetically specific cells while evaluating the animal's behavior. Chemogenetics (DREADD) uses orally or systemically administered chemicals to stimulate implanted genetically engineered receptors in specific brain cells to alter animal behavior. Chemogenetics is slower, but longer lasting, than optogenetic effects.

- CRISPR is a technique that provides a more rapid and less costly way to genetically engineer mice.
- Magnetogenetics uses genetic manipulations to make target cells (even deep within the brain) sensitive to remote magnetic stimulation in order to see how specific cell activation alters behavior, sensory processing, and cognitive function.
- DART attaches a drug to a limited number of biochemically identified cells to determine cell-specific drug mechanism of action.

STUDY QUESTIONS

1. Discuss the advantages of using laboratory animals in neuroscience research and the safeguards in place to ensure humane treatment.
2. Compare face validity, predictive validity, and construct validity. What is the difference between validity of an animal model and reliability?
3. Describe the three common measures of analgesia.
4. Why are tests of learning and memory challenging to interpret? Describe simple mazes as well as tests for spatial memory and working memory.
5. What are the anxiety-inducing situations that are central to the naturalistic tests of anxiety in rodents? Provide several examples. Why do you think some of these anxiety-induced situations are evolutionarily important for survival?
6. Compare the acute versus chronic stress models of depressive-like behaviors. What are the advantages and disadvantages?
7. Describe the three most important animal tests to evaluate drug reinforcement and potential dependence in humans.
8. Why are internal drug-induced cues important in the operant task evaluating discriminative stimuli? Given an example of how this test can be used.
9. What is translational research, and why is it so important? Describe three potential ways that translational research can be improved.
10. Describe stereotaxic surgery, and give examples of techniques that depend on its use.
11. Compare electrolytic lesioning with neurotoxin and specific neurotoxin lesioning.
12. What is microdialysis? In vivo voltammetry? How are they similar, and how are they different?
13. Why is electrophysiological stimulation and recording important to psychopharmacology?
14. What factors must be considered when interpreting radioligand binding data?
15. How is autoradiography performed when mapping receptor binding? In vivo receptor binding?
16. What is one significant difference between ICC and Western blot? How are antibodies used in these procedures?
17. Describe two important ways that RIAs differ from ELISAs.
18. What do ISH and microarrays measure? In what ways are they similar? Different?
19. Compare the use of 2-deoxyglucose in autoradiography and PET.
20. What information is provided by MRI? What two complimentary techniques depend on MRI technology, and what do they determine?
21. How is PET used in neuropharmacology?
22. What are the advantages of fMRI compared with PET? Briefly describe the importance of two techniques that depend on fMRI.
23. What is the EEG? Quantitative EEG? Event-related potential (ERP)?
24. Compare knockout mice, knockin mice, and transgenic mice. What role do they all play in psychopharmacology?
25. What are the goals of optogenetics? In a general way, tell how they are achieved. Compare the advantages and disadvantages of optogenetics, chemogenetics (DREADD), and magnetogenetics.

Go to the *Psychopharmacology* Companion Website at oup-arc.com/access/meyer-3e for animations, web boxes, flashcards, and other study aids.

CHAPTER 5

Mutant mice produced by genetic engineering (e.g., gene "knockout mice") may show phenotypic changes that emerge during development of the pups. (Bruno Cavignaux/Photononstop/Alamy Stock Photo.)

Catecholamines

THE PREGNANT LABORATORY MOUSE gives birth to her litter of six pups, all of which appear normal. If you performed a CT scan or MRI on the head of each pup, the brain would look to be of normal size and shape. Other bodily organs and tissues, including the skeletal muscles, also seem to be fine. During the first week of postnatal life, the pups are nursing and are gaining weight at a rate that you expected. As a new student who recently joined the lab to obtain research experience, you're happy that things are going so well. But soon thereafter something seems to be going very wrong. First, the pups have stopped gaining weight. Moreover, they have developed a hunched posture, they move very sluggishly, and their fur doesn't look well groomed. As the pups gradually wean from their mother to a state of independence in which they must feed themselves, they surprisingly fail to eat at all despite having continuous access to the standard food that normal lab mice eagerly consume. In fact, all six pups die before they reach 1 month of age.

Remarkably, if you were to perform a postmortem analysis of all of the thousands of chemicals that constitute a mouse brain and compare the results from these unfortunate animals with those obtained from normal healthy mice, you would find that the pups that had died lacked only a single chemical in their brains, namely the neurotransmitter **dopamine** (**DA**). Mice that lacked DA from embryonic development onward were first generated in the 1990s in the laboratory of Richard Palmiter at the University of Washington. In this chapter, we will learn much more about the neurobiology, pharmacology, and functions of this intriguing substance, including more details about Palmiter's dopamine-deficient mice. We will similarly cover a well-known sister transmitter to DA, namely **norepinephrine** (**NE**).

DA, NE, and the related substance **epinephrine** (**EPI**) make up a small but important group of neurotransmitters and hormones called **catecholamines**. The term *catecholamine* is derived from the fact that the members of this group all share two chemical similarities: a core structure of catechol and a nitrogen-containing group called an amine (**FIGURE 5.1**). ■

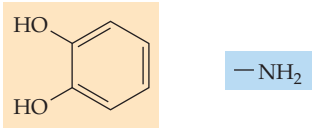


FIGURE 5.1 Structural features of catecholamines
A catechol nucleus and an amine group are found in all catecholamines.

The catecholamines, in turn, belong to a wider group of transmitters called either **monoamines** (transmitters that possess one amine group) or **biogenic amines** (*biogenic* refers to compounds made by living organisms). EPI and NE are sometimes called adrenaline and noradrenaline, respectively. It is important to note that the adjective forms for these substances are **adrenergic** and **noradrenergic**, although the term *adrenergic* is sometimes used broadly to refer to NE- as well as EPI-related features. The adjective form for DA is **dopaminergic**. Varying amounts of these substances are found within the central nervous system, the peripheral nervous system, and the inner part of the adrenal glands (adrenal medulla). The adrenal medulla secretes EPI and NE into the bloodstream, where they act as hormones. You will recall from Chapter 3 that stimulation of catecholamine secretion from the adrenal glands is a vital part of the physiological response to stress.

The main emphasis in this chapter is on DA and NE, as the neurotransmitter function of EPI is relatively minor. We begin by considering the basic neurochemistry of the catecholamines, including their synthesis, release, and inactivation. This will be followed by a discussion of the neural systems for DA and NE, including the anatomy of these systems, the receptors for DA and NE, some of the drugs that act on these receptors, and several behavioral functions in which catecholamine transmitters are key participants.

Catecholamine Synthesis, Release, and Inactivation

The overall level of catecholamine neurotransmission depends on a complex interplay between neurotransmitter synthesis, release, and inactivation. Understanding each of these steps enables pharmacologists to design drugs that can modulate catecholamine activity for either therapeutic or experimental purposes.

Tyrosine hydroxylase catalyzes the rate-limiting step in catecholamine synthesis

Classical transmitters (see Chapter 3) like the catecholamines are manufactured in one or more biochemical steps. These synthetic pathways offer neurons a mechanism for regulating the amount of transmitter available for release. At the same time, they offer us the opportunity to intervene with drugs that alter transmitter synthesis in specific ways. For example, we may administer a precursor that will be converted biochemically into a particular neurotransmitter. One application of this approach is seen in neurological disorders in which the neurons that make a certain transmitter have been damaged. Precursor therapy represents an attempt to boost transmitter synthesis and release in the remaining undamaged cells. Alternatively, we may give subjects a drug that blocks a step in the biochemical pathway, thereby causing a depletion of the transmitter synthesized by that pathway. Neurotransmitter depletion is not as widely used clinically, but nevertheless, it can be valuable in certain experimental settings.

The synthesis of catecholamine neurotransmitters occurs in several steps, as shown in **FIGURE 5.2**. The biochemical pathway begins with the amino acid **tyrosine**. Like other amino acids, tyrosine is obtained from dietary protein and is transported from the blood into the brain. Each of the steps in catecholamine formation depends on a specific enzyme that acts as a catalyst (an agent that increases the rate of a chemical reaction) for that step. Neurons that use DA as their transmitter contain only the first two enzymes, **tyrosine hydroxylase** (**TH**) and **aromatic amino acid decarboxylase** (**AADC**), and thus the biochemical pathway stops at DA. In contrast, neurons that need to synthesize NE also possess the third enzyme, which is called **dopamine β-hydroxylase** (**DBH**).[1]

The conversion of tyrosine to dihydroxyphenylalanine (DOPA) by TH occurs at a slower rate than subsequent reactions in the biochemical pathway. Consequently, TH is the **rate-limiting enzyme** in the pathway because it determines the overall rate of DA or NE formation. The activity of TH is regulated by a variety of factors, including how much DA or NE is present within the nerve terminal. High catecholamine levels tend to inhibit TH, thus serving as a negative feedback mechanism. Another important factor is the rate of cell firing, because neuronal activity has a stimulatory effect on TH. The mechanism by which cell firing stimulates TH activity is through phosphorylation of the enzyme. TH can be phosphorylated by all of the second-messenger-associated protein kinases mentioned in Chapter 3, namely protein kinase A (PKA), protein kinase G (PKG), protein kinase C (PKC), and calcium/calmodulin kinase II (CaMKII) (Tekin et al., 2014). The enzyme can even be phosphorylated by kinases of the MAP (mitogen-activated protein) kinase system, including ERK (extracellular signal-related kinase). Although the exact effect of phosphorylation depends

[1]It is worth noting some of the basics of how enzymes are named. Hydroxylases like TH and DBH add a hydroxyl group (–OH) to the molecule they're acting on. A decarboxylase like AADC removes a carboxyl group (–COOH) from the molecule. These reactions can be seen by following the biochemical pathway shown in Figure 5.2.

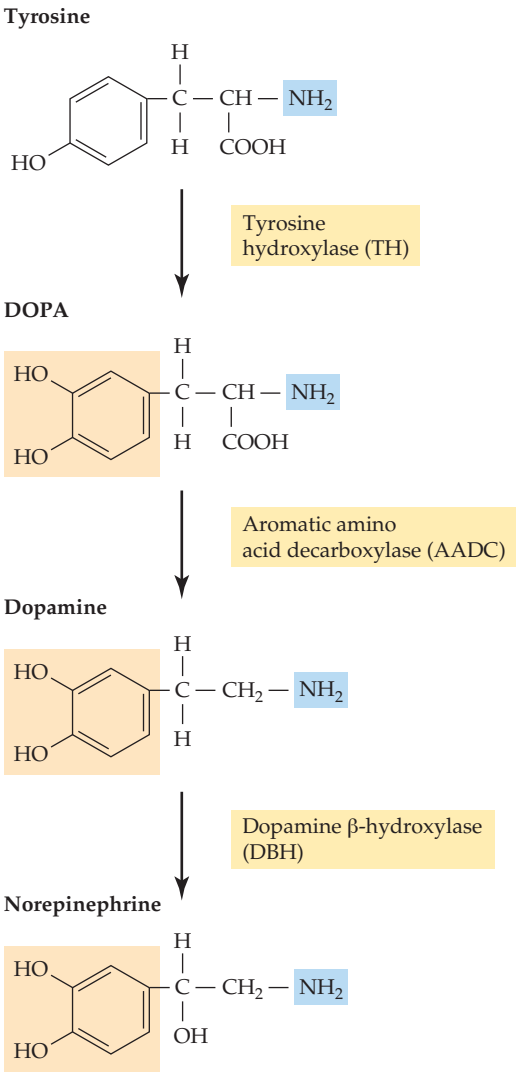


FIGURE 5.2 Multistep pathway of catecholamine synthesis Catecholamines are synthesized from the precursor amino acid tyrosine. Tyrosine hydroxylase and aromatic amino acid decarboxylase are found in all catecholaminergic neurons, whereas dopamine β-hydroxylase is present only in cells that use norepinephrine as their neurotransmitter.

on the particular kinase and on the location within the TH protein where the phosphate group is added, the general result is an increase in enzyme activity and, therefore, catecholamine synthesis.

These elegant mechanisms of phosphorylation activation and catecholamine feedback inhibition enable dopaminergic and noradrenergic neurons to carefully control their rate of neurotransmitter formation. When the levels are too high, TH is inhibited and catecholamine synthesis is slowed. But when the neurons are activated and firing at a high rate, such as during stress, TH is stimulated and catecholamine synthesis accelerates to keep up with the increased demand. Although the enzymes involved in synthesizing catecholamines (as well as other classical transmitters like acetylcholine and serotonin) can be found throughout the neurons using those transmitters, the rate of synthesis is greatest at the nerve endings near the sites of transmitter release. As mentioned in Chapter 3, this is important for the refilling of recycling vesicles.

As would be expected from our earlier discussion, catecholamine formation can be increased by the administration of biochemical precursors such as tyrosine and **L-DOPA**. Furthermore, certain cognitive functions such as working memory can be modestly but measurably enhanced by tyrosine administration under conditions of high cognitive demand or stress (reviewed by Hase et al., 2015; Jongkees et al., 2015). Such enhancement is thought to occur because dopaminergic and noradrenergic neurons are activated under the aforementioned conditions, and therefore tyrosine supplementation provides the necessary substrate to keep up with the demand for increased neurotransmitter synthesis. Clinically, L-DOPA has been the primary therapeutic agent used in the treatment of Parkinson's disease for many years (see Chapter 20). Drugs that reduce catecholamine synthesis by inhibiting one of the synthetic enzymes are not as clinically important, but they have had widespread use in both animal and human research. The best example is a drug known as **α-methyl-para-tyrosine** (**AMPT**). This compound blocks TH, thereby preventing overall catecholamine synthesis and causing a general depletion of these neurotransmitters. In a group of psychiatric studies reviewed by Booij and colleagues (2003), AMPT treatment caused a return of depressive symptoms in many patients who had been successfully treated with antidepressant medication. These findings suggest that at least in patients that exhibited increased symptomatology, recovery was dependent on the maintenance of adequate catecholamine levels in the brain.

Catecholamines are stored in and released from synaptic vesicles

Once catecholamines have been synthesized, they are transported into synaptic vesicles for later release (**FIGURE 5.3**). Vesicular packaging is important not only because it provides a means of releasing a predetermined amount of neurotransmitter (usually several thousand molecules per vesicle), but also because it protects the neurotransmitter from degradation by enzymes within the nerve terminal (see the next section). A specific protein in the vesicle membrane is responsible for vesicular catecholamine uptake. This protein recognizes several different monoamine transmitters and therefore is called the **vesicular monoamine transporter** (**VMAT**). There are actually two related VMATs: VMAT1 is found in the adrenal medulla, whereas VMAT2 is present in the brain. Both of these vesicular transporters are blocked by an interesting drug called **reserpine**, which comes from the

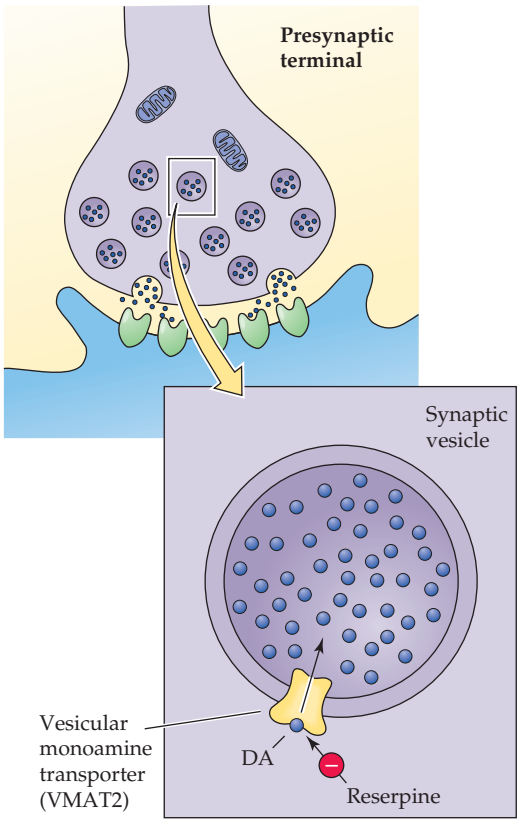


FIGURE 5.3 Catecholaminergic neurons use a vesicular monoamine transporter protein That protein, VMAT2, transports neurotransmitter molecules from the cytoplasm of the cell to the interior of the synaptic vesicles. This transport system is blocked by reserpine, which causes a marked depletion of catecholamine levels resulting from a lack of protection of the transmitter from metabolizing enzymes located outside of the vesicles.

roots of the plant *Rauwolfia serpentina* (snake root). Blocking the vesicular transporter means that DA and NE are no longer protected from breakdown within the nerve terminal. As a result, both transmitters temporarily drop to very low levels in the brain. The behavioral consequence of this neurochemical effect is sedation in animals and depressive symptoms in humans. Many years ago, a study by the eminent Swedish pharmacologist Arvid Carlsson and his colleagues (Carlsson et al., 1957) showed that the sedative effects of reserpine could be reversed by restoration of catecholamines with DOPA, the immediate biochemical precursor of DA (**FIGURE 5.4**). Carlsson's work, which played a key role in the development of the catecholamine theory of depression (see Chapter 18), resulted in his being a corecipient of the 2000 Nobel Prize in Physiology or Medicine.

Release of catecholamines normally occurs when a nerve impulse enters the terminal and triggers one or more vesicles to release their contents into the synaptic cleft through the process of exocytosis (see Chapter 3). Certain drugs, however, can cause a release of catecholamines independently of nerve cell firing. The most important of these compounds are the psychostimulants **amphetamine** and **methamphetamine**. In contrast to the behavioral sedation associated with reserpine-induced catecholamine depletion, catecholamine release leads to behavioral activation. In laboratory animals such as rats and mice, this activation may be shown by increased locomotor activity. At high doses, locomotor activation is replaced by **stereotyped behaviors** consisting of intense sniffing, repetitive head and limb movements, and licking and biting. Researchers believe that locomotion and stereotyped behaviors represent a continuum of behavioral activation that stems from increasing stimulation of DA receptors in the nucleus accumbens and striatum. In humans, amphetamine and methamphetamine produce

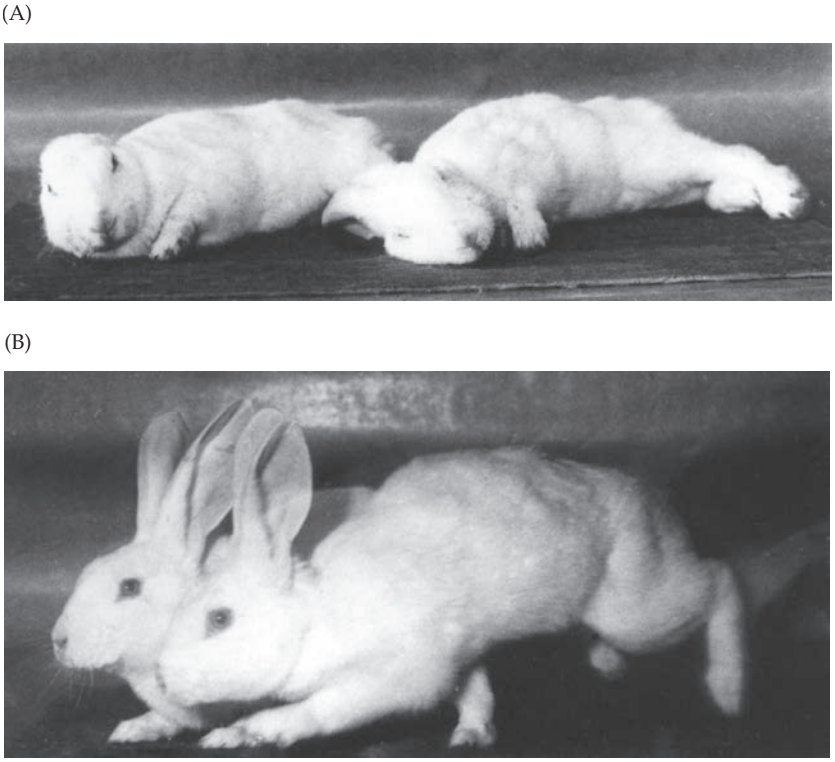


FIGURE 5.4 Role of catecholamine depletion in the behavioral depressant effects of reserpine Rabbits injected with reserpine (5 mg/kg IV) showed extreme behavioral sedation (A) that was reversed (B) by subsequent treatment with DOPA (200 mg/kg IV). (From Carlsson, 1960; photographs courtesy of Arvid Carlsson.)

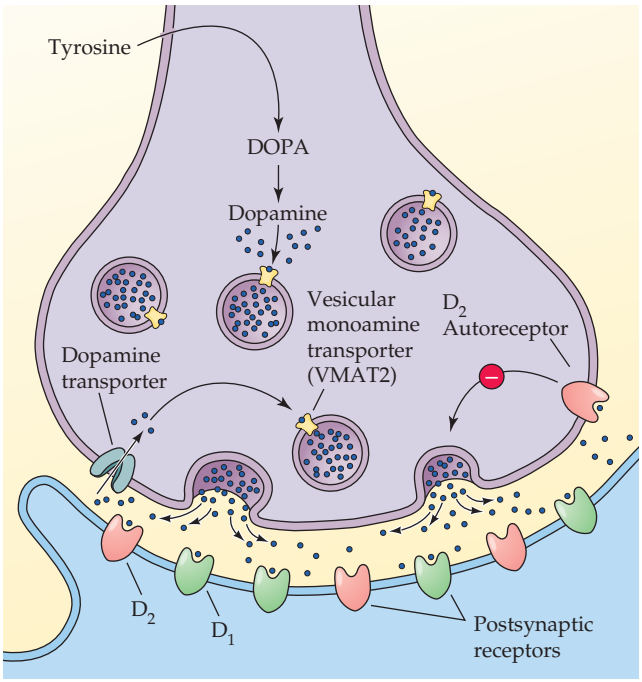


FIGURE 5.5 A typical dopaminergic neuron The membrane of its terminals possesses autoreceptors. When these receptors are stimulated, they inhibit subsequent DA release by the cell.

increased alertness, heightened energy, euphoria, insomnia, and other behavioral effects (see Chapter 12).

Catecholamine release is inhibited by autoreceptors located on the cell bodies, terminals, and dendrites of dopaminergic and noradrenergic neurons. Terminal autoreceptors and other features of a typical dopaminergic neuron are illustrated in **FIGURE 5.5**. Current evidence indicates that at least in the case of DA, these autoreceptors inhibit neurotransmitter release through two combined mechanisms: (1) by inhibiting the action of voltage-gated Ca^{2+} channels in the nerve terminal membrane and (2) by enhancing the opening of a specific type of voltage-gated K^+ channel in the terminal (Ford, 2014). The first mechanism would reduce DA release by directly reducing the amount of activity-mediated Ca^{2+} influx needed for vesicular exocytosis, whereas the second mechanism could indirectly reduce Ca^{2+} influx by shortening the duration of action potentials entering the terminal. Consequently, if a dopaminergic cell fires several action potentials in a row, we can imagine that DA released by the first few impulses stimulates the terminal autoreceptors and reduces the amount of DA released by the later action potentials. On the other hand, the somatodendritic autoreceptors exert a somewhat different effect on DA and NE neurons. As mentioned in Chapter 3, these autoreceptors inhibit release indirectly by reducing the rate of firing of the cell.

Firing pattern of the neuron is yet another factor that influences catecholamine release. This has been studied extensively in midbrain dopaminergic neurons, which fire in two different patterns. In **single-spiking mode**, the cell generates action potentials ("spikes") that appear at irregular intervals but with a typical average frequency of 4 to 5 Hz (4–5 spikes per second). Excitatory input to the cell can switch it from single-spiking mode to **burst mode**, which is characterized by trains of 2 to 20 spikes at a frequency of approximately 20 Hz. The difference between these firing modes can be seen in **FIGURE 5.6**, which shows representative firing patterns of dopaminergic neurons within the ventral tegmental area (VTA; see section below entitled Organization and Function of the Dopamine System for more information on this brain area) in a rat under conditions of quiet wakefulness, slow-wave sleep, rapid-eye-movement (REM) sleep, and feeding. Burst firing can be seen during REM sleep and feeding, which is the result of greater dopaminergic neuronal activation. DA released when the cell is in single-spiking mode is often called **tonic release**, whereas DA released in burst mode is called **phasic release**. Importantly, the extracellular level of DA in areas of release following a burst of spikes is greater than would be expected from the number of spikes in the burst, mainly because release of neurotransmitter is occurring faster than it can be cleared and/or metabolized (Chergui et al., 1994). This finding means that activation of burst firing in dopaminergic neurons is

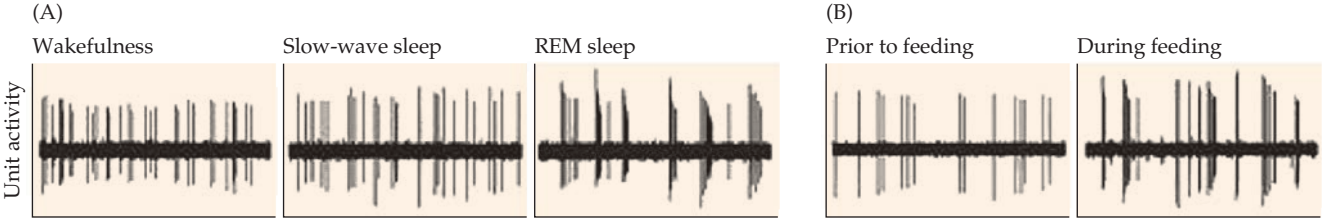


FIGURE 5.6 Spike firing patterns of dopaminergic neurons in the rat VTA under different behavioral states Unit activity (i.e., firing of an individual nerve cell) was recorded from VTA dopaminergic neurons in rats (A) during quiet wakefulness, slow wave sleep, and REM sleep, and (B) before and during feeding of a palatable food. The electrophysiological traces show short bursts of action potentials (burst mode) during REM sleep and feeding, which contrast with single action potentials (single-spiking mode) during the other behavioral states. (From Dahan et al., 2007.)

especially important for producing large increases in DA transmission in the brain.

Although the DA nerve terminal illustrated in Figure 5.5 looks like a typical axon bouton, it is worth noting that in many parts of the nervous system, both DA and NE axons form *en passant* ("in passing") synapses in which the fibers exhibit repeated swellings (termed **varicosities**) along their lengths that are filled with synaptic vesicles and that represent the sites of neurotransmitter release. The photomicrograph shown in **FIGURE 5.7** was produced using an antibody against DBH that allowed staining of the noradrenergic varicosities in the lateral geniculate nucleus (part of the thalamus) of a 21-day-old rat (Latsari et al., 2004). One can readily see the numerous varicosities along each axon that release NE into the extracellular fluid, not at specific synapses. This is an example of volume transmission (see Chapter 3).

As will be discussed below, the DA and NE systems possess several different subtypes of receptors. Here we will just mention that the DA autoreceptor is of the D_2 receptor subtype, and the NE autoreceptor is of the α_2 subtype. For technical reasons, it was extremely difficult for many years to determine how important these autoreceptors are for the normal functioning of catecholamine neurons and how those receptors might influence the cellular and behavioral responses to various pharmacological agents. However, several years ago Bello and colleagues (2011) developed a powerful genetic method for deleting the D_2 receptor gene *just* from the DA neurons in mice, thus eliminating the DA autoreceptors but allowing normal expression of postsynaptic D_2 receptors (i.e., D_2 receptors in non-DA neurons). Results showed that the DA neurons from the mutant mice released more DA, that the mutant mice were more spontaneously active than controls (we will see later that DA plays a key role in regulating locomotor activity), and that the animals were more sensitive to the behavioral effects of cocaine, a drug that exerts many of its effects through the DA system (see Chapter 12 for more details). These findings confirm that D_2 autoreceptors are, indeed, functionally active under normal circumstances and that they influence behavioral responses to dopaminergic agents.

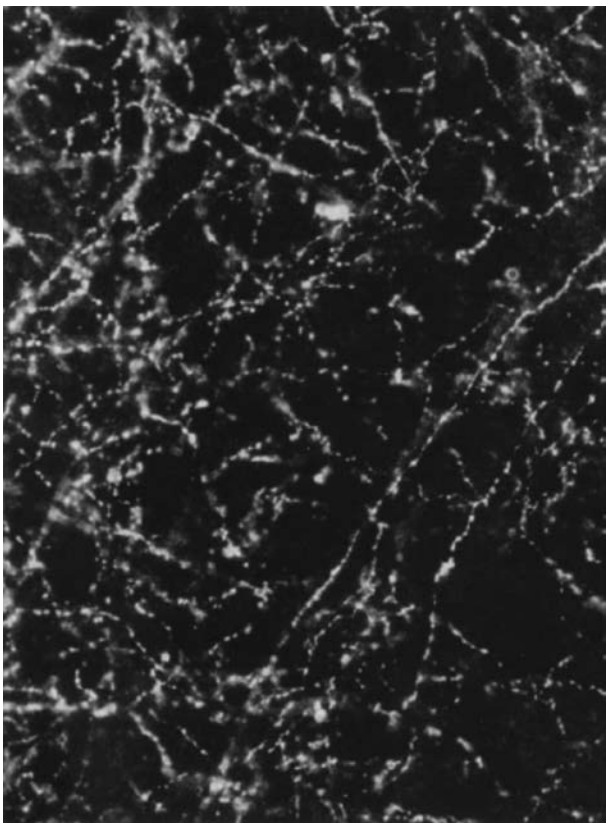


FIGURE 5.7 Varicosities along the lengths of noradrenergic fibers The fibers shown are innervating the lateral geniculate nucleus of a 21-day-old rat. The tissue was stained using immunocytochemistry with an antibody to DBH, and noradrenergic fibers were visualized with dark-field microscopy, which reverses the light and dark areas of the image (i.e., darkly stained fibers appear light). (From Latsari et al., 2004.)

Drugs that stimulate autoreceptors, like the neurotransmitter itself, inhibit catecholamine release. In contrast, autoreceptor antagonists tend to enhance the rate of release by preventing the normal inhibitory effect of the autoreceptors. We can see these effects illustrated dramatically in the case of the noradrenergic α_2-autoreceptor system. Withdrawal from opioid drugs such as heroin and morphine activates the noradrenergic system, which is one of the factors leading to withdrawal symptoms such as increased heart rate, elevated blood pressure, and diarrhea. For this reason, α_2-agonists such as **clonidine** (**Catapres**) are often used to treat the symptoms of opioid withdrawal because of their ability to stimulate the autoreceptors and inhibit noradrenergic cell firing. In contrast, experimental administration of the α_2-antagonist **yohimbine**, which blocks the autoreceptors and thus increases noradrenergic cell firing and NE release, was found to provoke withdrawal symptoms and drug craving in opioid-dependent patients (Stine et al., 2002) (**FIGURE 5.8**). Norepinephrine may also be involved in producing feelings of anxiety, especially in patients suffering from a mental illness called *panic disorder* (see Chapter 17). Consequently, yohimbine induces anxiety in such patients and may even trigger a panic attack (Charney et al., 1998). Such an effect obviously provides no therapeutic benefit, but it has yielded useful research information related to a possible role for NE in panic disorder and other anxiety-related disturbances.

Catecholamine inactivation occurs through the combination of reuptake and metabolism

Inactivation of catecholamines depends on the two different kinds of processes first mentioned in Chapter 3.

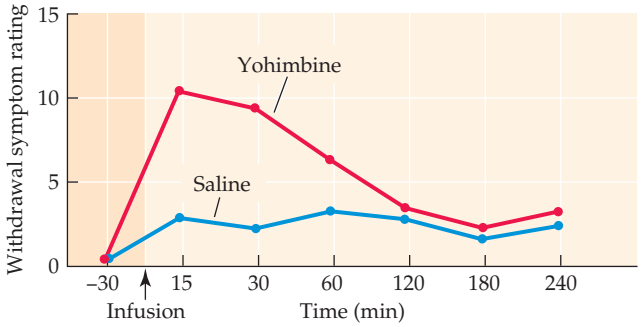


FIGURE 5.8 Noradrenergic activity contributes to symptoms of opioid withdrawal When opioid-dependent patients were infused intravenously either with the α_2-receptor antagonist yohimbine (0.4 mg/kg) or a saline control solution, yohimbine but not saline caused a rapid increase in experimenter-rated withdrawal symptoms over the next 60 minutes. (After Stine et al., 2002.)

The first process is reuptake. Much of the released DA and NE is taken up again into the nerve terminal by means of specific transporter proteins in the nerve cell membrane. That is, dopaminergic neurons contain a **DA transporter** (see Figure 5.5), whereas noradrenergic neurons contain a slightly different protein that is logically called the **NE transporter**. After the neurotransmitter molecules are returned to the terminal, some of them are repackaged into vesicles for rerelease, and the remainder are broken down and eliminated. It is important to keep in mind that neurotransmitter transporters differ from the autoreceptors discussed earlier in terms of both structure and function.

The importance of reuptake for catecholamine functioning can be seen when the DA or NE transporter is missing. For example, mutant mice lacking a functional gene for the DA transporter do not show the typical behavioral activation in response to psychostimulants like cocaine or amphetamine, whereas genetic deletion of the NE transporter gene causes increased sensitivity to these same drugs (F. Xu et al., 2000). A role for the NE transporter in cardiovascular function was demonstrated by a case study of identical twins carrying a mutation of the NE transporter gene (Shannon et al., 2000). These patients exhibited abnormally high NE levels in the bloodstream, along with heart rate and blood pressure abnormalities. These findings are consistent with animal studies showing that transporter-mediated uptake plays a vital role in the normal regulation of catecholamine activity.

Since the transporters are necessary for clearance of released catecholamines from the extracellular fluid, transporter-blocking drugs enhance DA or NE transmission by increasing the amount of neurotransmitter available to activate the receptors for these transmitters. This is an important mechanism of action of several kinds of psychoactive drugs, including the **tricyclic antidepressants**, which inhibit the reuptake of both NE and the non-catecholamine transmitter serotonin (5-HT) (see Chapter 18). There are also a few compounds in clinical use that more selectively inhibit NE uptake by blocking the NE transporter. These include **reboxetine** (**Edronax**), which is an antidepressant drug, and **atomoxetine** (**Strattera**), which is used in the treatment of ADHD. Finally, another important transporter-blocking drug is **cocaine**, which inhibits the reuptake of all three major monoamine transmitters: DA, NE, and 5-HT (see Chapter 12).

Although reuptake can quickly terminate the synaptic actions of catecholamines, there must also be processes of metabolic breakdown to prevent excessive neurotransmitter accumulation. The breakdown of catecholamines primarily involves two enzymes: **catechol-*O*-methyltransferase** (**COMT**) and **monoamine oxidase** (**MAO**). There are two types of MAO: MAO-A and MAO-B. The relative importance of each one depends on the species, the brain area, and which neurotransmitter is being metabolized. NE is metabolized primarily by MAO-A in both humans and rodents, whereas DA is metabolized primarily by MAO-B in humans and by MAO-A in rodents (Napolitano et al., 1995). Although COMT doesn't have two distinctly different forms of the enzyme like MAO, researchers have found variation in the COMT gene that causes individuals to have varying rates of DA metabolism. This genetic variation, its influence on brain DA, and functional implications are described in **Web Box 5.1**.

The action of COMT and MAO, either individually or together, gives rise to several catecholamine **metabolites** (breakdown products). We mention only the most important ones here. In humans, DA has only one major metabolite, which is called **homovanillic acid** (**HVA**). In contrast, NE breakdown gives rise to several important compounds, including **3-methoxy-4-hydroxy-phenylglycol** (**MHPG**) and **vanillymandelic acid** (**VMA**). Metabolism of NE within the brain primarily leads to MHPG, whereas VMA is the more common metabolite in the peripheral nervous system. The brain metabolites HVA and MHPG make their way into the cerebrospinal fluid for subsequent clearance from the brain into the bloodstream and, along with VMA, are eventually excreted in the urine. Levels of these substances in the various fluid compartments (i.e., blood and urine for all three metabolites, and cerebrospinal fluid for HVA and MHPG) provide a rough indication of catecholaminergic activity in the nervous system. Such measurements have sometimes been used to help discern the possible involvement of these neurotransmitters in mental disorders such as depression and schizophrenia (see Chapters 18 and 19).

Not surprisingly, drugs that inhibit catecholamine-metabolizing enzymes lead to an accumulation of these

transmitters. Historically, this has been most important in the case of **MAO inhibitors** such as **phenelzine** (**Nardil**) or **tranylcypromine** (**Parnate**), both of which are nonselective MAO inhibitors (i.e., they block both MAO-A and MAO-B) that have long been used in the treatment of clinical depression (see Chapter 18 for additional discussion of the use of MAO inhibitors in depression). Drugs that selectively inhibit either MAO-A or MAO-B have also been developed (see review by Finberg, 2014). For example, **moclobemide** is a selective MAO-A inhibitor that has been approved in several countries outside of the United States for the treatment of depression and social anxiety. **Selegiline** (**Eldepryl**) and **rasagiline** (**Azilect**) are inhibitors of MAO-B that are used clinically to elevate brain DA levels in Parkinson's disease (see Chapter 20). Finally, COMT inhibitors such as **entacapone** (**Comtan**) and **tolcapone** (**Tasmar**) are administered as supplemental therapies to enhance the effectiveness of L-DOPA in treating Parkinson's disease. This is done not so much to prevent the metabolism of DA but rather to block the metabolism of L-DOPA by COMT before the precursor reaches the brain.

Section Summary

- The major catecholamine transmitters in the brain are DA and NE.
- Catecholamines are synthesized in several steps from the amino acid tyrosine. The first, and also rate-limiting, step in this biochemical pathway is catalyzed by the enzyme TH.
- Catecholamine synthesis can be enhanced by administration of biochemical precursors such as tyrosine and L-DOPA, the latter of which is the main therapeutic agent used in the treatment of Parkinson's disease.
- Once they have been synthesized, catecholamines are stored in synaptic vesicles for subsequent release.
- Catecholamine release is controlled, in part, by the rate of cell firing. For example, dopaminergic neurons can fire either in single-spike or burst mode, leading to tonic or phasic release of DA. Burst firing causes especially large amounts of DA to be available to postsynaptic cells, primarily because the rate of DA release is greater than the rate at which the neurotransmitter can be cleared and/or metabolized.
- The process of release is controlled by inhibitory autoreceptors located on the cell body, dendrites, and terminals of catecholamine neurons. Terminal autoreceptors function by inhibiting voltage-gated Ca^{2+} channels and enhancing voltage-gated K^+ channels in the terminal membrane.
- DA autoreceptors are of the D_2 subtype, whereas NE autoreceptors are of the α_2 subtype.
- Catecholamines are inactivated by reuptake from the extracellular fluid mediated by specific DA and NE transporters, and also by enzymatic degradation. MAO-A, MAO-B, and COMT are enzymes that are important in catecholamine metabolism.
- The major catecholamine metabolites are HVA for DA, and MHPG and VMA for NE.
- A number of drugs can modify catecholaminergic function by acting on the processes of synthesis, release, reuptake, or metabolism. Some of these compounds are used either clinically to treat various disorders (e.g., depression, ADHD, and Parkinson's disease) or experimentally to study the DA or NE system.

Organization and Function of the Dopaminergic System

The dopaminergic system originates in several cell groups located primarily in the brainstem. Ascending fibers from these cells innervate a number of forebrain areas where they release DA onto five different receptor subtypes.

Two important dopaminergic cell groups are found in the midbrain

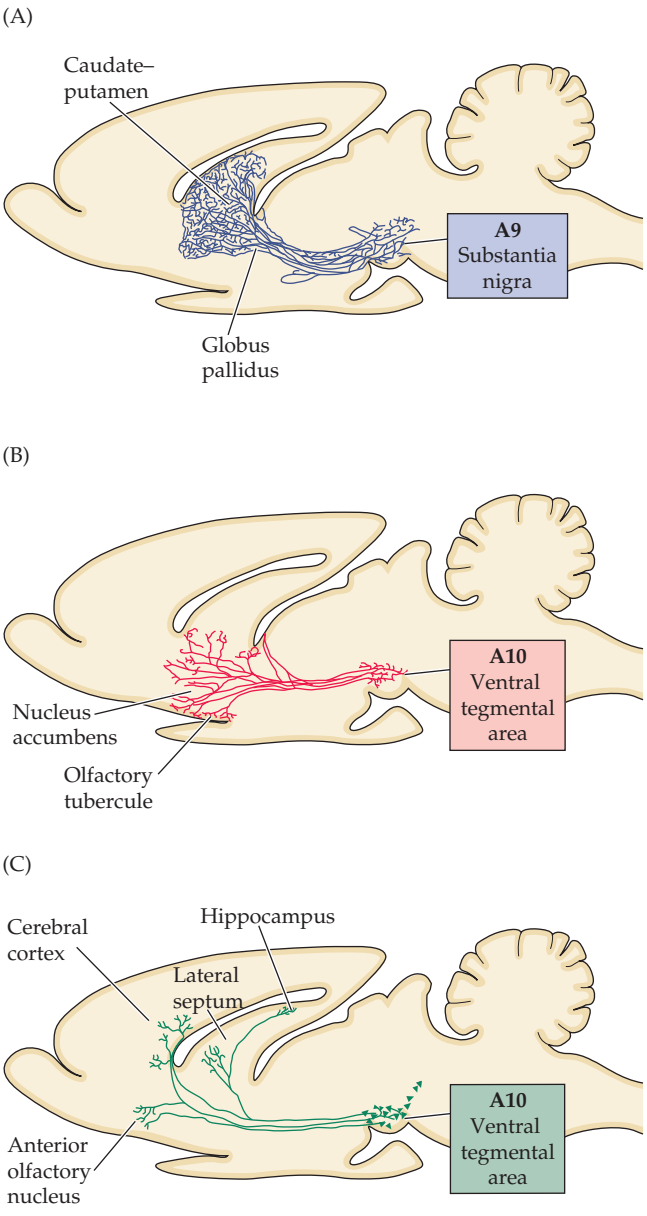
In the early 1960s, Swedish researchers first began to map the location of DA- and NE-containing nerve cells and fibers in the brain using a fluorescence method (Dahlström and Fuxe, 1964). They developed a classification system in which the catecholamine cell groups (clusters of neurons that stained for either DA or NE) were designated with the letter *A* plus a number from 1 to 16. According to this system, cell groups A1 to A7 are noradrenergic, whereas groups A8 to A16 are dopaminergic. In this book, we will focus on only a few catecholaminergic cell groups that are of particular interest to psychopharmacologists. To identify the various systems arising from these cells, we will use both the Swedish classification system and standard anatomical names.

Several dense clusters of dopaminergic neuronal cell bodies are located near the base of the mesencephalon (midbrain). Particularly important is the A9 cell group, which is associated with a structure called the **substantia nigra**, and the A10 group, which is found in a nearby area called the **ventral tegmental area** (**VTA**). Axons of dopaminergic neurons in the substantia nigra ascend to a forebrain structure known as the caudate–putamen or dorsal striatum. Note

that the caudate and putamen are separate, though closely related, structures in humans and non-human primates, whereas the two structures are fused in rodents (hence "caudate–putamen"). Nerve tracts in the central nervous system are often named by combining the site of origin of the fibers with their termination site. Hence, the pathway from the substantia nigra to the dorsal striatum is called the **nigrostriatal tract** (**FIGURE 5.9A**).

Two other important ascending dopaminergic systems arise from cells of the VTA. Some of the axons from these neurons travel to various structures of the limbic system, including the nucleus accumbens (the major component of the ventral striatum), septum, amygdala, and hippocampus. These diverse projections constitute the **mesolimbic dopamine pathway** (*meso* represents mesencephalon, which is the site of origin of the fibers; *limbic* stands for the termination of fibers in structures of the limbic system) (**FIGURE 5.9B**). Other DA-containing fibers from the VTA go to the cerebral cortex, particularly the prefrontal cortex (PFC). This group of fibers is termed the **mesocortical dopamine pathway** (**FIGURE 5.9C**). Together, the mesolimbic and mesocortical pathways are very important to psychopharmacologists because they have been implicated in the neural mechanisms underlying drug abuse (see Chapter 9) and also schizophrenia (see Chapter 19).

A few other sites of dopaminergic neurons can be mentioned briefly. For example, a small group of cells in the hypothalamus gives rise to the **tuberohypophyseal dopamine pathway**. This pathway is important in controlling the secretion of the hormone prolactin by the pituitary gland. There are also DA-containing neurons within sensory structures such as the olfactory bulbs and the retina.



Ascending dopamine pathways have been implicated in several important behavioral functions

One of the key functions of DA innervation of the dorsal striatum via the nigrostriatal tract is to facilitate voluntary movement. Loss of this function is illustrated dramatically in the case of Parkinson's disease, which involves a massive loss of DA neurons in the substantia nigra and consequent DA denervation of the dorsal striatum. Parkinson's disease is characterized by progressive motor dysfunction, typically beginning with tremors and advancing to postural disturbances, akinesia (lack of movement), and rigidity (see Chapter 20 for a more extensive discussion of this disorder). Information about the motor functions of DA in humans has also been derived from individuals with genetic mutations that interfere with DA neurotransmission. Some of these mutations and the clinical symptoms they produce are discussed in **BOX 5.1**.

In experimental animals, a range of symptoms, including those seen in Parkinson's disease, can be produced by administering **neurotoxins** that damage or destroy midbrain DA neurons and lesion their ascending pathways. Two such compounds are

FIGURE 5.9 The ascending DA system can be divided into three pathways The nigrostriatal pathway (A) originates in the substantia nigra (A9 cell cluster) and innervates the caudate–putamen (dorsal striatum). The mesolimbic pathway (B and C) originates in the VTA (A10 cell cluster) and innervates various limbic system structures such as the nucleus accumbens, hippocampus, lateral septum, and amygdala (not shown here). The mesocortical pathway (C) also originates in the VTA and innervates the cerebral cortex.

BOX 5.1 Clinical Applications

Mutations That Affect Dopamine Neurotransmission

Later in this section and also in Box 5.2, we will discuss ways in which researchers create mutant strains of mice to study the role of DA in behavioral functioning and the molecular mechanisms of action of dopaminergic drugs. Such technology has not yet been applied to human beings, not only because of technical obstacles but also because of ethical objections to performing genetic engineering in people. Nevertheless, we can learn a lot about the dopaminergic system in humans by studying the consequences of naturally occurring mutations of DA-related genes.

To date, almost all clinically significant mutations associated with the dopaminergic system involve either DA synthesis or uptake (reviewed by Ng et al., 2014; 2015). Segawa and colleagues (1976) were among the first to identify a clinical syndrome caused by mutations in the genes that govern these processes. The researchers reported a genetic disorder characterized by progressive dystonia, which is a type of movement disorder in which the patient experiences involuntary muscle contractions causing repetitive twisting movements, postural problems, tremors, and/or rigidity. Although there are many different types and causes of dystonia, in this instance the symptoms usually appear in childhood (around 6 to 8 years of age) and show diurnal (across the day) variation, with the least symptomatology upon awakening and worsening of symptom severity as the day goes on (Gordon, 2007; Segawa, 2010). Later research showed that what is now called Segawa's disease is produced by mutations in the gene coding for the enzyme guanosine triphosphate cyclohydrolase 1 (GCH1). GCH1 plays an indirect but important role in DA synthesis because this enzyme catalyzes the first step in the formation of tetrahydrobiopterin, a necessary cofactor used by TH in converting tyrosine to DOPA (see Figure 5.2). Even with the mutated gene, patients with Segawa's disease usually have some GCH1 activity and therefore can synthesize a certain amount of DA. However, levels of tetrahydrobiopterin (and therefore presumably DA as well) decline over the course of the day, which explains why symptoms are the least severe in the early morning but become more pronounced as the day progresses. The key role of DA in this disorder is shown by the fact that symptoms are greatly improved if the patient is treated with L-DOPA. As is evident from Figure 5.2, this drug bypasses the TH step, thus allowing normal amounts of DA to be synthesized by the brain. Segawa's disease is quite rare, with an incidence of only 0.5 cases per 100,000 people. But there are many different mutations of the GCH1 gene that can give rise to the disorder (Ng et al., 2015).

What about mutations directly in the biochemical pathway shown in Figure 5.2? Although also rare, such mutations occasionally do occur, and when they do, the effects are more severe than in the case of *GCH1* mutations. Loss-of-function mutations of the TH gene give rise to two different clinical phenotypes (Ng et al., 2015). Patients with type A, which is the more common phenotype, display a syndrome of hypokinesis (reduced movement), rigidity, and dystonia. Symptom onset occurs in infancy or childhood. Symptoms of the type B phenotype begin very early (within 3 months after birth) and include severe Parkinson's disease–like motor impairment, hypotonia (poor muscle tone) and dystonia, and cognitive deficits. Analysis of cerebrospinal fluid (CSF) shows very low levels of the DA metabolite HVA but normal levels of the serotonin metabolite 5-hydroxyindoleacetic acid, which demonstrates the selectivity of the disorder for the dopaminergic system. As in the case of Segawa's disease, treatment with L-DOPA (sometimes augmented with other drugs to enhance the production or inhibit the metabolism of the DA that is produced) is the most effective remedy for reducing symptoms (Ng et al., 2014a).

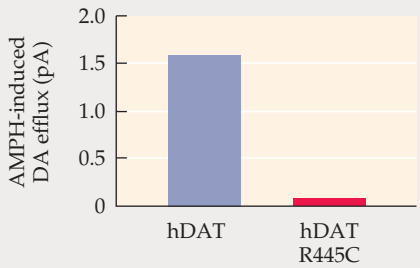
Clinically significant mutations in the gene for AADC, the enzyme that converts DOPA to DA, have also been reported in a small number of patients. It is no surprise that such patients develop severe motor problems similar to those seen in patients with *TH* mutations (Ng et al., 2015). L-DOPA is not the typical therapy for patients with AADC deficiency, because lack of that enzyme precludes synthesis of much DA from its precursor. Rather, patients are typically treated with DA receptor agonists (because the postsynaptic receptors are still present and functional) and/or MAO inhibitors to slow down DA metabolism (Ng et al., 2014a). Another key medication is pyridoxine (vitamin B_6), which is converted by the body to pyridoxal phosphate, a necessary cofactor for AADC. The mechanism of the clinical benefit of pyridoxine is not fully understood, but one notion is that cofactor excess optimizes any residual enzyme activity that may be present in the patient.

Recent studies have identified a small number of patients with loss-of-function mutations in the gene for the DA transporter, the membrane protein

BOX 5.1 Clinical Applications (*continued*)

expressed by dopaminergic neurons that mediates reuptake and reuse of DA after its release. Some mutant transporters show a complete loss of functionality (see **Figure**), whereas others show only a partial loss. In laboratory animals, decreased DA transporter function (produced, for example, by administration of a DA reuptake inhibitor like cocaine) leads to elevated extracellular levels of DA and consequent enhancement of dopaminergic transmission. We have seen that severe motor impairments are produced either by genetic loss of DA synthesis or degeneration of midbrain dopaminergic neurons, as occurs in Parkinson's disease. This would lead one to predict that reduced DA reuptake should cause excessive motor activity, and indeed that is exactly what is observed in mutant mice in which the DA transporter has been knocked out (see Box 5.2). Surprisingly, however, patients with DA transporter mutations exhibit a syndrome of *impaired* movement characterized by the development of Parkinsonian symptoms and dystonia (Hansen et al., 2014; Kurian et al., 2009; Ng et al., 2014b). Different mutations of the gene seem to cause variability in age of onset and symptom profile. Some patients display symptom onset in infancy and rapid progression of symptom severity, whereas others show later onset in childhood, adolescence, or adulthood with a slower disease progression. The symptoms of infants with early onset disorder can resemble those seen in cerebral palsy, leading researchers to speculate that some cases of presumed cerebral palsy may be misdiagnosed. Researchers are still unclear about why patients with loss-of-function DA transporter mutations show decreased motor function instead of the hyperactivity observed in transporter knockout mice. Two theories proposed thus far are (1) depletion of DA in the dopaminergic neurons due to lack of neurotransmitter uptake for repackaging and release, and/or (2) possible progressive degeneration of DA neurons as seen in adult-onset Parkinson's disease. While some evidence for the latter theory has been presented for one patient with early-onset disease (Hansen et al., 2014), we don't yet know whether a degenerative process can account for the symptomatology in other patients with this disorder. Moreover, unlike genetic disorders that compromise DA synthesis, the syndrome associated with DA transporter loss of function does not respond well to currently available drug therapies (Ng et al., 2014a).

We have seen that genetic deficiencies in DA synthesis or reuptake lead to significant motor problems that usually begin during development (early infancy in the case of TH or AADC deficiency, childhood in the case of GCH1 deficiency, and typically either infancy or childhood for loss of DA transporter function). No adequate treatments are available for patients who lack functional DA reuptake, where patients with DA synthesis disorders can be treated successfully as long as DA neurotransmission can be adequately restored. It is worth noting that mutant mice that have been genetically engineered to completely lack TH activity inevitably die either in utero or relatively soon after birth if they are left untreated (Kobayashi and Nagatsu, 2005). Although this fact may seem discrepant with findings in human patients, it is possible that fetuses that carry the most severe loss-of-function TH mutations also die before birth and are spontaneously aborted without diagnosis, and that some human patients would not survive beyond infancy without L-DOPA treatment. We do not know whether the additional (e.g., motivational) symptoms of 6-OHDA lesions observed in animal models would be present in TH-deficient human patients if they did not receive treatment. These interesting questions reveal some of the complexities that arise when human clinical research is compared with studies using animal models.



Loss of function of mutant DA transporter obtained from a clinical patient Either the wild-type human DA transporter (hDAT) or the mutant transporter obtained from a patient with deficient DA uptake (hDAT R445C) was expressed in non-neuronal cells and studied in vitro. Both types of cells were prefilled with DA by microinjection, after which they were exposed to amphetamine. As discussed in more detail in Chapter 12, amphetamine and related drugs cause a rapid release of DA by reversing the direction of transport by the DA transporter protein. The figure shows a virtually complete lack of DA release by the mutant compared with the wild-type transporter. Separate tests of actual DA uptake by this and additional mutant transporters identified in other clinical patients revealed little or no uptake, consistent with the results shown in the figure. (After Ng et al., 2014b, CC-BY 3.0.)

6-hydroxydopamine (**6-OHDA**) and **1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine** (**MPTP**). Although the two substances produce similar damage to the dopaminergic system with similar functional consequences, here we focus on the effects produced by 6-OHDA administration. The use of MPTP as a DA neurotoxin will be covered later in Chapter 20. To lesion the central dopaminergic system with 6-OHDA, one must inject the drug directly into the brain since it does not readily cross the blood–brain barrier. The toxin is taken up mainly by the catecholaminergic neurons (thus sparing neurons that use other neurotransmitters) because of its close structural similarity to DA. Once the toxin is inside the neuron, the nerve terminals are severely damaged, and sometimes the entire cell dies. Animals with bilateral 6-OHDA lesions of the ascending dopaminergic pathways show severe behavioral dysfunction. They exhibit sensory neglect (they pay little attention to stimuli in the environment), motivational deficits (they show little interest in eating food or drinking water), and motor impairment (like patients with Parkinson's disease, they have difficulty initiating voluntary movements). It is also possible to damage the nigrostriatal DA pathway on only one side of the brain, as illustrated in **FIGURE 5.10**. In this case, the lesioned animals display a postural asymmetry characterized by leaning and turning toward the damaged side of the brain because of the dominance of the untreated side. The severe abnormalities seen following either bilateral or unilateral lesions of the DA system indicate how important this neurotransmitter is for many behavioral functions, not just for initiation of movement.

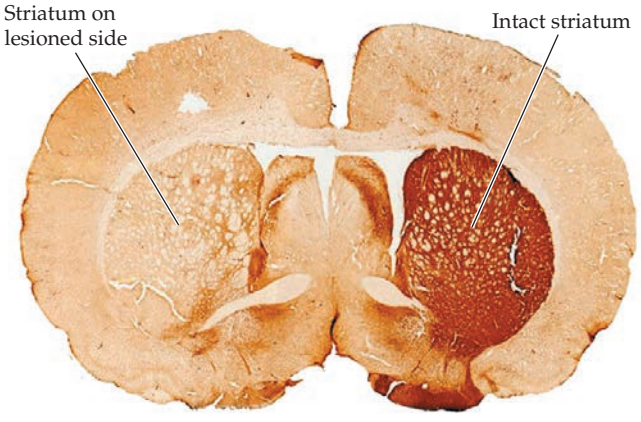


FIGURE 5.10 Unilateral damage to the nigrostriatal pathway Shown here is a photomicrograph of a tissue section from the brain of a rat that had received a unilateral 6-OHDA lesion of the medial forebrain bundle, which contains the axons of the nigrostriatal pathway. The section, which was stained using an antibody against tyrosine hydroxylase, depicts the loss of dopaminergic fibers and terminals in the striatum on the side of the lesion. (Courtesy of Michael Zigmond and Ann Cohen.)

At the beginning of this chapter we mentioned some of the characteristics of mice genetically engineered to lack DA. These mice constitute another model that illustrates many of the same functions of the dopaminergic system learned from bilateral 6-OHDA-lesioned animals. Two studies from different laboratories published over 20 years ago showed that if the gene for TH is disrupted embryonically in mice (*Th*-knockout mice), the animals die either in utero or shortly after they're born (Kobayashi et al., 1995; Zhou et al., 1995). Although disruption of the TH gene prevents the synthesis of all catecholamines, researchers noted that a similar early lethality occurred when the DBH gene, which is necessary for NE but not for DA synthesis, was knocked out instead of the TH gene (Thomas et al., 1995).[2] This finding raised the possibility that lack of NE, not DA, was responsible for the early mortality of *Th*-knockout mice. To determine more selectively the possible role of DA in behavioral and physiological development, Palmiter's lab created the so-called **dopamine-deficient** (**DD**) **mouse**. This was done very cleverly by genetically knocking out *Th* but restoring the gene in *Dbh*-expressing (i.e., noradrenergic) cells. In this manner, the ability to synthesize catecholamines was lacking only in the dopaminergic cells. There is some similarity between DD mice and genetically normal animals that have been given 6-OHDA, although two important differences should be mentioned. First, the dopaminergic neurons are physically undamaged in the DD mice; they just can't synthesize DA. Second, in contrast to animals given a 6-OHDA lesion in adulthood, DD mice lack DA throughout development because the genetic manipulations are performed at an early embryonic stage.

As described in the chapter opener, DD mice appear normal from birth through the first week of postnatal life. Afterward, however, they stop gaining weight and exhibit severe aphagia (lack of feeding behavior), adipsia (lack of drinking), and hypoactivity (Zhou and Palmiter, 1995). Remarkably, all of these behaviors can be restored within minutes by an injection of L-DOPA, which, of course, bypasses the TH-mediated step and is converted directly to DA in the dopaminergic neurons (see Figure 5.2). But once the effects of the L-DOPA wear off within a few hours, the mice show a tremendous reduction in movement and seem to have no interest in eating food or drinking despite the fact that they can physically grasp food and swallow liquids that are placed into their mouths. Space limitations prevent us from fully describing all of the subsequent research

[2]Examination of the *Dbh*-knockout mice showed defects in the heart, suggesting that the death of the animals was caused, at least in part, by a failure of normal heart development.

that has been performed with DD mice; however, one key finding worth noting is that many of the behavioral deficits observed in these mutant animals can be rescued by selectively restoring DA synthesis just in the caudate–putamen (dorsal striatum). Among the rescued behaviors are locomotor activity, eating and drinking behavior, nest building, and performance in many kinds of learning and memory tasks (Darvas and Palmiter, 2009; Palmiter, 2008). In summary, the findings obtained from DD mice taken together with evidence from nigrostriatal DA lesion studies point to a critical role for the dorsal striatum in many of the activational, motivational, and cognitive functions of DA.

The mesocortical and mesolimbic dopaminergic pathways also have well-described roles in behavioral regulation. The mesocortical input to the PFC helps regulate various cognitive functions such as attention and working memory (reviewed in Clark and Noudoost, 2014; Ranganath and Jacob, 2016). Indeed, DA hypoactivity in the PFC is thought to underlie some of the cognitive deficits in patients with schizophrenia (see Chapter 19). In contrast, the mesolimbic dopaminergic system, particularly the part of the system that innervates the nucleus accumbens, has a different set of functions. One of these functions is to activate behavioral arousal and locomotor behavior. Accordingly, microinjection of DA into specific parts of the nucleus accumbens produces locomotor hyperactivity (Campbell et al., 1997). A second key function of the mesolimbic pathway involves the mediation of natural rewards such as food and sex, and also artificial rewards such as those produced by addictive drugs and (for some people) gambling. The role of DA in reward is described in detail in Chapter 9. Until recently, it was unclear whether the same or different VTA dopaminergic neurons participate in locomotor activation versus reward. Recent studies by Howe and Dombeck (2016) now indicate that different cells underlie these two functions.

Yet another line of research has shown that some VTA dopaminergic neurons are activated by stressful or aversive (i.e., unpleasant) stimuli. How can these results be reconciled with DA's role in reward? Once again, it appears that rewarding and aversive stimuli activate different subsets of VTA neurons (Chandler et al., 2014; Lammel et al., 2014). Moreover, these subsets are also differentiated by both their inputs and outputs. As illustrated in **FIGURE 5.11**, the VTA dopaminergic neurons involved in reward receive input from the laterodorsal tegmentum (LDT) in the dorsal midbrain and project to the nucleus accumbens (NAc) as part of the mesolimbic pathway, whereas the neurons that respond to aversive stimuli receive input from the lateral habenula (LHb) and project to the medial PFC (mPFC) as part of the mesocortical pathway. Of course, both subsets of cells receive other inputs that provide additional information about whether a particular stimulus is rewarding or aversive.

There are five main subtypes of dopamine receptors organized into D_1- and D_2-like families

In the previous chapter, we discussed the concept of receptor subtypes. The neurotransmitter DA uses five main subtypes, designated D_1 to D_5, all of which are metabotropic receptors. That is, they interact with G proteins and they function, in part, through second messengers. Various studies have shown that the D_1 and D_5 receptors are very similar to each other, whereas the D_2, D_3, and D_4 receptors represent a separate family. The D_1 and D_2 receptors were discovered first, and they are the most common subtypes in the brain. Both types

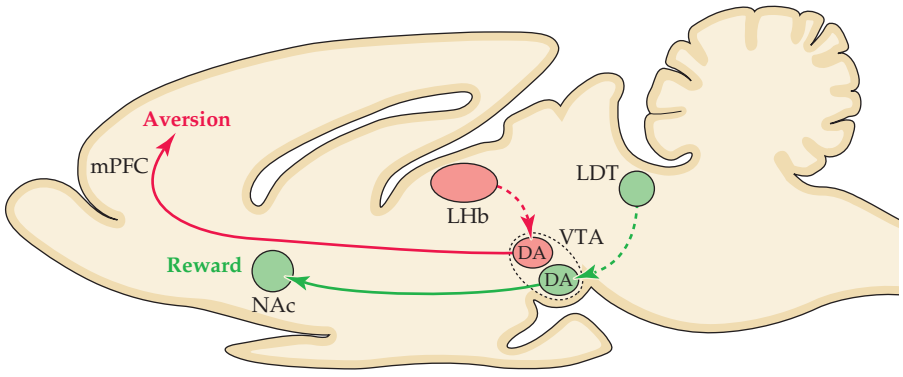


FIGURE 5.11 Different subsets of VTA dopaminergic neurons mediate the effects of rewarding versus aversive stimuli The diagram depicts a sagittal section through a rodent brain illustrating the features of VTA dopaminergic neurons that help encode rewarding versus aversive stimuli. The reward-mediating pathway (green) includes input to the VTA from the laterodorsal tegmentum (LDT) and dopaminergic output from the VTA to the nucleus accumbens (NAc). The aversion-mediating pathway (red) includes input to the VTA from the lateral habenula (LHb) and dopaminergic output from the VTA to the medial prefrontal cortex (mPFC). (From Chandler et al., 2014, CC-BY 3.0.)

of receptors are found in large numbers in the dorsal striatum and the nucleus accumbens, major termination sites of the nigrostriatal and mesolimbic DA pathways, respectively. Thus, D_2 receptors not only function as autoreceptors, as mentioned earlier, they also play an important role as normal postsynaptic receptors. Interestingly, these receptors are additionally found on cells in the pituitary gland that make the hormone prolactin. Activation of D_2 receptors by DA from the hypothalamus leads to inhibition of prolactin secretion, whereas the blockade of these receptors stimulates prolactin release. We will see in Chapter 19 that nearly all current antischizophrenic drugs are D_2 receptor antagonists—a characteristic that is believed to play a key role in the therapeutic efficacy of these compounds.

In the early stages of research on DA receptors, investigators discovered that D_1 and D_2 have opposite effects on the second-messenger substance cyclic adenosine monophosphate (cAMP) (Kebabian and Calne, 1979). More specifically, D_1 receptors stimulate the enzyme adenylyl cyclase, which is responsible for synthesizing cAMP (see Chapter 3). Consequently, the rate of cAMP formation is increased by stimulation of D_1 receptors. In contrast, D_2 receptor activation inhibits adenylyl cyclase, thereby decreasing the rate of cAMP synthesis (**FIGURE 5.12**). These opposing effects can occur because the receptors activate two different G proteins: G_s in the case of D_1 receptors, and G_i in the case of D_2 receptors. Resulting changes in the level of cAMP within the postsynaptic cell alter the excitability of the cell (i.e., how readily it will fire nerve impulses) in complex ways that are beyond the scope of this discussion. A second important mechanism of D_2 receptor function involves the regulation of membrane ion channels for potassium (K^+). In some cells, D_2 receptor stimulation activates a G protein that subsequently enhances K^+ channel opening. As we saw in Chapter 3, opening of such channels causes hyperpolarization of the cell membrane, thus decreasing the excitability and rate of firing of the cell.

D_1 and D_2 receptors differ not only with respect to their signaling mechanisms but also in their affinity for DA. D_2 receptors have a significantly higher affinity for DA than D_1 receptors (Marcellino et al., 2012). This means that less DA is required to occupy a given percentage (for example, 50%) of D_2 compared with D_1 receptors. This difference has led to the hypothesis that tonic DA release primarily activates the higher-affinity D_2 receptors, whereas the large increase in synaptic DA levels produced by phasic release additionally activates the lower-affinity D_1 receptors (Grace et al., 2007).

Dopamine receptor agonists and antagonists affect locomotor activity and other behavioral functions

Many studies of DA pharmacology have used compounds that directly stimulate or block DA receptors. **Apomorphine** is a widely used agonist that stimulates both D_1 and D_2 receptors. At appropriate doses, apomorphine treatment causes behavioral activation similar to that seen with classical stimulants like amphetamine and cocaine. There is also a new use for apomorphine—treating erectile dysfunction in men (marketed under the trade name Uprima). At present, the best-known remedy for this disorder is, of course, Viagra. You will recall that the mechanism of action of Viagra, which involves inhibiting the breakdown of cyclic guanosine monophosphate (cGMP) in the penis, was discussed in Chapter 3. In contrast, apomorphine seems to increase penile blood flow, which is necessary for an erection, by acting through DA receptors in the brain. This effect of apomorphine has actually been known for some time, but clinical application for this purpose was previously thwarted by undesirable side effects, particularly nausea, as well as poor bioavailability when the drug is taken orally. These problems have been overcome to some extent through the development of a lozenge that is taken sublingually (under the tongue), thereby bypassing the digestive system and delivering the drug directly into the bloodstream.

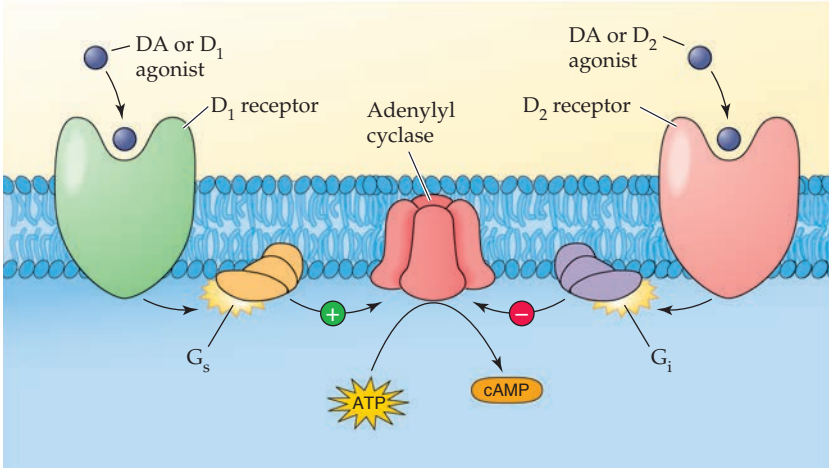


FIGURE 5.12 Signaling mechanisms of D_1 and D_2 receptors
Activation of D_1 receptors stimulates the enzyme adenylyl cyclase and enhances DA synthesis, whereas activation of D_2 receptors inhibits adenylyl cyclase and decreases DA synthesis. These effects are produced by the activation of different G proteins in the postsynaptic cell membrane, namely, G_s for the D_1 receptor, and G_i for the D_2 receptor.

Psychopharmacologists also make use of drugs that are more selective for members of the D_1 or D_2 receptor family. Receptor-selective agonists and antagonists are extremely important in helping us understand which behaviors are under the control of a particular receptor subtype. The most commonly used agonist for D_1 receptors is a compound known as **SKF 38393**.[3] Administration of this compound to rats or mice elicits self-grooming behavior. **Quinpirole** is a drug that activates D_2 and D_3 receptors, and its effect is to increase locomotion and sniffing behavior. These responses are reminiscent of the effects of amphetamine or apomorphine, although quinpirole is not as powerful a stimulant as the former compounds.

The typical effect of administering a DA receptor antagonist is suppression of spontaneous exploratory and locomotor behavior. At higher doses, such drugs elicit a state known as **catalepsy**. Catalepsy refers to a lack of spontaneous movement, which is usually demonstrated experimentally by showing that the subject does not change position when placed in an awkward, presumably uncomfortable posture (**FIGURE 5.13**). Nevertheless, the subject is neither paralyzed nor asleep, and in fact it can be aroused to move by strong sensory stimuli such as being picked up by the experimenter. Catalepsy is usually associated with D_2 receptor blockers such as **haloperidol**, but it can also be elicited by giving a D_1 blocker such as **SCH 23390**. Given the important role of the nigrostriatal DA pathway in movement, it is not surprising that catalepsy is particularly related to the inhibition of DA receptors in the striatum. We mentioned earlier that D_2 receptor antagonists are used in the treatment of schizophrenia. The therapeutic benefit of these drugs is thought to derive from their blocking of DA receptors mainly in the limbic system. It should be clear from the present discussion, however, that the same drugs are also likely to produce inhibition of movement and other troublesome motor side effects because of simultaneous interference with dopaminergic transmission in the striatum. The various effects of DA receptor agonists and antagonists have given researchers a lot of useful information about the behavioral functions of DA. A newer approach is to manipulate the genes for individual components of the dopaminergic system and determine the behavioral consequences of such manipulations. Results from this approach are discussed in **BOX 5.2**.

[3]Many drugs used in research never receive common names like cocaine or reserpine. In such instances, the drug is designated with an abbreviation for the pharmaceutical company at which it was developed, along with an identifying number. In the present instance, *SKF* stands for Smith Kline & French, which is now part of GlaxoSmithKline.

FIGURE 5.13 Catalepsy can be produced by DA receptor antagonists This is particularly true of drugs that block D_2 receptors. Shown here is a 10-day-old rat pup that had received a subcutaneous injection of the D_2 antagonist haloperidol (1 mg/kg) 1 hour earlier. Pups treated in this manner often spent an entire 3-minute testing period immobile with their forepaws resting on the elevated bar, whereas control pups not given haloperidol got off the bar within a few seconds. (Photograph by Jerrold Meyer.)

We conclude this section by considering the consequences of administering a D_2 receptor antagonist repeatedly rather than just once or twice. When haloperidol is given on a long-term basis to rats, the animals develop a syndrome called **behavioral supersensitivity**. This means that if the haloperidol treatment is stopped to unblock the D_2 receptors and then subjects are given a DA agonist like apomorphine, they respond more strongly than controls not pretreated with haloperidol. Since the experimental and control animals both received the same dose of apomorphine, this finding suggests that somehow the DA receptors in the experimental group are more sensitive to the same pharmacological stimulation. A similar effect occurs after DA depletion by 6-OHDA. The similarity between the effects of haloperidol and 6-OHDA is because both treatments persistently reduce DA stimulation of D_2 receptors. Haloperidol accomplishes this by blocking the receptors, whereas 6-OHDA achieves the same result by causing a long-lasting depletion of DA. Various studies suggest that the supersensitivity associated with haloperidol or 6-OHDA treatment is related, at least in part, to an increase in the density of D_2 receptors on postsynaptic cells in the striatum. This phenomenon, which is called **receptor up-regulation**, is considered to be an adaptive response whereby the lack of normal neurotransmitter (in this case DA) input causes the neurons to increase their sensitivity by making more receptors.

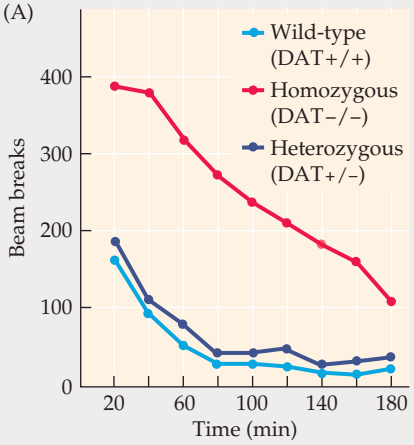
BOX 5.2 The Cutting Edge

Using Molecular Genetics to Study the Dopaminergic System

Many researchers use techniques from molecular biology to investigate how neurotransmitter systems function, how they control various behaviors, and the mechanisms of psychoactive drug action. One of the most powerful tools in this field of molecular pharmacology is the production of genetically engineered animals, which means animals in which one or more genes have been altered experimentally (rather than animals with genetic changes like mutations that can occur spontaneously in nature; see Chapter 4). Until recently, almost all genetically engineered animal strains were mice; however, the relevant techniques are constantly improving, so other species, including rats and monkeys, can now also be genetically modified. Several different kinds of mutations can be created, depending on the aim of the experiment. To briefly review the information presented in Chapter 4, genetically engineered mutant animal lines include (1) gene knockout animals, in which the target gene is modified so that it no longer yields a functional protein product; (2) gene "knockin" animals, which are usually produced by making a point mutation in the gene that alters the identity of one particular amino acid in the protein product; (3) gene "overexpressing" animals, engineered by inserting one or more additional copies of the target gene into the recipient's genome; and (4) transgenic animals, in which one or more genes from a different species have been inserted into the recipient's genome. An example of that last approach is the insertion of a mutant form of a human gene that causes disease (e.g., rare mutations that cause inherited forms of Alzheimer's or Parkinson's disease) into mice to provide an animal model for testing potential new treatments for that disease. Note that we are using the traditional definition of the term *transgenic*, although sometimes the word is used more loosely to describe any genetically modified strain of organism.

The dopaminergic system is one of the most intensively studied neurotransmitter systems using genetic engineering approaches, and the results of those studies have provided important clues about how this system regulates behavioral functions. Genetically engineered mice have additionally been useful for dissecting the molecular actions of dopaminergic drugs and providing novel animal models for several neuropsychiatric disorders, particularly attention deficit hyperactivity disorder (ADHD) and schizophrenia. Our earlier discussion of DD mice provided an elegant example of applying genetic engineering technology to the dopaminergic system. Other strains of mutant mice have been generated that lack either the DA transporter or one of the DA receptors. It turns out that each gene knockout produces a unique behavioral phenotype, which refers to the behavior of the mutant strain compared with that of wild-type animals (animals carrying the form of the gene found in nature). Due to space limitations, we will focus on manipulations of the DA transporter and the D_1 and D_2 receptor genes, which have been the most heavily studied components of the dopaminergic system.

The most obvious characteristic of DA transporter knockout mice is that they are extremely hyperactive (Giros et al., 1996) (**Figure A**). This effect is understandable in light of the fact that without a transporter on the cell membrane, the dopaminergic neurons cannot remove DA from the extracellular fluid. Consequently, the postsynaptic DA receptors are exposed to excessive amounts of transmitter, and this has an activating effect on the animal's behavior. Other features of DA transporter knockout mice include cognitive deficits and impulsivity, traits that are often seen in children suffering from ADHD. Moreover, hyperactivity in these animals



(A) Dopamine transporter (DAT) knockout mice show increased locomotor activity Mutant mice lacking a functional DAT (homozygous DAT–/–) were compared with wild-type mice (DAT+/+) and with heterozygous mice (DAT+/–) that carry one functional copy of the DAT gene. Mice were tested in a photocell apparatus, and the number of photobeam breaks was recorded every 20 minutes for a total of 3 hours. All groups showed a gradual habituation to the apparatus, as indicated by decreasing beam breaks over time, but the activity of the DAT knockout mice (DAT–/–) was consistently higher than that of the other two groups. (After Giros et al., 1996.)

BOX 5.2 The Cutting Edge (*continued*)

is reduced by administration of amphetamine or methylphenidate, psychostimulants that serve as first-line medications for ADHD pharmacotherapy (see Chapter 12). Based on these findings, some researchers have proposed DA transporter knockout mice to be an animal model of ADHD (Leo and Gainetdinov, 2013). However, there have also been criticisms of this model, leading to the development of a DA transporter knockin strain that recapitulates a mutant form of the human transporter gene that has been linked to ADHD vulnerability (Mergy et al., 2014).

Holmes and coworkers (2004) have reviewed the behavioral effects seen in various DA receptor knockout mice. D_1 receptor knockout mice, the first to be generated, are similar to DA-deficient mice in that they appear normal at birth. However, by the age of weaning, they begin to show reduced growth and may even die if their normally dry food isn't moistened to make it more palatable. In adulthood, these animals reportedly show complex behavioral abnormalities, including increased locomotor activity and reduced habituation in an open field, deficits in motor coordination in the rotarod test, and reduced self-grooming behavior (recall that D_1 receptor agonists given to wild-type rodents stimulate grooming behavior). Dopamine, particularly acting through D_1 receptors, has been implicated in various cognitive processes, including attention and working memory. It is interesting to note, therefore, that D_1 receptor knockout mice also exhibit deficits in several kinds of cognitive tasks (Holmes et al., 2004). Contrasting results with respect to motor function are observed after disruption of the D_2 receptor gene. D_2 knockout mice show impairment in spontaneous movement, coordination, and postural control (also see Nakamura et al., 2014, for a more recent study comparing D_1 to D_2 knockout mice). Nevertheless, the degree of impairment seems to depend strongly on which genetic strain of mouse is used to create the mutation. This is partly because different strains already vary considerably in their behavior before any genes are knocked out, and indeed, the same issue of background strain has arisen in the analysis of behavioral changes in D_1 receptor knockout mice. The necessity of having functional activity in at least one of the two main DA receptors is shown by the observation that animals with a double knockout of both D_1 and D_2 receptor genes die during the second or third week of postnatal life (Kobayashi et al., 2004). This effect is due mainly to poor feeding behavior, as was seen in DD mice. However, in this case, the animals cannot be rescued by treatment with L-DOPA, since their neurochemical deficit lies in a lack of postsynaptic DA responsiveness rather than in a lack of presynaptic DA synthesis.

Mice overexpressing D_2 receptors have been proposed to be a partial animal model of schizophrenia, based on the fact that nearly all antipsychotic drugs block this DA receptor subtype and that at least some postmortem studies have found increased D_2 receptor binding in the striatum of patients with schizophrenia (Chapter 19). Such mice exhibit specific cognitive and motivational deficits, which fit some of the symptoms seen in patients with schizophrenia (Moran et al., 2014; O'Tuathaigh et al., 2014). However, it is important to be cautious in our interpretation of these findings, as schizophrenia is an extremely complex disorder with a variety of symptom profiles as well as neurochemical and genetic abnormalities.

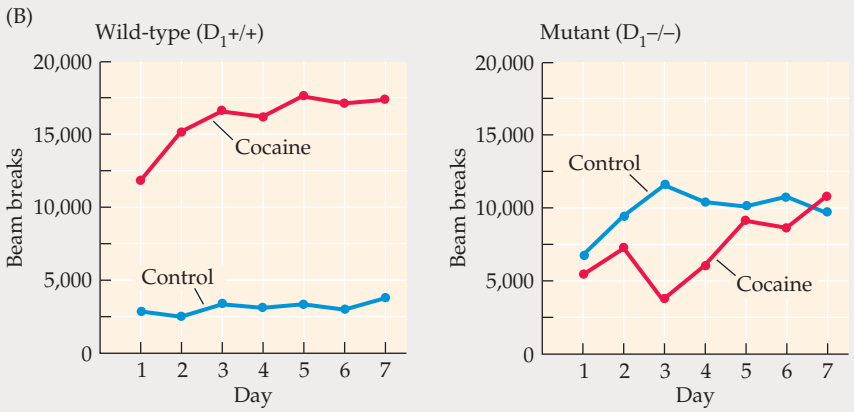
Pharmacologists have naturally taken advantage of these mutant strains of mice to study how disruptions in various DA system genes affect reactions to different psychoactive drugs. Several studies have looked at behavioral responses to psychostimulant drugs such as cocaine, amphetamine, and methamphetamine because DA is already known to play an important role in the effects of these compounds. DA transporter knockout mice and mice lacking either the D_1 or D_2 receptor show reduced or, in some cases, no increase in locomotion after psychostimulant treatment (Holmes et al., 2004; M. Xu et al., 2000) (**Figure B**). Conversely, mutant mice that overexpress the DA transporter exhibit an exaggerated locomotor response to amphetamine (Salahpour et al., 2008). Taken together, these findings demonstrate that both the DA transporter on the presynaptic side and the D_1 receptor on the postsynaptic side play pivotal roles in the behavior-stimulating effects of cocaine and amphetamine (see Chapter 12).

Other studies have examined behavioral responses to ethanol (alcohol) in various strains of DA receptor knockout mice. Ethanol can produce either locomotor stimulation or inhibition, depending on dose (low doses tend to be stimulatory, whereas higher doses are sedating) and other factors. Knocking out the D_2 receptor in mice enhances the stimulatory effects of low-dose ethanol but blunts the sedating effects of higher doses (Holmes et al., 2004). Voluntary ethanol consumption is also reduced in D_1 and D_2 knockout mice, suggesting that these two receptor subtypes play an important role in the rewarding effects of this substance.

(*Continued*)

BOX 5.2 The Cutting Edge (*continued*)

The findings discussed in this box show that by acting through different receptor subtypes, a single transmitter such as DA may influence many different behaviors and physiological functions and may also play a complex role in the responses to psychoactive drugs. In some cases, genetically engineered mice have largely confirmed theories that researchers had previously formulated using more traditional pharmacological approaches. In other cases, however, the use of such animals has provided new and exciting insights into the interactions between neurotransmitters, drugs, and behavior.



(B) D_1 receptor knockout mice show decreased reaction to a psychostimulant drug Mutant mice lacking D_1 receptors (D_1–/–) are insensitive to the locomotor-stimulating effects of cocaine. Wild-type (D_1+/+) and homozygous mutant mice were injected twice daily for 7 consecutive days with either cocaine (20 mg/kg intraperitoneally) or a saline control solution. The animals were tested in a photocell apparatus for 30 minutes after each injection to record their locomotor activity. Cocaine greatly increased locomotor activity in the wild-type but not the mutant mice on all test days. (After M. Xu et al., 2000.)

Section Summary

- The dopaminergic neurons of greatest interest to neuropsychopharmacologists are found near the base of the midbrain in the substantia nigra (A9 cell group) and the VTA (A10 cell group).
- Neurons in the substantia nigra send their axons to the dorsal striatum, thus forming the nigrostriatal tract. The dorsal striatum is composed of two separate structures, called the caudate and putamen, in humans and non-human primates, whereas these structures are fused in rodents.
- The nigrostriatal pathway plays an important role in the control of movement and is severely damaged in Parkinson's disease. Two different animal models have not only confirmed the involvement of nigrostriatal DA in the initiation of voluntary motor activity (e.g., locomotion), but also implicated this pathway in the regulation of motivation (e.g., motivation to eat and drink) and in cognitive function. These animal models involve either biochemical lesioning of the nigrostriatal tract (e.g., using the neurotoxin 6-OHDA) or the genetic engineering of DD mice.
- Dopaminergic neurons in the VTA form two major dopaminergic systems: the mesolimbic system, which has terminations in several limbic system structures (e.g., nucleus accumbens, septum, amygdala, hippocampus), and the mesocortical system, which terminates in the cerebral cortex (particularly the PFC). The nucleus accumbens is a major constituent of the ventral striatum.
- The mesolimbic and mesocortical DA systems have been implicated in mechanisms of drug abuse, as well as in schizophrenia. A specific subset of VTA dopaminergic neurons activated by rewarding stimuli receive input from the laterodorsal tegmentum and project to the nucleus accumbens, whereas a separate subset that is activated by aversive stimuli receive input from the lateral habenula and project to the medial PFC.
- Researchers have identified five main DA receptor subtypes, designated D_1 to D_5, all of which are metabotropic receptors. These subtypes fall into two families, the first consisting of D_1 and D_5, and the second consisting of D_2, D_3, and D_4. The most common subtypes are D_1 and D_2, both of which are found in large numbers in the striatum and the nucleus accumbens.
- D_1 and D_2 receptor subtypes can be differentiated partly on the basis that D_1 receptors stimulate adenylyl cyclase, thus increasing the rate of cAMP synthesis, whereas D_2 receptors decrease the rate of cAMP synthesis by inhibiting adenylyl cyclase. Activation of D_2 receptors can also enhance the opening of K^+ channels in the cell membrane, which hyperpolarizes the membrane and therefore reduces cell excitability. In addition, D_2 receptors

TABLE 5.1 Drugs That Affect the Dopaminergic System

Drug	Action
Dihydroxyphenylalanine (L-DOPA)	Converted to dopamine (DA) in the brain
Phenelzine	Increases catecholamine levels by inhibiting monoamine oxidase (MAO)
α-Methyl-*para*-tyrosine (AMPT)	Depletes catecholamines by inhibiting tyrosine hydroxylase
Reserpine	Depletes catecholamines by inhibiting vesicular uptake
6-Hydroxydopamine (6-OHDA)	Damages or destroys catecholaminergic neurons
Amphetamine*	Releases catecholamines
Cocaine and methylphenidate*	Inhibit catecholamine reuptake
Apomorphine	Stimulates DA receptors generally (agonist)
SKF 38393	Stimulates D_1 receptors (agonist)
Quinpirole	Stimulates D_2 and D_3 receptors (agonist)
SCH 23390	Blocks D_1 receptors (antagonist)
Haloperidol	Blocks D_2 receptors (antagonist)

*These drugs are not selective for catecholamines, as they also affect serotonin release or reuptake.

have a greater affinity for DA than D_1 receptors, thereby leading to the hypothesis that tonic DA release primarily activates D_2 receptors, whereas significant activation of D_1 receptors requires the additional amount of DA released by phasic activity of the dopaminergic neurons.

- Some of the drugs that affect the dopaminergic system, including DA receptor agonists and antagonists, are presented in **TABLE 5.1**. In general, enhancement of dopaminergic function has an activating effect on behavior, whereas interference with DA causes suppression of normal behaviors, ranging from temporary sedation and catalepsy to the profound deficits observed after treatment with a DA neurotoxin such as 6-OHDA.
- When D_2 receptor transmission is persistently impaired either by chronic antagonist administration or by denervation (e.g., 6-OHDA lesions), animals become supersensitive to treatment with a D_2 agonist. This response is mediated at least in part by up-regulation of D_2 receptors by postsynaptic neurons in areas such as the striatum.

Organization and Function of the Noradrenergic System

The noradrenergic system has both a central and peripheral component. The cell bodies of the central noradrenergic system are found in the brainstem, and their ascending fibers innervate a wide range of forebrain structures, whereas peripheral noradrenergic neurons are an important component of the sympathetic nervous system. NE released from these cells acts on adrenergic receptors located either in the central nervous system or in peripheral target organs.

Norepinephrine is an important transmitter in both the central and peripheral nervous systems

The NE-containing neurons within the brain are located in the parts of the brainstem called the *pons* and the *medulla*. Of particular interest is a structure known as the **locus coeruleus** (**LC**), a small area of the pons that contains a dense collection of noradrenergic neurons corresponding roughly to the A6 cell group (according to the numbering system described previously). At first glance, the LC might not seem to be a very impressive structure; the rat LC contains a few more than 3000 nerve cells out of the millions of neurons present in the entire rat brain. Of course, more LC cells are found in the larger brains of primates, but even the human brain only contains approximately 50,000 noradrenergic neurons in the LC (Sharma et al., 2010). Location and size of the LC in the human brain are shown in the histological sections and magnetic resonance image (MRI) of **FIGURE 5.14**. Despite its small size, the LC sends fibers to almost all areas of the forebrain, thereby providing nearly all of the NE in the cortex, limbic system, thalamus, and hypothalamus (**FIGURE 5.15**). The LC also provides noradrenergic input to the cerebellum and the spinal cord.

Norepinephrine additionally plays an important role in the peripheral nervous system. Many neurons that have their cell bodies in the ganglia of the sympathetic branch of the autonomic nervous system (see

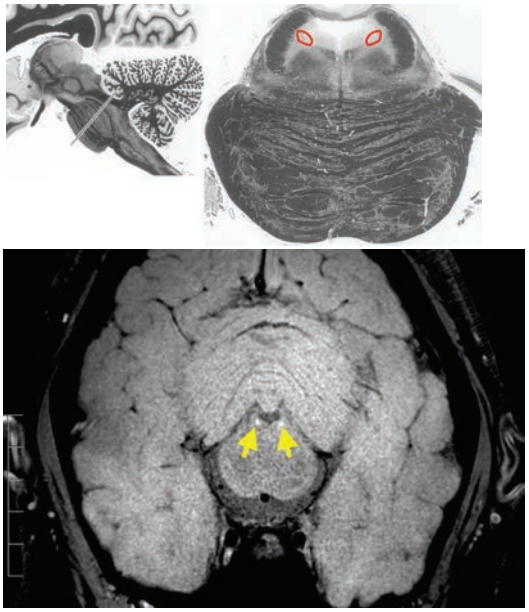


FIGURE 5.14 Location of the locus coeruleus (LC) in the human brain (A) Histological section from a human brain at the level of the pons. The image at the left shows the plane of section of the brain, whereas the image at the right shows the resulting section with the LC on each side of the brain outlined in red. The tissue was treated with a myelin stain, which accounts for why cell-rich, myelin-poor areas like the LC are so lightly stained. (B) Structural magnetic resonance image (MRI) in which the LC is marked by the yellow arrows. The plane of the MRI is oriented with the front of the head toward the top, thus producing an image with a different plane than the histological section shown above it. (Top images from Warner, 2001; bottom image from Samuels and Szabadi, 2008.)

Chapter 2) use NE as their transmitter. These cells, which send out their fibers to various target organs throughout the body, are responsible for the autonomic actions of NE. We mentioned earlier in the chapter that NE (as well as EPI) functions as a hormone secreted by the adrenal glands directly into the bloodstream. In this way, NE can reach an organ such as the heart through two routes: it can be released from sympathetic noradrenergic neurons at axonal varicosities that come into close proximity with cardiac cells, and it can be released from the adrenal glands and travel to the heart through the circulatory system. On the other hand, blood-borne NE does not reach the brain, because it is effectively excluded by the blood–brain barrier.

Blood-borne NE and EPI work together with NE released as a transmitter from sympathetic nerve endings to mediate most of the physiological changes that make up the "fight-or-flight" response described almost 100 years ago by the great physiologist Walter Cannon (1922). These changes include alterations in blood vessel constriction that prepare the organism for rapid action (i.e., dilation of vessels in skeletal muscle, heart, and brain, but constriction of blood vessels in other organs), elevated blood pressure, increased heart rate and contractile force of heart muscle, increased oxygen intake caused by dilation of the airways and elevated respiration rate, reduced digestive activity caused by relaxation of intestinal smooth muscle, pupil dilation to let more light into the eyes, increased liberation of metabolic fuels from the liver and adipose tissue (sugar and fats, respectively), and decreased secretion of gastric juices and saliva (reviewed by Tank and Wong, 2015). Below we describe the adrenergic receptor subtypes responsible for these effects and the use of drugs targeting these receptors for the treatment of various medical conditions.

Norepinephrine and epinephrine act through α- and β-adrenergic receptors

Receptors for NE and EPI are called *adrenergic receptors* (an alternate term is **adrenoceptors**). Like DA receptors, adrenergic receptors all belong to the general family of metabotropic receptors. However, they serve a broader role by having to mediate both neurotransmitter (mainly NE) and hormonal (mainly EPI) actions of the catecholamines.

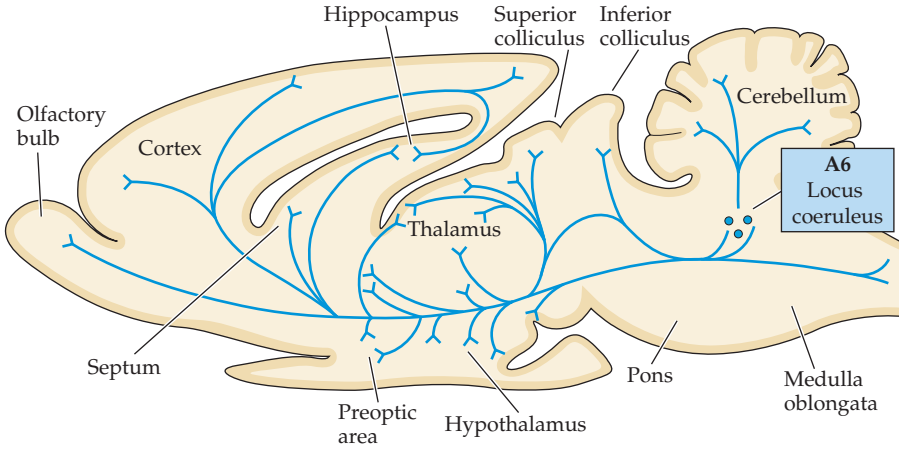


FIGURE 5.15 The locus coeruleus (LC) sends fibers to much of the brain The LC contains a dense cluster of noradrenergic neurons designated the A6 cell group. These cells send their fibers to almost all regions of the forebrain, as well as to the cerebellum and spinal cord.

Early studies by Ahlquist (1948; 1979) and other investigators suggested the existence of two adrenoceptor subtypes, which were designated alpha (α) and beta (β). Since the time of Ahlquist's pioneering research, many experiments have shown that the α- and β-adrenoceptors actually represent two families, each composed of several receptor subtypes. For present purposes, we will distinguish between α_1- and α_2-receptors, and also between β_1 and β_2. Postsynaptic adrenoceptors are found at high densities in many brain areas, including the cerebral cortex, thalamus, hypothalamus, and cerebellum, and in various limbic system structures such as the hippocampus and amygdala. In addition, α_2-autoreceptors are located on noradrenergic nerve terminals and on the cell bodies of noradrenergic neurons in the LC and elsewhere. These autoreceptors cause an inhibition of noradrenergic cell firing and a reduction in NE release from the terminals.

Like dopamine D_1 receptors, both β_1- and β_2-adrenoceptors stimulate adenylyl cyclase and enhance the formation of cAMP. In contrast, α_2-receptors operate in a manner similar to that of D_2 receptors. That is, α_2-receptors reduce the rate of cAMP synthesis by inhibiting adenylyl cyclase, and they can also cause hyperpolarization of the cell membrane by increasing K^+ channel opening. Yet another kind of mechanism is used by receptors of the α_1 subtype. These receptors operate through the phosphoinositide second-messenger system, which, as we saw in Chapter 3, leads to an increased concentration of free calcium (Ca^{2+}) ions within the postsynaptic cell.

The central noradrenergic system plays a significant role in arousal, cognition, and the consolidation of emotional memories

Neurochemical, pharmacological, and electrophysiological studies in experimental animals indicate that the central noradrenergic system is involved in a large number of behavioral functions, only some of which can be discussed here because of space limitations. We have chosen to focus on the role of this system in arousal, cognition, and the consolidation of memories of emotional experiences. Later chapters will present evidence for involvement of NE in various psychiatric disorders, particularly mood and anxiety disorders.

A large body of literature has demonstrated that the firing of LC noradrenergic neurons and the activation of adrenergic receptors in the forebrain are important for both behavioral and electrophysiological (i.e., EEG) arousal[4] (Berridge et al., 2012b). One of the earliest indications of this relationship comes from studies in the 1970s and 1980s showing that LC neurons fire more rapidly during waking than during sleep and that changes in the firing of these cells come *before* the transition between these behavioral states. The ability of LC noradrenergic neurons to trigger arousal was further supported by the demonstration that selective stimulation of these cells at the right frequency caused sleeping mice to awaken, whereas inhibition of the cells reduced wakefulness in the animals (Carter et al., 2010). Projections from the LC to the medial septal, medial preoptic, and lateral hypothalamic areas have been implicated in the wakefulness-promoting effects of NE (Berridge et al., 2012b). In one experiment, for example, researchers microinjected the α_1-receptor agonist **phenylephrine** and/or the general β-receptor agonist **isoproterenol** directly into the lateral hypothalamic area of rats (Schmeichel and Berridge, 2013). The animals were then monitored to determine the amount of time they spent awake or asleep. As illustrated in **FIGURE 5.16A**, phenylephrine infusion dose-dependently increased the amount of time spent awake. The infusion also reduced the amount of time spent in both slow-wave and rapid-eye-movement (REM) sleep (data not shown). Isoproterenol caused a much weaker response, with a modest increase in waking and a reduction in REM but not slow-wave sleep (**FIGURE 5.16B**). Interestingly, combined treatment with low doses of the two compounds produced an additive effect on waking (**FIGURE 5.16C**). These results show that in the lateral hypothalamic area, α_1-receptors play the most important role in NE-mediated arousal and wakefulness, although β-receptor activation can add to the effects of moderate α_1-receptor stimulation.

In Chapter 3, we described the presence of orexin neurons in the lateral hypothalamic area that represent another cluster of cells that can exert a powerful arousing effect. This raises the possibility that the mutual arousing properties of orexin and noradenergic neurons could involve an interaction between these cell groups. Based on the fact that noradrenergic agonists infused into the lateral hypothalamic area cause rapid arousal, it seems logical that NE might produce its effects by activating the orexin cells. However, not only is this *not* the case (Schmeichel and Berridge, 2013), but the functionally important connection is in the opposite direction. Carter and coworkers (2013) reviewed evidence that orexin works, at least in part, by activating LC noradrenergic neurons. The key finding in that research is that inhibition of the LC prevented the wakefulness-promoting effects of stimulating the orexin neurons. In summary, the findings from orexin studies described in Chapter 3 combined with those discussed above on NE have revealed part of an intricate circuit that is critically important for transitioning from sleep to wakefulness and for the maintenance of an appropriate state of arousal when interacting with the environment.

[4]During states of drowsiness and slow-wave sleep, EEG measurements taken from the cortex show a predominant pattern of slow, high-voltage, synchronized wave forms. During a state of aroused wakefulness, the cortical EEG instead shows a predominance of fast, low-voltage, desynchronized waves.

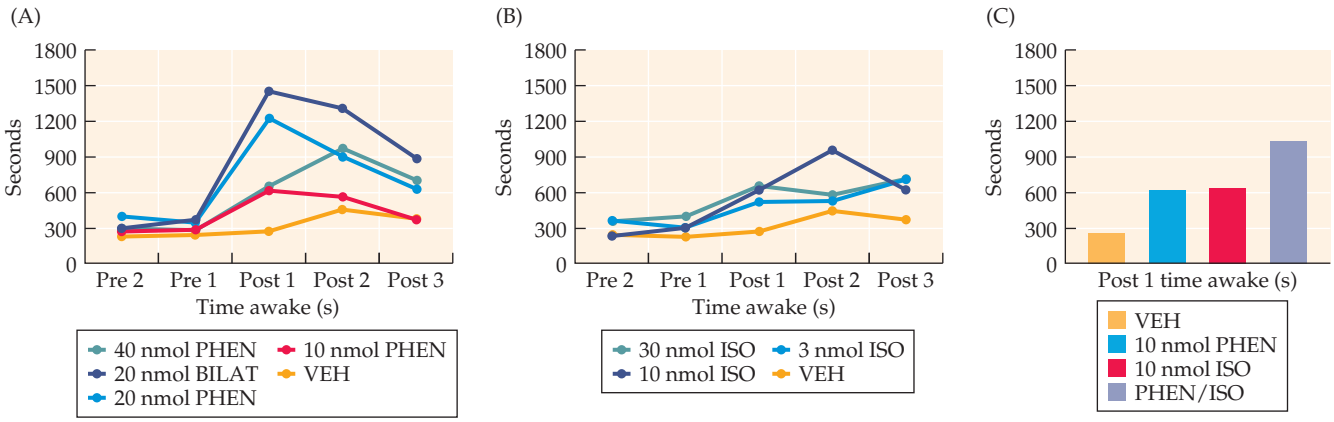


FIGURE 5.16 Wakefulness-promoting effects of adrenergic agonists infused into the lateral hypothalamic area Rats were given infusions of varying doses of the α_1-agonist phenylephrine (PHEN) and/or the β-agonist isoproterenol (ISO) directly into the lateral hypothalamic area. Control injections were of the vehicle (VEH) used to dissolve the active drugs. Behavioral state (awake or asleep) was determined for two 30-minute intervals prior to drug administration (Pre 1 and Pre 2) as well as three 30-minute postdrug intervals (Post 1, Post 2, and Post 3). All animals received unilateral infusions except for one group denoted as BILAT. (A) Infusions of PHEN alone markedly increased the amount of time spent awake, with the largest effect seen in the 20-nanomole (20 nmol) BILAT group. (B) Infusions of ISO alone exerted a modest effect on time spent awake, with a significant increase only seen in the 30 nmol ISO group. (C) Infusions of 10 nmol PHEN or ISO individually produced nonsignificant increases in time spent awake; however, infusion of both drugs together at the same doses produced a larger, statistically significant increase in waking time. (From Schmeichel and Berridge, 2013.)

Another brain area to which the LC projects is the PFC, which expresses α_1-, α_2-, and β-adrenoceptors. We mentioned earlier that the mesocortical dopaminergic projection to the PFC is important for attention and working memory, and the same is true for the noradrenergic input to this region (Chamberlain and Robbins, 2013; Clark and Noudoost, 2014). The relationship between NE release in the PFC and cognitive function can be described as an inverted U-shaped function in which the optimum amount of release is neither too low nor too high. Abnormally low release that is caused, for example, by damage to the LC noradrenergic neurons or administration of drugs that suppress LC neuronal firing leads to behavioral sedation (loss of NE-mediated arousal) and poor cognitive function. Cognition is also impaired by excessive noradrenergic activity that can be caused by stress or by high doses of adrenergic agonist drugs.

The facilitatory effects on PFC-related cognitive processes are mediated primarily by activation of α_2-receptors. Thus, administration of a selective α_2-receptor agonist such as clonidine or **guanfacine** has been shown to enhance working memory under a variety of conditions including animals with NE depletion, aged animals that suffer from a natural reduction in catecholamine levels, and animals tested under particularly demanding task conditions (Ramos and Arnsten, 2007; Arnsten, 2011). **FIGURE 5.17** presents an example of the facilitating effects of guanfacine (GFC) in a study of working memory in aged monkeys. The study used a two-choice spatial

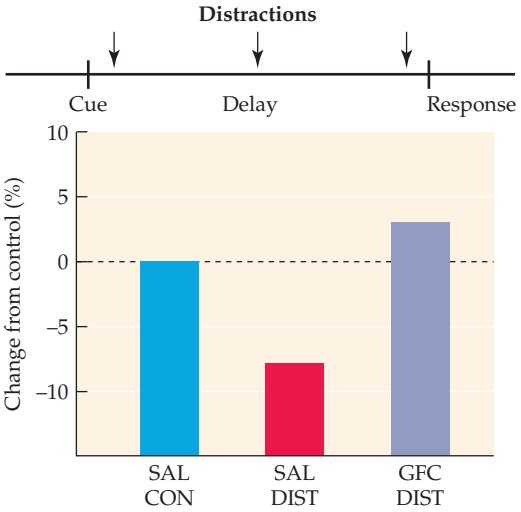


FIGURE 5.17 Guanfacine facilitation of working memory in aged monkeys Working memory was tested in aged rhesus monkeys using a two-choice spatial delayed response task. This task requires that the animals remember the location of a food treat with a delay period interposed between presentation of the treat and the opportunity to make a response. Distracting stimuli can be presented during the delay period to determine their influence on response accuracy. The first two bars demonstrate that in monkeys given saline vehicle (SAL), distracters impaired working memory performance (SAL/DIST) compared to the no-distracter control condition (SAL/CON). The third bar shows that guanfacine administration abolished the deleterious effect of the distracters (GFC/DIST) and returned performance to the control condition or slightly better. (From Arnsten and Jin, 2012.)

delayed response task in which monkeys observed the experimenter placing a food treat into one of two food wells, after which the wells were covered and a screen was imposed between the wells and the animal for a variable length of time (i.e., a delay). After the delay, the monkeys were permitted access to the wells but had to remember which well contained the treat in order to make the correct response and receive the reward. The animals typically perform quite well when the delay is very short (under 10 seconds), but their performance declines at longer delays. Just like humans, aged monkeys perform more poorly on this task than young monkeys; that is, older monkeys are more severely affected by increasing delay length. Moreover, task difficulty can be increased further by imposing "distracter" stimuli during the delay period on some trials. The figure illustrates the decline in task performance produced by the distracters and the ability of guanfacine to enhance working memory, thereby restoring performance on the task. These and other findings have led to the clinical use of guanfacine to treat attention deficit hyperactivity disorder (ADHD) and Tourette's syndrome, both of which are thought to involve abnormal PFC functioning (Arnsten and Jin, 2012; Connor et al., 2014).

In contrast to the facilitative effects of α_2-receptors, activation of α_1-receptors in the PFC has a deleterious effect on cognitive functions mediated by this brain area. These differing effects raise the question of how an organism's own NE might regulate PFC functioning. The answer lies in the fact that NE has a lower affinity for α_1-receptors than for the specific subtype of α_2-receptor found in high levels in the PFC. Taking all of this information together, Ramos and Arnsten (2007) proposed that NE facilitates PFC functioning and PFC-dependent cognitive tasks under normal (i.e., nonstressful) conditions mainly by activating the high-affinity α_2-adrenoceptors in this brain area. On the other hand, the heightened release of NE associated with stress would increase the amount of α_1-adrenoceptor activation, which presumably overrides the beneficial effects of the α_2-receptors and leads to cognitive impairment. **FIGURE 5.18** illustrates this hypothesis, which predicts that cognitive functions such as working memory function are optimized at intermediate levels of PFC noradrenergic activity.

Finally, there is substantial evidence from animal studies that NE, EPI, and glucocorticoid hormones modulate the consolidation of emotional memories. One type of behavioral task often used to study the mechanisms of memory consolidation is the one-trial **passive avoidance learning** paradigm (sometimes also called *inhibitory avoidance learning*). This paradigm uses a two-chamber apparatus with one side brightly lit and the other side dark. The two sides are separated by a partition with an opening in it to allow

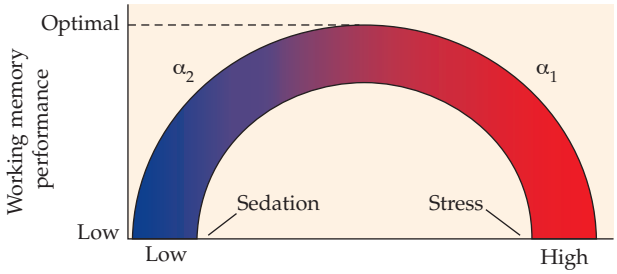
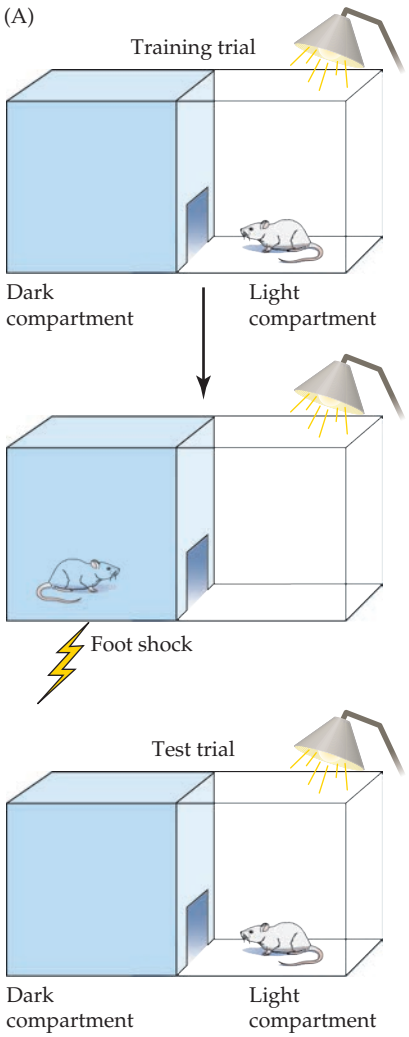


FIGURE 5.18 Modulation of working memory by adrenergic receptor subtypes in the prefrontal cortex The figure depicts an inverted U-shaped curve relating working memory function to noradrenergic activity in the prefrontal cortex (PFC). Abnormally low levels of noradrenergic activity in the PFC, which can be caused by certain drugs or by locus coeruleus lesions, result in behavioral sedation and impaired working memory. These effects are due to understimulation of α_2-adrenoceptors, since they can be reversed by selective activation of this receptor subtype. Impaired working memory can also result from abnormally high levels of noradrenergic activity in the PFC, which can be caused by stress. Optimal working memory is, therefore, predicted to occur at intermediate levels of PFC noradrenergic activity. (After Berridge and Spencer, 2016 and Berridge et al., 2012a.)

passage of the animal. For the training (learning) trial, a rat or a mouse is first placed in the brightly lit side, which is naturally aversive to the animal. However, when the subject spontaneously moves from the lit to the dark side, it receives a foot shock on the dark side (**FIGURE 5.19A**). This single experience is sufficient to produce long-lasting avoidance of the dark side, which is typically measured on a test trial one or more days after the learning. The memory of the event is quantified by measuring the increased length of time (latency) that it takes for a trained animal to move from the lit to the dark side compared with unshocked controls. A major advantage of such a one-trial learning paradigm is that memory consolidation (i.e., transfer of information from short-term to long-term memory) for the task occurs entirely during the single period of time after the learning trial. Another feature of this paradigm is that the foot shock induces a certain amount of fear and stress in the animals and thus can be considered a paradigm for emotional learning.

The stress associated with one-trial passive avoidance learning is well known to produce increased EPI and glucocorticoid secretion from the adrenal glands (see Chapter 3), as well as activation of central noradrenergic neurons and increased NE release within the brain. Although all of these processes are believed to play some role in passive avoidance memory consolidation, we will focus here on EPI. A number of studies have shown that EPI injections at the appropriate dose facilitate memory both in laboratory animals and



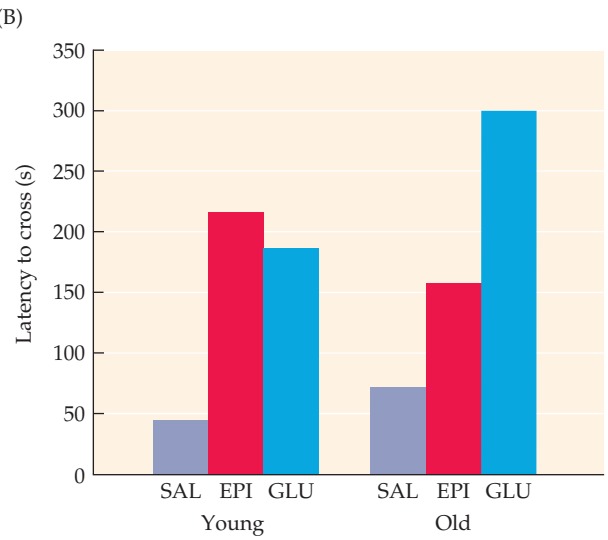


FIGURE 5.19 Memory enhancement by epinephrine and glucose using a one-trial passive avoidance learning paradigm (A) Emotional learning and memory in animals such as rats and mice are often studied using the one-trial passive avoidance learning paradigm illustrated here and described in the text. (B) Epinephrine (EPI; 0.1 mg/kg), glucose (GLU; 250 mg/kg), or saline vehicle (SAL) were injected SC to young (3–5 months of age) or old (24–26 months of age) rats immediately after the training trial. Latency to cross from the lit to the dark compartment was tested 48 hours later (maximum latency = 300 seconds). The SAL-treated rats in both groups showed a relatively short latency to cross, which was interpreted to mean a weak memory of the training. EPI and GLU were equally efficacious in increasing the crossing latency in the young rats, suggesting a large improvement in memory consolidation. In contrast, only GLU significantly increased the crossing latency in the old rats, suggesting that this treatment approach may work better for improving memory consolidation in an aged population. (B after Morris et al., 2010.)

humans (Gold, 2014). However, we also know that EPI in the bloodstream doesn't get into the brain because it cannot cross the blood–brain barrier. So the hormone must be doing something outside of the brain that ultimately facilitates the brain's memory mechanisms. One of the principal theories is that peripheral EPI signals to the brain by activating β-receptors on sensory nerve endings of the vagus nerve (cranial nerve X), which communicates information regarding peripheral organs to the brain (Tank and Wong, 2015). Indeed, information transmitted by the vagus could reach the LC and engage memory mechanisms by stimulating the release of central NE. Another interesting theory is that the memory-enhancing effects of EPI are mediated by its ability to increase blood glucose levels (Gold, 2014; Messier, 2004). This increase is caused by EPI activation of β-receptors in the liver, thereby causing a breakdown of liver glycogen (an important storage form of glucose) and release of glucose into the bloodstream. Unlike EPI, blood-borne glucose readily enters into the brain, and in fact glucose is the primary metabolic fuel used by the brain for producing energy. Researchers have demonstrated not only that glucose can improve memory just like EPI, but that preventing the ability of EPI to increase blood glucose levels blocks at least some of its memory-enhancing properties (Gold, 2014).

Epinephrine exerts a number of undesirable side effects, which diminishes its usefulness as a potential cognitive enhancer in the clinic. But because glucose is a foodstuff (also called dextrose), it does have potential to be a medication. One possible application is in aging, where memory deficits are present even in healthy individuals (i.e., people without Alzheimer's disease or any other kind of age-related dementia). **FIGURE 5.19B** compares the memory-enhancing effects of EPI and glucose in both young and old rats tested in the one-trial passive avoidance paradigm (Morris et al., 2010). Notice that at the doses used, glucose was even more efficacious than EPI in the old animals. Results such as these offer the possibility that glucose itself, or perhaps a drug that boosts blood glucose levels, might provide cognitive improvement not only in aged individuals (with or without dementia)

but also in individuals suffering from other disorders characterized by cognitive impairment (Gold and Korol, 2014).

Several medications work by stimulating or inhibiting peripheral adrenergic receptors

Adrenergic agonists or antagonists are frequently used in the treatment of nonpsychiatric medical conditions because of the widespread distribution and important functional role of adrenergic receptors in various peripheral organs (**TABLE 5.2**). For example, general adrenergic agonists that activate both α- and β-receptors have sometimes been used in the treatment of bronchial asthma. Stimulating the α-receptors causes constriction of the blood vessels in the bronchial lining, thus reducing congestion and edema (tissue swelling) by restricting blood flow to the tissue. On the other hand, β-receptor stimulation leads to relaxation of the bronchial muscles, thereby providing a wider airway. Although general adrenergic agonists can be effective antiasthma medications, they also cause several adverse side effects. For this reason, asthma is more commonly treated with a selective β_2-adrenoceptor agonist such as **albuterol** (**Ventolin**). Such drugs are packaged in an inhaler that delivers the compound directly to the respiratory system. The β-receptors found in the airways are of the β_2 subtype, in contrast to the heart, which contains mainly β_1-receptors. Consequently, albuterol is effective in alleviating the bronchial congestion of patients with asthma without producing adverse cardiovascular effects.

Even over-the-counter cold medications are based on the properties of peripheral adrenergic receptors. Thus, the α_1-receptor agonist phenylephrine is the key ingredient in several decongestant medications available in most pharmacies. This compound, which may be taken either in tablet form or in a nasal spray, constricts the blood vessels and reduces inflamed and swollen nasal membranes resulting from colds and allergies. In the form of eye drops, it is also used to stimulate α-receptors of the iris to dilate the pupil during eye examinations or before surgery of the eyes.

Alpha$_2$-receptor agonists such as clonidine are commonly used in the treatment of hypertension (high blood pressure). The therapeutic benefit of these drugs is a result of their ability to inhibit activity of the sympathetic nervous system while stimulating the parasympathetic system. The consequence of these actions is to reduce the patient's heart rate and blood pressure. Unfortunately, sedation and feelings of sleepiness are common side effects of clonidine treatment. On the other hand, these effects and others have been exploited therapeutically with the development of **dexmedetomidine** (**Precedex**), an α_2-agonist with combined sedative, anxiolytic (antianxiety), and analgesic (pain-reducing) effects that is particularly useful for surgical patients in the intensive care unit. The sedative and anxiolytic effects of dexmedetomidine are believed to be mediated by α_2-autoreceptors in the LC, whereas the analgesic effect probably occurs at the level of the spinal cord.

Adrenergic receptor antagonists likewise have varied clinical uses. For example, the α_2-antagonist yohimbine helps in the treatment of certain types of male sexual impotence. This compound increases parasympathetic and decreases sympathetic activity, which is thought to stimulate penile blood inflow and/or to inhibit blood outflow.

TABLE 5.2 Location and Physiological Actions of Peripheral α- and β-Adrenergic Receptors

Location	Action	Receptor subtype
Heart	Increased rate and force of contraction	β
Blood vessels	Constriction	α
	Dilation	β
Smooth muscle of the trachea and bronchi	Relaxation	β
Uterine smooth muscle	Contraction	α
Bladder	Contraction	α
	Relaxation	β
Spleen	Contraction	α
	Relaxation	β
Iris	Pupil dilation	α
Adipose (fat) tissue	Increased fat breakdown and release	β

The α_1-antagonist **prazosin** (**Minipress**) and the general β-adrenoceptor antagonist **propranolol** (**Inderal**) are both used clinically in the treatment of hypertension. Prazosin causes dilation of blood vessels by blocking the α_1-receptors responsible for constricting these vessels. In contrast, the main function of propranolol is to block the β-receptors in the heart, thereby reducing the heart's contractile force. The discovery that β_1 is the major adrenoceptor subtype in the heart has led to the introduction of β_1-selective antagonists such as **metoprolol** (**Lopressor**). These compounds exhibit fewer side effects than the more general β-antagonist propranolol. Beta-receptor antagonists like propranolol and metoprolol are also useful in the treatment of cardiac arrhythmia (irregular heartbeat) and angina pectoris (feelings of pain and constriction around the heart caused by deficient blood flow and oxygen delivery to the heart).

Finally, it should be mentioned that propranolol and other β-antagonists have also been applied to the treatment of generalized anxiety disorder, which is one of the most common types of anxiety disorder (see Chapter 17). Many patients with generalized anxiety disorder suffer from physical symptoms such as palpitations, flushing, and tachycardia (racing heart). Beta-blockers do not alleviate anxiety per se, but instead they may help the patient feel better by reducing some of these distressing physical symptoms of the disorder. A summary of compounds that affect the noradrenergic system centrally and/or peripherally is presented in **TABLE 5.3**.

Section Summary

- The most important cluster of noradrenergic neurons is the A6 cell group, which is located in a region known as the locus coeruleus. These neurons innervate almost all areas of the forebrain and mediate many of the important behavioral functions of NE.
- NE and EPI released into the bloodstream as hormones work together with NE released from sympathetic neurons to mediate the physiological reactions that make up the "fight-or-flight" response. These reactions include (1) vascular changes that direct more blood flow to skeletal muscles, heart, and brain; (2) increases in blood pressure, heart rate, and cardiac muscle contractile force; (3) respiratory changes that produce increased oxygen intake; (4) reduced digestive activity; (5) pupil dilation; (6) increased availability of metabolic fuels due to liberation of sugar from the liver and fats from adipose tissue; and (7) decreased secretion of gastric juices and saliva.
- NE and EPI activate a group of metabotropic receptors called adrenoceptors. They are divided into two broad families, α and β, which are further subdivided into α_1, α_2, β_1, and β_2. Both β-receptor subtypes enhance the synthesis of cAMP, whereas α_2-receptors inhibit cAMP formation.

Table 5.3 Drugs That Affect the Noradrenergic System

Drug	Action
Phenelzine	Increases catecholamine levels by inhibiting monoamine oxidase (MAO)
α-Methyl-*para*-tyrosine (AMPT)	Depletes catecholamines by inhibiting tyrosine hydroxylase
Reserpine	Depletes catecholamines by inhibiting vesicular uptake
6-Hydroxydopamine (6-OHDA)	Damages or destroys catecholaminergic neurons
Amphetamine*	Releases catecholamines
Cocaine and methylphenidate*	Inhibit catecholamine reuptake
Nisoxetine	Selectively inhibits norepinephrine (NE) reuptake
Phenylephrine	Stimulates α_1-receptors (agonist)
Clonidine	Stimulates α_2-receptors (agonist)
Albuterol	Stimulates β-receptors (partially selective for β_2)
Prazosin	Blocks α_1-receptors (antagonist)
Yohimbine	Blocks α_2-receptors (antagonist)
Propranolol	Blocks β-receptors generally (antagonist)
Metoprolol	Blocks β_1-receptors (antagonist)

* These drugs are not selective for catecholamines, as they also affect serotonin release or reuptake.

- Another mechanism of action of α_2-receptors involves hyperpolarization of the cell membrane by stimulating K^+ channel opening. In contrast, α_1-receptors increase the intracellular concentration of Ca^{2+} ions by means of the phosphoinositide second-messenger system.
- The noradrenergic system is involved in many behavioral functions, including arousal, cognition, and the consolidation of emotional memories (e.g., related to fear-inducing situations).
- The arousal- and wakefulness-promoting effects of NE are primarily mediated by pathways from the LC to the medial septal, medial preoptic, and lateral hypothalamic areas. Both α_1- and β-receptors are involved in these effects. LC neurons constitute one of the components of an arousal circuit that also includes orexin cells of the lateral hypothalamic area.
- The cognitive-enhancing effects of NE (e.g., on attention and working memory) are mediated by activation of high-affinity α_2-adrenoceptors in the PFC. Activation of lower-affinity α_1-receptors has the opposite effect, namely a decrease in cognitive function. Consequently, the relationship between NE release in the PFC and cognition can be described as an inverted U-shaped function: optimum cognitive function occurs at moderate amounts of NE release, whereas either too little or too much release can cause cognitive impairment.
- The facilitatory role of α_2-adrenoceptors in cognition has led to the use of the α_2-agonist guanfacine for the treatment of ADHD and Tourette's syndrome.
- Both central NE and peripherally released EPI play roles in memory consolidation. The mechanism by which EPI promotes memory is still being studied, with one theory focusing on activation of β-receptors on the vagus nerve and a competing theory focusing on a role for EPI-mediated elevations in plasma glucose. Administration of glucose has been shown to mimic the facilitatory effects of EPI in some studies, including studies of memory enhancement in aged animals.
- Adrenergic agonists are used therapeutically for various physiological and psychological disorders. These include the α_1-agonist phenylephrine, which helps relieve nasal congestion; the α_2-agonist clonidine, which is used in the treatment of hypertension and drug withdrawal symptoms; and β_2-agonists such as albuterol, which is an important medication for relieving bronchial congestion in people suffering from asthma.

STUDY QUESTIONS

1. Which neurotransmitters make up the category called catecholamines? What are the distinguishing chemical features of this category?
2. Describe the steps involved in the biosynthesis of dopamine and norepinephrine. Name the enzyme that catalyzes each biochemical reaction, and indicate which reaction is the rate-limiting step in catecholamine synthesis.
3. Discuss the factors that regulate the rate of catecholamine synthesis.
4. List the names of the proteins that transport catecholamines in synaptic vesicles. Which of these proteins is expressed in the brain, and which is expressed in the adrenal medulla?
5. How do amphetamine and methamphetamine affect catecholamine release, and what are the behavioral effects of these drugs in laboratory rats and mice?
6. What is meant by single-spiking versus burst firing mode as applied to the firing patterns of midbrain dopaminergic neurons? How do these different firing patterns influence dopamine release at the nerve terminal?
7. Describe how catecholamine release is regulated by autoreceptors, including differences in the location and mechanism of action of terminal versus somatodendritic autoreceptors. What adrenergic subtypes function as noradrenergic autoreceptors? Name an adrenergic autoreceptor agonist and an antagonist, and indicate what effects these drugs have on noradrenergic cell firing.
8. What are the two basic mechanisms by which catecholamine transmission is terminated?
9. Name the major metabolites of DA and NE.

(*Continued*)

STUDY QUESTIONS (*continued*)

10. Name and discuss the clinical uses of drugs that alter either catecholamine reuptake or catecholamine metabolism.
11. Describe the two major dopaminergic pathways that originate in the midbrain and project to forebrain structures. Include in your answer the derivation of each pathway's name.
12. The midbrain dopaminergic cell groups have been shown to play important roles in motor function, motivation, and cognition. Much of this information has been obtained using either neurotoxins to damage/kill the cells or genetic engineering methods to produce a biochemical DA deficiency (i.e., DD mice). Compare and contrast these methodological approaches, and then discuss the behavioral characteristics of laboratory animals that have been generated using one or the other technique.
13. Describe (a) the behavioral functions of the mesolimbic versus the mesocortical dopaminergic pathways, and (b) the involvement of different subsets of VTA dopaminergic neurons in responding to rewarding versus aversive stimuli.
14. How many different subtypes of DA receptors exist? How are these subtypes grouped into families? Discuss the differences between D_1 and D_2 receptors with respect to signaling mechanisms and affinity for DA.
15. Name the drugs presented in the text that act as either agonists or antagonists for D_1 and D_2 receptors. Then describe the behavioral effects in rats or mice of administering each drug.
16. What is behavioral supersensitivity? In the case of D_2 receptor supersensitivity, how is this phenomenon produced pharmacologically, and what is the hypothesized mechanism?
17. Discuss the major sources of NE in the forebrain and in the peripheral nervous system. What is the "fight-or-flight" response, and how do EPI and NE mediate this response?
18. Describe the adrenergic receptor subtypes and their signaling mechanisms.
19. Discuss the involvement of the central noradrenergic system in arousal and cognition. Include in your answer information derived from pharmacological manipulations of this system and the role of specific adrenergic receptor subtypes.
20. What is the evidence that peripheral EPI plays a role in the consolidation of emotional memories? What are the hypothesized mechanisms underlying this effect of EPI?
21. Describe the uses of specific medications that work by either stimulating or blocking peripheral adrenergic receptors.
22. Discuss the human disorders produced by spontaneously occurring mutations in the biochemical mechanisms for DA synthesis or reuptake. What are the symptoms produced in each disorder, and what pharmacological treatments are used to counteract those symptoms? Include in your answer the rationale for selection of each kind of pharmacological symptom based on the mutation involved.

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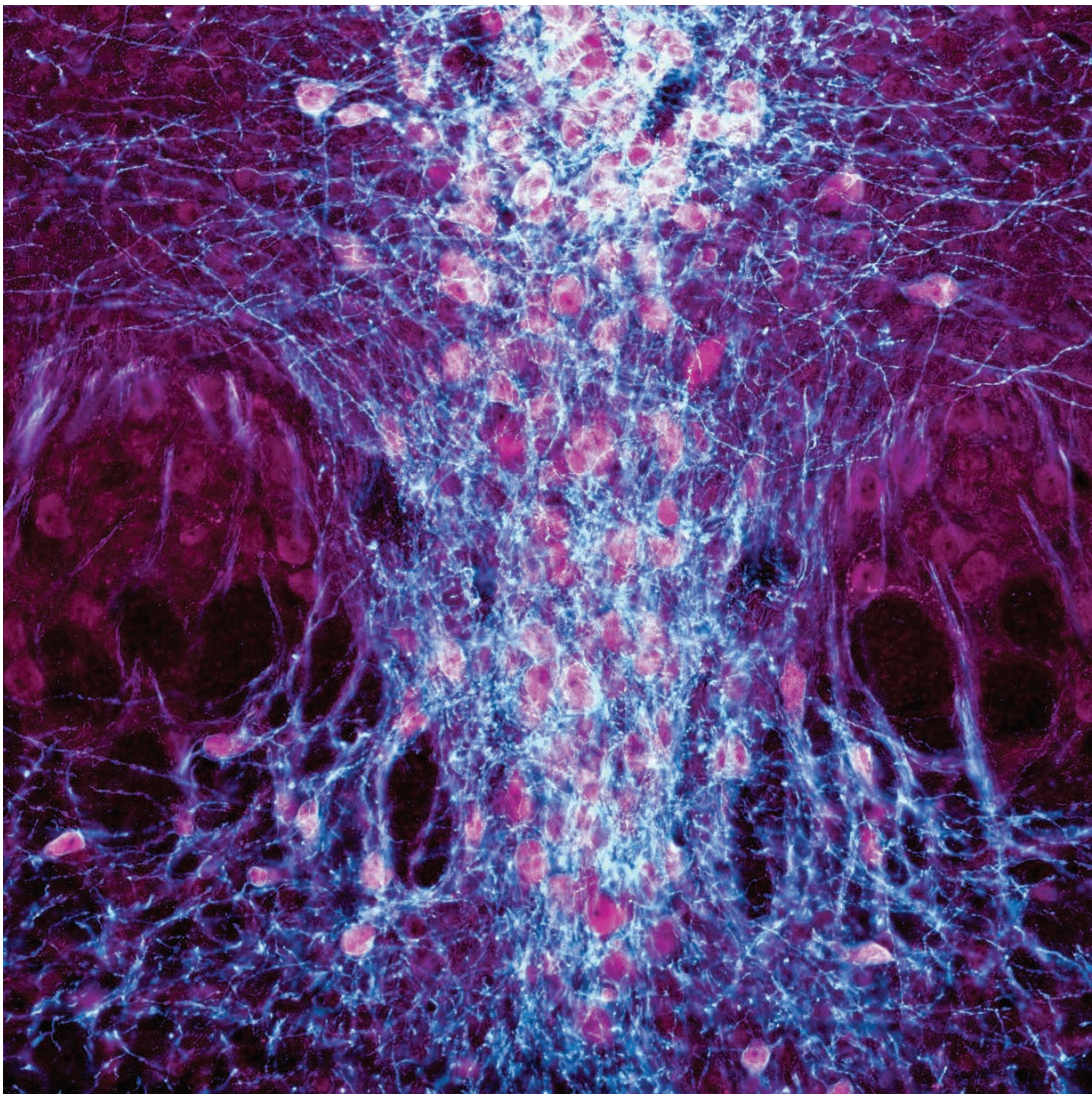
CHAPTER 6



The serotonergic neurons of the dorsal raphe nucleus (shown here in pink) are located in the midbrain and send their axons to many forebrain areas. (© Tessa Hirschfeld-Stoler.)

Serotonin

THE PATIENT WAS A 34-YEAR-OLD MARRIED WOMAN who was brought to the hospital's emergency room by her husband at 3:00 in the morning. Half an hour earlier, he had found his wife lying on the floor of their home, and he immediately brought her to the hospital. Doctors in the emergency room noted symptoms of agitation, mental confusion and disorientation, sweating, and muscular rigidity. The patient also had a mild fever of 38.3°C (100.9°F) and exhibited rapid but shallow breathing. Over time, her neuromuscular symptoms worsened, and she spiked a fever of 42.3°C (108°F). Despite receiving extensive supportive care from hospital staff, the woman died of cardiopulmonary arrest 20 hours after she was admitted.

This real case report was published in 2005 by Sener and colleagues. What caused this unfortunate woman's demise? Her husband determined that his wife had intentionally consumed large amounts of two prescription medications, presumably for the purpose of committing suicide. The medications—paroxetine and moclobemide—act to boost levels of the neurotransmitter **serotonin** (paroxetine works by blocking reuptake of serotonin, whereas moclobemide slows the rate of serotonin metabolism by inhibiting monoamine oxidase A [MAO-A]). Clinicians have known for some time that excessive doses of serotonergic agents, particularly in combination, as occurred in the present case, can lead to a dangerous and even life-threatening set of symptoms called the **serotonin syndrome** (Alusik et al., 2014). Although the serotonin syndrome is rare, this neurotransmitter—more technically known as **5-hydroxytryptamine**, or **5-HT**—has been featured in the popular culture as the culprit in just about every human malady or vice, including depression, anxiety, obesity, impulsive aggression and violence, and even drug addiction. Can a single neurotransmitter really have such far-reaching behavioral consequences?

The answer is not a simple one—5-HT probably does influence many different behavioral and physiological systems, yet the ability of this chemical to either destroy us (if imbalanced) or to cure all that ails us (if brought back into equilibrium) has unfortunately been oversold by a sensationalist media aided and abetted by a few publicity-seeking scientists. In this chapter, we will learn about the neurochemistry, pharmacology, and functional characteristics of this fascinating neurotransmitter. ■

Serotonin Synthesis, Release, and Inactivation

The level of serotonergic transmission depends on the relative contributions of synthesis, release, and inactivation of the transmitter.

Serotonin synthesis is regulated by enzymatic activity and precursor availability

Serotonin was discovered and first isolated not from the brain, but rather from its presence in the bloodstream and its ability to cause vasoconstriction (see Whitaker-Azmitia, 1999, and Göthert, 2013, for the history of serotonin's discovery). Indeed, the name *serotonin* is a combination of *sero*, referring to serum (the liquid that remains after blood has been allowed to clot), and *tonin*, referring to the substance's effect on vascular tone (contraction of smooth muscles that constrict blood vessels). Later in this chapter you will learn about the cells in the gut that are the source of blood-borne 5-HT.

Serotonin is synthesized from the amino acid **tryptophan**, which comes from protein in our diet. As shown in **FIGURE 6.1**, the biochemical pathway comprises two steps. The first step is catalyzed by the enzyme **tryptophan hydroxylase** (**TPH**), which converts tryptophan to **5-hydroxytryptophan** (**5-HTP**). 5-HTP is then acted upon by **aromatic amino acid decarboxylase** (**AADC**) to form 5-HT. In 2003, researchers discovered that there are two forms of the TPH gene, designated *TPH1* and *TPH2*. *TPH2* is expressed by **serotonergic neurons** (neurons that use 5-HT as their neurotransmitter), whereas *TPH1* is expressed by certain types of non-neuronal cells, including 5-HT–secreting enterochromaffin cells located in the gut and melatonin-secreting cells in the pineal gland.

Many features of the 5-HT synthesis pathway are similar to those of the pathway described in Chapter 5 involving the formation of dopamine (DA) from the amino acid tyrosine. Just as the initial step in the synthesis of DA (i.e., tyrosine to DOPA) is the rate-limiting step, the conversion of tryptophan to 5-HTP is rate limiting in the 5-HT pathway. Furthermore, just as tyrosine hydroxylase is found only in neurons that synthesize catecholamines, TPH in the brain is a specific marker for the serotonergic neurons. Another important point is that the second enzyme in the pathway, AADC, is the same for both catecholamines and 5-HT.

Synthesis of serotonin in the brains of animals can be stimulated by giving them large doses of tryptophan, but administration of 5-HTP is even more effective because it is converted so rapidly and efficiently to 5-HT. There is also an interesting link between food intake and 5-HT synthesis that was first discovered many years ago by John Fernstrom and Richard Wurtman (1972). Imagine a group of rats that have been fasted overnight and then fed a protein-rich meal. The level

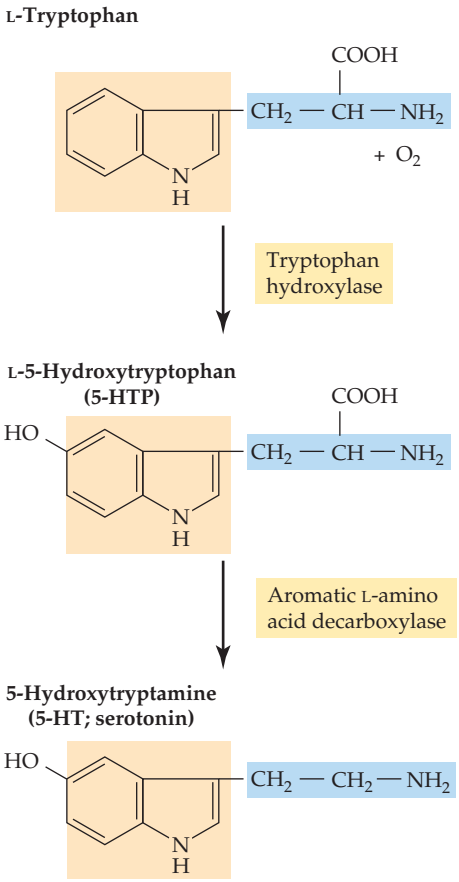


FIGURE 6.1 Synthesis of serotonin Serotonin (5-HT) is synthesized from the amino acid tryptophan in two steps, catalyzed by the enzymes tryptophan hydroxylase and aromatic amino acid decarboxylase.

of tryptophan in their blood goes up, and thus you probably would expect brain 5-HT to rise as well, since an injection of pure tryptophan produces such an effect. Surprisingly, however, Fernstrom and Wurtman found that consumption of a protein-rich meal did not cause increases in either tryptophan or 5-HT in the brain, even though tryptophan levels in the bloodstream were elevated. The researchers explained this result by showing that tryptophan competes with a group of other amino acids (called large neutral amino acids; LNAA) for transport from the blood to the brain across the blood–brain barrier (**FIGURE 6.2**). Consequently, it's the *ratio* between the amount of tryptophan in the blood and the overall amount of its competitors that counts. Most proteins contain larger amounts of these competitor amino acids than tryptophan; thus, when these proteins are consumed, the critical ratio either stays the same or even goes down.

Even more surprising was an additional finding of Fernstrom and Wurtman. When these researchers fed previously fasted rats a meal low in protein but high in carbohydrates, that experimental treatment led to increases in brain tryptophan and 5-HT levels. How

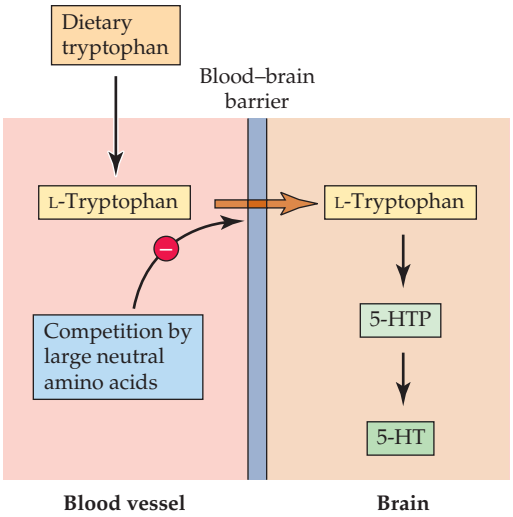


FIGURE 6.2 Tryptophan entry into the brain and 5-HT synthesis Transport and synthesis are regulated by the relative availability of tryptophan versus large neutral amino acids that compete with it for transport across the blood–brain barrier. In rats, a high-protein, low-carbohydrate meal does not increase brain tryptophan levels or 5-HT synthesis rate, because of this competitive process. However, the ratio of circulating tryptophan to large neutral amino acids is elevated after a high-carbohydrate, low-protein meal, thereby enhancing entry of tryptophan into the brain and stimulating 5-HT synthesis.

could this be the case? You might already know that eating carbohydrates (starches and sugars) triggers release of the hormone **insulin** from the pancreas. One important function of this insulin response is to stimulate the uptake of glucose from the bloodstream into various tissues, where it can be metabolized for energy. But glucose is not the only substance acted on by insulin. This hormone also stimulates the uptake of most amino acids from the bloodstream; tryptophan, however, is relatively unaffected. Because of this difference, a low-protein, high-carbohydrate meal will increase the tryptophan-to-LNAA ratio, allowing more tryptophan to cross the blood–brain barrier and more 5-HT to be made in the brain. Yet another nutrient-based method for increasing this ratio is to have subjects consume a diet enriched with high-tryptophan proteins such as the milk protein α-lactalbumin (Markus et al., 2000).

Pharmacological depletion of 5-HT has been widely used to assess the role of this neurotransmitter in various behavioral functions. One method often used in rodent studies consists of administering the drug **para-chlorophenylalanine** (**PCPA**), which selectively blocks 5-HT synthesis by irreversibly inhibiting TPH. One or two high doses of PCPA can reduce brain 5-HT levels in rats by 80% to 90% for as long as 2 weeks, until the serotonergic neurons make new TPH molecules that haven't been exposed to the inhibitor. Because PCPA can cause adverse side effects in humans, researchers have developed an alternative method for decreasing brain 5-HT in human studies. Based in part on the rat studies of Fernstrom and Wurtman, this method involves the administration of an amino acid "cocktail" containing a large quantity of amino acids except for tryptophan. Ingestion of this cocktail causes large reductions in both plasma and cerebrospinal fluid levels of tryptophan over a period of roughly 6–12 hours (reviewed by Hulsken et al., 2013). Tryptophan depletion occurs for two reasons: (1) the surge of amino acids in the bloodstream stimulates protein synthesis by the liver, which reduces the level of plasma tryptophan to below its starting point; and (2) the large neutral amino acids in the cocktail inhibit entry of the remaining tryptophan into the brain. Brain 5-HT synthesis and metabolism are also reduced by tryptophan depletion, but no information is yet available concerning the influence of this procedure on actual 5-HT release and synaptic signaling.

Manipulation of tryptophan availability (and therefore brain 5-HT) has been used to assess the role of 5-HT in mood and cognition. First we will discuss the results of increasing trytophan availability by administration of pure tryptophan (sometimes called **tryptophan loading**), consumption of a diet supplemented with α-lactalbumin or other high-tryptophan proteins, or consumption of a diet rich in carbohydrates but low in protein. In healthy subjects, the effects of elevating tryptophan on mood and cognition seem to depend on the magnitude of increase in the tryptophan-to-LNAA ratio. Modest increases in this ratio usually have no effect on mood or cognition, larger increases can positively enhance mood and cognition (e.g., memory or attention), and extremely high increases can actually cause negative effects on mood (e.g., increased anger, tension, and depression) and cognition (reviewed by Silber and Schmitt, 2010; Hulsken et al., 2013).

Quite a few studies have investigated the effects of reducing brain 5-HT by means of acute tryptophan depletion with the tryptophan-free amino acid cocktail. In general, this procedure produces little change in mood in healthy participants. However, many patients previously diagnosed with clinical depression and who are currently taking an antidepressant that acts on the serotonergic system experience a relapse in their symptoms when subjected to acute tryptophan depletion (Ruhé et al., 2007; Hulsken et al., 2013). This effect is not seen in patients being treated with antidepressants that work on the noradrenergic instead of the serotonergic system (see Chapter 18 for a detailed discussion of the hypothesized roles of 5-HT and norepinephrine in the etiology of depression). Thus, in contrast to mood regulation in nondepressed individuals, symptom improvement in depressed patients who have responded to serotonergic medications may depend on continued

activity of this neurotransmitter system. A thorough review of the effects of acute tryptophan depletion on cognition concluded that this procedure impairs memory consolidation of verbal information but has little or no influence on working memory or attention (Mendelsohn et al., 2009). Other pharmacological approaches such as administration of serotonergic receptor agonists and antagonists have revealed additional information regarding the involvement of 5-HT in learning and memory. Some of this research is presented in the final section of this chapter.

Similar processes regulate storage, release, and inactivation of serotonin and the catecholamines

The main features of a serotonergic neuron are depicted in **FIGURE 6.3**. Serotonin (5-HT) is transported into synaptic vesicles using VMAT2 (vesicular monoamine transporter 2), which is the same vesicular transporter found in dopaminergic and noradrenergic neurons. As with the catecholamines, storage of 5-HT in the vesicles plays a critical role in protecting the transmitter from enzymatic breakdown in the nerve terminal. Consequently, the VMAT blocker reserpine depletes serotonergic neurons of 5-HT, just as it depletes catecholamines in dopaminergic and noradrenergic cells.

Serotonergic autoreceptors control release of 5-HT in the same way as the DA and NE autoreceptors discussed in Chapter 5. Terminal autoreceptors directly inhibit 5-HT release, whereas other autoreceptors on the cell body and dendrites of the serotonergic neurons (somatodendritic autoreceptors) indirectly inhibit release by slowing the rate of firing of the neurons. Somatodendritic autoreceptors are of the 5-HT_{1A} subtype, whereas the terminal autoreceptors are of either the 5-HT_{1B} or 5-HT_{1D} subtype, depending on the species (see later discussion of 5-HT receptors).

Release of 5-HT can be directly stimulated by a family of drugs based on the structure of amphetamine. These compounds include **para-chloroamphetamine**, which is mainly used experimentally; **fenfluramine**, which at one time was prescribed for appetite suppression in obese patients; and **3,4-methylenedioxymethamphetamine** (**MDMA**), which is a recreational and abused drug. Besides their acute behavioral effects, these drugs (particularly *para*-chloroamphetamine and MDMA) can exert toxic effects on the serotonergic system (**BOX 6.1**).

When we examine the processes responsible for inactivation of 5-HT after its release, many similarities to the catecholamine systems become apparent. After 5-HT is released, it is removed from the extracellular fluid by a reuptake process. As with DA and NE, this mechanism involves a protein on the nerve terminal known as the **5-HT transporter**, also known as **SERT** (see Figure 6.3). This protein turns out to be a key target of drug action. For example, the introduction of **fluoxetine**, better known as **Prozac**, in late 1987 spawned a whole new class of antidepressant drugs based on the idea of inhibiting 5-HT reuptake. These compounds are, therefore, called **selective serotonin reuptake inhibitors** (**SSRIs**) (see Chapter 18). Certain abused drugs such as cocaine and MDMA likewise interact with SERT, but they are not selective in their effects because they also influence the transporters for DA and NE. Mutant mice lacking a functional SERT exhibit an astonishing array of behavioral and physiological abnormalities that presumably arise as the result of chronic enhancement of serotonergic activity (since the neurotransmitter cannot be taken up after it is released) during the animal's lifetime (Murphy and Lesch,

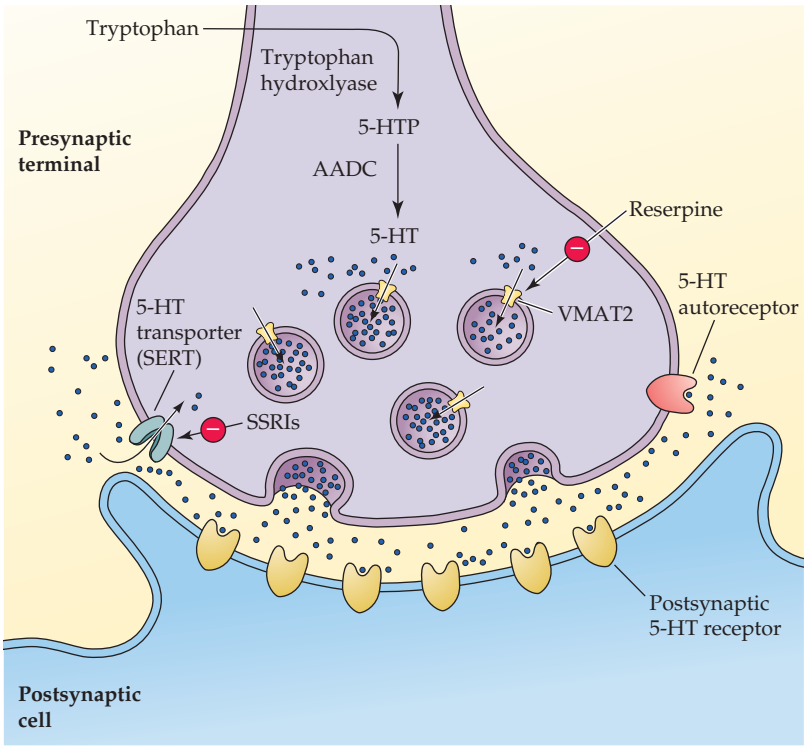


FIGURE 6.3 Features of a serotonergic neuron Serotonergic neurons express the enzymes tryptophan hydroxylase and aromatic L-amino acid decarboxylase (AADC), the vesicular monoamine transporter VMAT2, the 5-HT transporter (SERT), and 5-HT_{1B} or 5-HT_{1D} autoreceptors in their terminals. 5-HTP, 5-hydroxytryptophan; SSRIs, selective serotonin reuptake inhibitors.

BOX 6.1 History of Psychopharmacology

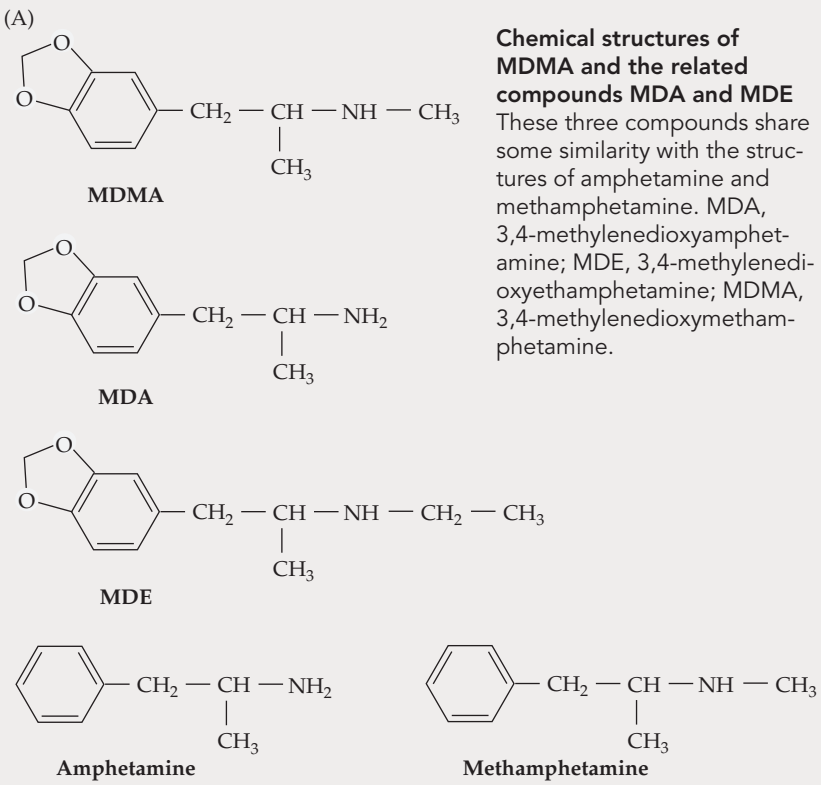
"Ecstasy"—Harmless Feel-Good Drug, Dangerous Neurotoxin, or Miracle Medication?

Over the years, pharmaceutical companies have synthesized many thousands of new compounds in the search for drugs that might be useful in the treatment of one or another medical disorder. Unfortunately, a vast majority of these compounds are discarded, either because they do not produce a useful biological effect or because they fail somewhere along the rigorous process of safety and efficacy testing (see Box 4.2). Of course, occasionally a drug is discovered that does produce an interesting set of biological actions that are ultimately exploited for therapeutic benefit, illicit recreational use, or sometimes both. The present discussion deals with one such compound—3,4-methylenedioxymethamphetamine (MDMA), which is known on the street by a variety of names including "ecstasy," "E," "X," "Adam," "love drug," and most recently "Molly." When someone purchases a pill that the seller says is ecstasy, it may or may not actually contain MDMA. For this reason, we will use the term *MDMA* when we are referring to the pure chemical compound (e.g., as might be used in a controlled animal study); however, we will use the term *ecstasy* when we are referring to the human clinical literature on this topic, because of the uncertainty associated with the purchase and consumption of street drugs that contain variable and unknown amounts of MDMA.

The early history of the discovery of MDMA and the route by which it became a recreational drug have been thoroughly reviewed by Freudenmann et al. (2006), Pentney (2001), and Rosenbaum (2002). Chemically, MDMA is a close relative of amphetamine and methamphetamine (**Figure A**), and it shares some but not all of their biological and behavioral characteristics. In particular, all three compounds acutely release catecholamines and 5-HT from their respective nerve terminals, although MDMA has a more potent effect on 5-HT release than is seen with amphetamine or methamphetamine. MDMA was first synthesized by the Merck pharmaceutical company in Germany in 1912. Although many textbooks and other sources claim that MDMA was produced as part of a project to find new anorectic (appetite-suppressing) compounds, original documents demonstrate that Merck was, in fact, seeking new hemostatic agents (drugs that would help stop bleeding) (Freudenmann et al., 2006). Although Merck went so far as to patent MDMA in 1914 and did perform some later unpublished studies on the drug, the company never marketed it for any therapeutic use.

Harold Hardman and his colleagues reported the first study on the effects of MDMA on experimental animals in 1973. This work had actually been conducted 20 years earlier under a classified contract from the U.S. Army to investigate the mechanisms of action of mescaline and other potential hallucinogenic compounds with a similar chemical structure (later declassification of the results allowed them to finally be published). More important, the first information regarding the influence of MDMA on humans was published by Alexander Shulgin and David Nichols in 1978. Shulgin was an American chemist who, while working for the Dow Chemical Company, had become interested in developing and characterizing new mind-altering drugs. He began the practice of taking the drugs himself and/or offering them to close colleagues and

(*Continued*)



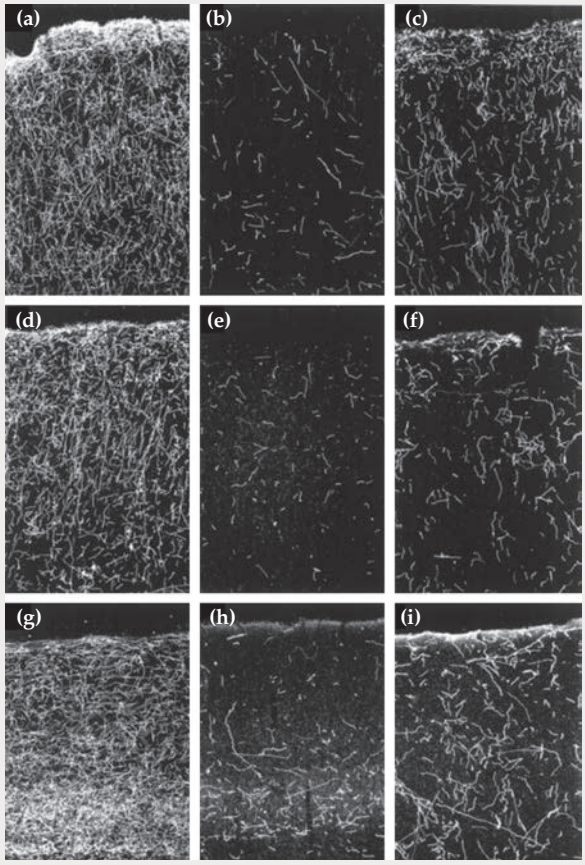
Chemical structures of MDMA and the related compounds MDA and MDE These three compounds share some similarity with the structures of amphetamine and methamphetamine. MDA, 3,4-methylenedioxyamphetamine; MDE, 3,4-methylenedioxyethamphetamine; MDMA, 3,4-methylenedioxymethamphetamine.

BOX 6.1 History of Psychopharmacology *(continued)*

friends (Benzenhöfer and Passie, 2010). After leaving Dow in 1966, Shulgin continued this work as a private researcher and consultant. In the Shulgin and Nichols 1978 article, which included a report on the effects of taking MDMA (75 to 150 mg orally, which is a common recreational dose even now), the authors stated that "the drug appears to evoke an easily controlled altered state of consciousness with emotional and sensual overtones. It can be compared in its effects to marijuana, to psilocybin devoid of the hallucinatory component, or to low levels of MDA" p. 77). It is noteworthy that the authors mention MDA (3,4-methylenedioxyamphetamine), which, although studied before MDMA, was later discovered to be a biologically active metabolite of the latter compound.

Following this "rediscovery" of MDMA, events started to move rapidly in several different directions. MDA, which produces psychological effects similar to those elicited by MDMA, had already been proposed as a potential adjunct to psychotherapy because of its purported ability to increase empathy between therapist and client (Naranjo et al., 1967). After experiencing the effects of MDMA himself, Shulgin apparently decided that this drug would be even better therapeutically than MDA. Consequently, in the 1970s, he began to distribute MDMA to many psychotherapists for use during their therapy sessions (Pentney, 2001). Note that both the distribution and use of MDMA were performed quietly because of the uncertain legal status of the compound at that time. During this same period, MDMA was (perhaps inevitably) gradually beginning to make its way onto the street. By the early 1980s, "Adam" or "ecstasy," depending on where one lived, was growing in popularity. Use was particularly heavy in Dallas, Austin, and other parts of Texas. Therefore, it was no surprise when MDMA began to attract the attention of the Drug Enforcement Administration (DEA), an arm of the U.S. Justice Department that determines the legal status of drugs and prosecutes users of illegal substances. Several research laboratories had already been studying the short- and long-term effects of MDMA in laboratory animals such as rats and monkeys and had found alarming evidence for large depletions of 5-HT in the forebrains of animals given high doses of this compound. Moreover, if brain slices were stained with an anti–5-HT antibody to allow visualization of serotonergic axons in the forebrain, most of the staining disappeared within a week after high-dose MDMA exposure. In a particularly striking study by Hatzidimitriou and coworkers (1999) using squirrel monkeys, some of the animals exhibited a marked reduction in staining even 7 years after receiving drug treatment (**Figure B**). The DEA held hearings concerning the status of MDMA in 1985,

(B)



Reduced serotonergic fiber density in the neocortex of squirrel monkeys treated with MDMA Serotonergic axons were stained with an antibody against 5-HT and then visualized using dark-field microscopy (this makes the background appear dark and the stained fibers appear light). The panels show fiber staining in tissue sections from the frontal cortex, parietal cortex, and primary visual cortex, respectively, of a control monkey that had received only saline (a, d, and g), a monkey that had received subcutaneous injections of 5mg/kg MDMA twice daily for 4 days and was then killed 2 weeks later (b, e, and h), and a monkey that had received the same MDMA treatment but was killed 7 years later (c, f, and i). MDMA exposure produced a massive reduction of 5-HT–immunoreactive fibers in all three cortical areas at 2 weeks post-treatment, and noticeable deficits were still apparent even after 7 years of recovery. (From Hatzidimitriou et al., 1999.)

and largely because of the testimony of the animal researchers, the agency assigned the drug Schedule I status. As discussed further in Chapter 9, Schedule I is the most restrictive schedule and is reserved for substances that have no recognized medical use and high abuse potential (e.g., heroin).

BOX 6.1 History of Psychopharmacology (*continued*)

In hindsight, we know that the scheduling of MDMA has not prevented its continued recreational use each year by thousands of young people, often at "raves" and other dances. These users presumably either don't care what happens to the serotonergic system in their brains or don't believe that the drug is as dangerous as was argued in that original DEA hearing over 30 years ago. So, who is right? Unfortunately, despite an enormous amount of research both on ecstasy users and on experimental animals, there is yet to be a consensus regarding the risk of taking this drug. There is considerable evidence that heavy MDMA exposure (i.e., long-term and/or at high doses) causes abnormalities in the serotonergic system and deficits in cognitive function (Parrott, 2013; Vegting et al., 2016); however, whether actual degeneration of serotonergic nerve fibers occurs, as opposed to biochemical abnormalities without anatomical damage, remains a point of controversy (Biezonski and Meyer, 2011; Meyer, 2013). It is also unclear whether more moderate use of the drug produces any lasting behavioral or neurochemical impairment (Mueller et al., 2016).

The final part of this historical survey of MDMA/ecstasy is perhaps the most fascinating. We mentioned earlier that beginning in the 1970s, MDMA was being administered in an unregulated way by many psychotherapists to their clients to assist in the therapeutic process. This practice mostly stopped once MDMA was given a Schedule I classification in 1985. However, around the year 2000, a private organization called the Multidisciplinary Association for Psychedelic Studies (MAPS) began to initiate the process of organizing, obtaining regulatory permissions, and raising funding for controlled studies into the use of low-dose MDMA treatment as an adjunct to psychotherapeutic treatment of patients with several different disorders, particularly severe post-traumatic stress disorder (PTSD). Two clinical trials have been published thus far, and the results showed significant improvement in the clinical response when MDMA was added to a standard psychotherapy procedure. In fact, the available data indicate that MDMA-assisted psychotherapy may be more effective than prolonged exposure therapy, a widely used approach for treating PTSD (Amoroso and Workman, 2016). Clearly, more work needs to be done in this area, and these initial positive findings must be replicated by other research groups. Nevertheless, it is intriguing to consider that we may have now come full circle with MDMA: originally discovered in a program to develop new therapeutic compounds for hemostasis, it was later found to be neurotoxic when used recreationally at high doses, and now considered potentially beneficial for patients with severe treatment-resistant PTSD when given at low, controlled doses in a therapeutic setting.

2008; Murphy et al., 2008) (**FIGURE 6.4**). Some of the functions that can be ascribed to 5-HT on the basis of the phenotype of SERT knockout mice are discussed in the last part of this chapter.

You will recall that DA and NE are metabolized by two different enzymes—monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT). Because 5-HT is not a catecholamine, it is not affected by COMT. However, its breakdown is catalyzed by MAO-A to yield the metabolite **5-hydroxyindoleacetic acid** (**5-HIAA**). The level of 5-HIAA in the brains of animals or in the cerebrospinal fluid of humans or animals is often used as a measure of the activity of serotonergic neurons. This practice is based on research showing that

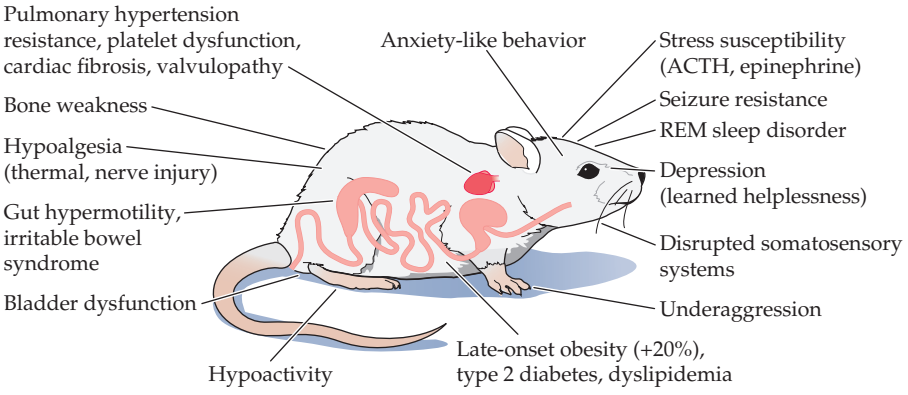


FIGURE 6.4 Behavioral and physiological phenotypes of SERT knockout mice Widespread SERT expression both in the brain (by serotonergic neurons and their axons and terminals) and in various peripheral tissues leads to dysfunction in many behavioral and physiological systems when normal 5-HT uptake is impaired by loss of this transporter. ACTH, adrenocorticotropic hormone; REM, rapid-eye-movement. (After Murphy and Lesch, 2008.)

when these neurons fire more rapidly, they make more 5-HT, and a corresponding increase in the formation of 5-HIAA occurs.

Section Summary

- The neurotransmitter 5-HT is synthesized from the amino acid tryptophan in two biochemical reactions. The first and rate-limiting reaction is catalyzed by the enzyme TPH.
- Brain 5-HT synthesis is controlled, in part, by tryptophan availability. Tryptophan entry into the brain from the bloodstream is determined by the plasma ratio of tryptophan to other large neutral amino acids (LNAA) that compete for transport across the blood–brain barrier.
- 5-HT synthesis is increased by several methods including direct tryptophan administration (tryptophan loading), consumption of proteins with very high tryptophan content, or consumption of a high-carbohydrate, low-protein meal. 5-HT synthesis is reduced by administration of an amino acid mixture lacking tryptophan, which causes acute tryptophan depletion.
- In healthy subjects, modest increases in the tryptophan-to-LNAA ratio produced by tryptophan loading usually have no effect on mood or cognition, whereas larger increases can positively enhance mood and cognition, and extremely high increases can exert negative effects on these processes. Acute tryptophan depletion generally does not affect mood in healthy participants but can cause symptom relapse in remitted depressed patients who are under treatment with serotonergic antidepressant drugs. Tryptophan depletion in healthy people can impair memory consolidation of verbal information.
- VMAT2 is responsible for loading 5-HT into synaptic vesicles for subsequent release.
- Serotonin release is inhibited by autoreceptors located on the cell body, dendrites, and terminals of serotonergic neurons. The terminal autoreceptors are of either the 5-HT_{1B} or 5-HT_{1D} subtype, depending on the species, whereas the somatodendritic autoreceptors are of the 5-HT_{1A} subtype.
- Several compounds have been identified that cause 5-HT release, one of which is MDMA. At high doses, this drug can produce serotonergic toxicity characterized by long-lasting depletion of 5-HT and other markers of serotonergic neurons.
- Serotonergic transmission is terminated by reuptake of 5-HT from the extracellular fluid. This process is mediated by the 5-HT transporter, which is an important target of several antidepressant drugs.
- Serotonin is ultimately metabolized by MAO-A to form the major breakdown product 5-HIAA.

Organization and Function of the Serotonergic System

There is a widespread distribution of 5-HT in the brain, which contributes to its participation in a variety of behavioral and physiological functions.

The serotonergic system originates in the brainstem and projects to all forebrain areas

The Swedish researchers who first mapped the catecholamine systems in the 1960s (see Chapter 5) used the same experimental techniques to study the distribution of neurons and pathways using 5-HT. But in this case, they designated the 5-HT–containing cell groups with the letter *B* instead of *A*, which they had used for the dopaminergic and noradrenergic cell groups. It turns out that almost all of the serotonergic neurons in the CNS are found along the midline of the brainstem (medulla, pons, and midbrain), loosely associated with a network of cell clusters called the **raphe nuclei**.[1] Of greatest interest to neuropharmacologists are the **dorsal raphe nucleus** and the **median raphe nucleus**, located in the area of the caudal midbrain and rostral pons. Together, these nuclei give rise to most of the serotonergic fibers in the forebrain. Virtually all forebrain regions receive serotonergic innervation, including the neocortex, striatum and nucleus accumbens, thalamus and hypothalamus, and limbic system structures such as the hippocampus, amygdala, and septal area (Tork, 1990) (**FIGURE 6.5**).

As with other neurotransmitters, the serotonergic fibers innervating a particular brain area are not uniformly distributed. **FIGURE 6.6** illustrates this principle using a photomicrograph of serotonergic fibers in the mouse neocortex and hippocampus (Donovan et al., 2002). Note that in the cortex, the fibers are particularly dense at the cortical surface (layer 1), which is a layer that has very few nerve cells but has a high density of dendrites and synaptic connections. In contrast, there are very few serotonergic fibers within the white matter separating the hippocampus from the cortex above and also very few fibers in the two major cell layers of the hippocampus, the pyramidal cell layer and the dentate gyrus granule cell layer. The paucity of serotonergic fibers within these cell layers tells us that the serotonergic inputs to the cells in these layers are not targeting

[1]The term *raphe* is Greek for "seam" or "suture." In biology, the term is applied to structures that look as if they are joined together in a line. This is applicable to the raphe nuclei, which are aligned with each other along the rostral–caudal axis of the brainstem.

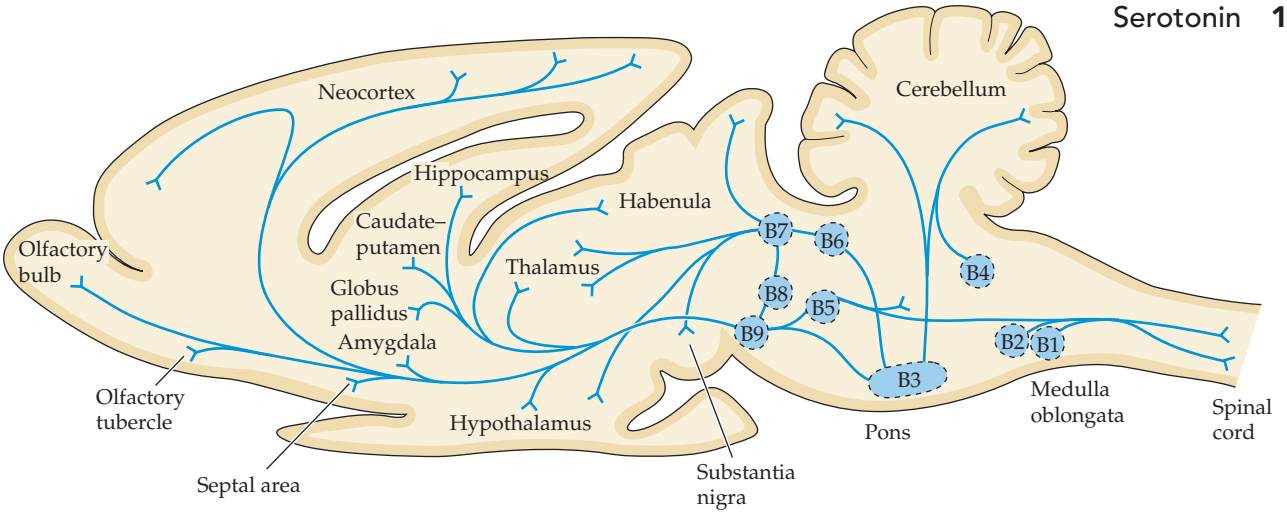


FIGURE 6.5 Anatomy of the serotonergic system The B7 cell group corresponds to the dorsal raphe, and the B8 cell group corresponds to the median raphe.

the cell bodies but instead are targeting dendrites that have branched out away from the cells.

The firing of dorsal raphe serotonergic neurons varies with behavioral state and in response to rewards and punishments

Firing patterns of the dorsal raphe neurons have been well characterized, particularly in cats. When a cat is awake, each cell fires at a relatively slow but very regular rate (tonic firing), almost like a ticking clock (**FIGURE 6.7**). When the cat enters slow-wave sleep, which is the stage of sleep in which large-amplitude, slow electroencephalographic (EEG) waves can be recorded in the cortex, the serotonergic neurons slow down and become more irregular in their firing. Most intriguingly, the cells are almost completely shut down when the cat is in rapid-eye-movement (REM) sleep, a stage of sleep characterized by side-to-side eye movements and low-amplitude, fast EEG waves in the cortex (reviewed by Jacobs and Fornal, 1999). Thus, we can see that 5-HT neurons, like catecholamine and orexin neurons, are part of the complex circuitry that regulates and responds to phases of the sleep–wake cycle.

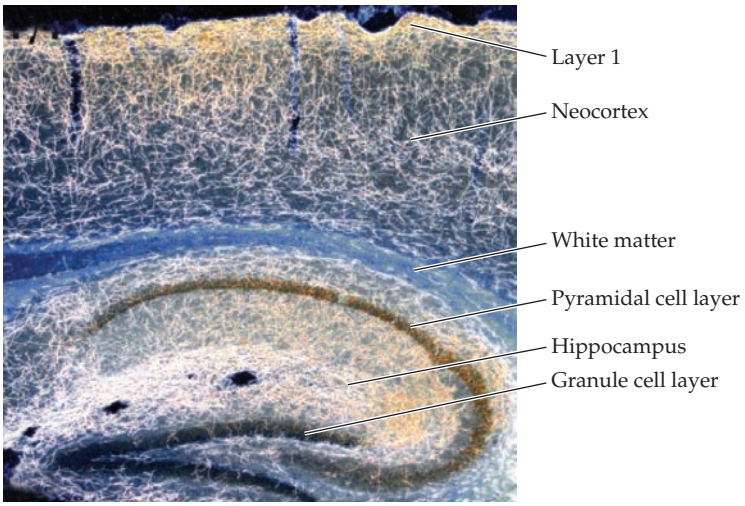


FIGURE 6.6 Photomicrograph of serotonergic fibers in the mouse neocortex and hippocampus The photomicrograph shows a parasagittal section from a young mouse brain that was stained using an antibody against the 5-HT transporter. This method permits visualization of serotonergic fibers (axons) because the transporter protein is present throughout the length of the axonal membrane. The photomicrograph was taken using dark-field microscopy, which reverses light and dark structures. Consequently, the stained axons appear brightly colored, whereas the cell bodies of the hippocampal pyramidal cell layer and dentate gyrus granule cell layer appear dark. Note that in some areas, such as the deep layers of the hippocampus and layer 1 of the cortex, the serotonergic axons are so numerous that individual fibers cannot be resolved at the present magnification. (From Donovan et al., 2002.)

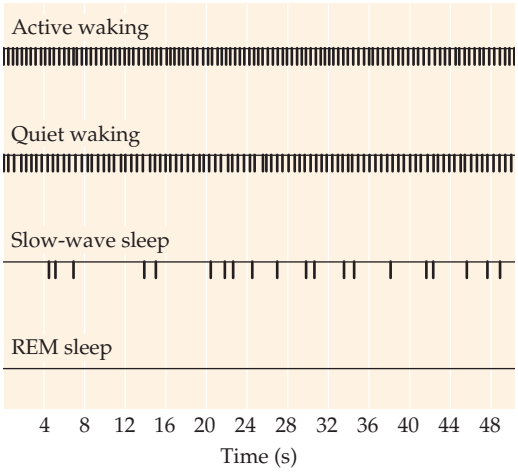


FIGURE 6.7 The firing rate of serotonergic neurons in the cat dorsal raphe is related to the animal's behavioral state During quiet waking, the cells fire at a steady rate of approximately 2 spikes (action potentials) per second. The firing rate is slightly increased during behavioral activity, but greatly diminished during slow-wave sleep. Dorsal raphe firing is essentially abolished during REM sleep. (After Jacobs and Fornal, 1993.)

Dorsal raphe serotonergic neurons also show phasic bursts of activity, a phenomenon that we discussed in Chapter 5 with respect to midbrain dopaminergic neurons. Jacobs and coworkers, who had also investigated tonic serotonergic activity in awake and sleeping cats, hypothesized that phasic activity is principally involved in facilitating motor output while simultaneously suppressing sensory processing (Jacobs and Fornal, 1999). While this may be one of the functions of the dorsal raphe serotonergic neurons, more recent research performed in mice and rats has additionally demonstrated robust responding of these cells to rewards and punishments (Ranade and Mainen, 2009; Liu et al., 2014; Cohen et al., 2015; Li et al., 2016b). Although the meaning of these studies is still being discussed, the findings point to the need to move beyond some of the overly simplistic ideas about serotonergic neural activity that had dominated the field in previous years (Dayan and Huys, 2015).

Phasic firing of the dorsal raphe serotonergic neurons must be triggered by activity in excitatory synaptic inputs to the cells. Many of these excitatory inputs use glutamate as a neurotransmitter and originate from the prefrontal cortex, lateral habenula, hypothalamus, and various brainstem areas (Soiza-Reilly and Commons, 2014). There are also excitatory inputs using acetylcholine as a transmitter that come from two important cholinergic nuclei in the dorsolateral pons: the laterodorsal and pedunculopontine nuclei (Hernandez-Lopez et al., 2013) (see Chapter 7). Neurons almost always receive inhibitory as well as excitatory inputs that, working together, regulate the firing rate of the cells. In the case of the dorsal raphe serotonergic neurons, many of the major inhibitory inputs use the transmitter γ-aminobutyric acid (GABA). These inhibitory connections come from a number of different brain areas, including the hypothalamus, substantia nigra, ventral tegmental area, and periaqueductal gray, and locally from GABAergic interneurons within the dorsal raphe itself (Soiza-Reilly and Commons, 2014). Interestingly, there is evidence for presynaptic interactions between glutamate and GABA. For example, GABA released onto glutamate nerve terminals alters glutamate release from those terminals, thereby affecting serotonergic neuronal firing. This complex interplay likely helps modulate the involvement of 5-HT, not only in the behaviors discussed above, but also in many other behaviors discussed in the last section of the chapter.

The discussion of neurotransmitter regulation of serotonergic neuron firing has focused on glutamate, acetylcholine, and GABA because of the extensive study of those particular connections. However, it's important to recognize that there are numerous other neurotransmitters that help regulate serotonergic neuron activity, including DA from the ventral tegmental area, NE from the locus coeruleus, and orexin from the hypothalamus (Maejima et al., 2013).

There is a large family of serotonin receptors, most of which are metabotropic

One of the remarkable properties of 5-HT is the number of receptors that have evolved for this transmitter. At the present time, pharmacologists have identified at least fourteen 5-HT receptor subtypes. Among these is a group of five different 5-HT_1 receptors (designated 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F}), along with a smaller group of three 5-HT_2 receptors (5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}) and additional receptors designated 5-HT_3, 5-HT_4, 5-HT_{5A}, 5-HT_{5B} (not expressed in humans), 5-HT_6, and 5-HT_7. All of these receptors are metabotropic, except for the 5-HT_3 receptor, which is an excitatory ionotropic receptor. The attentive reader will have noticed that there is no 5-HT_{1C} subtype listed. One of the serotonergic receptors initially had that designation; however, this receptor was later determined to belong to the 5-HT_2 family by structural and functional criteria, and therefore it was renamed 5-HT_{2C}. The following discussion will focus largely on 5-HT_{1A} and 5-HT_{2A} receptors, which are the best-known receptor subtypes in terms of their cellular and behavioral effects. We will subsequently touch on two other subtypes that have successfully been targeted for therapeutic purposes.

5-HT_{1A} RECEPTORS 5-HT_{1A} receptors are found in many brain areas, but they are particularly concentrated in the hippocampus, the septal area, parts of the amygdala, and the dorsal raphe nucleus. In the forebrain, these receptors are located postsynaptic to 5-HT–containing nerve terminals. As mentioned earlier, 5-HT_{1A} receptors additionally function as somatodendritic autoreceptors in the dorsal and median raphe nuclei. 5-HT_{1A} receptors work through two major mechanisms. First, the receptors reduce cAMP synthesis by inhibiting adenylyl cyclase (**FIGURE 6.8A**). The second mechanism involves increased opening of K^+ channels and membrane hyperpolarization, which we have seen is a property shared by D_2 dopamine receptors and α_2-adrenergic receptors. You will recall from Chapter 2 that this hyperpolarization leads to a decrease in firing of either the postsynaptic cell (in the case of 5-HT_{1A} receptors located postsynaptically) or the serotonergic neuron itself (in the case of the 5-HT_{1A} autoreceptors).

Several drugs act as either full or partial agonists at 5-HT_{1A} receptors, including **buspirone**, **ipsapirone**, and **8-hydroxy-2-(di-n-propylamino) tetralin** (**8-OH-DPAT**). Some of the behavioral effects produced by administering a 5-HT_{1A} agonist are described in the last part of this chapter. The most widely used 5-HT_{1A} receptor antagonist is the experimental drug **WAY 100635**, which was originally developed by the Wyeth-Ayerst pharmaceutical company (hence the "WAY" designation). Administration of this compound in the absence

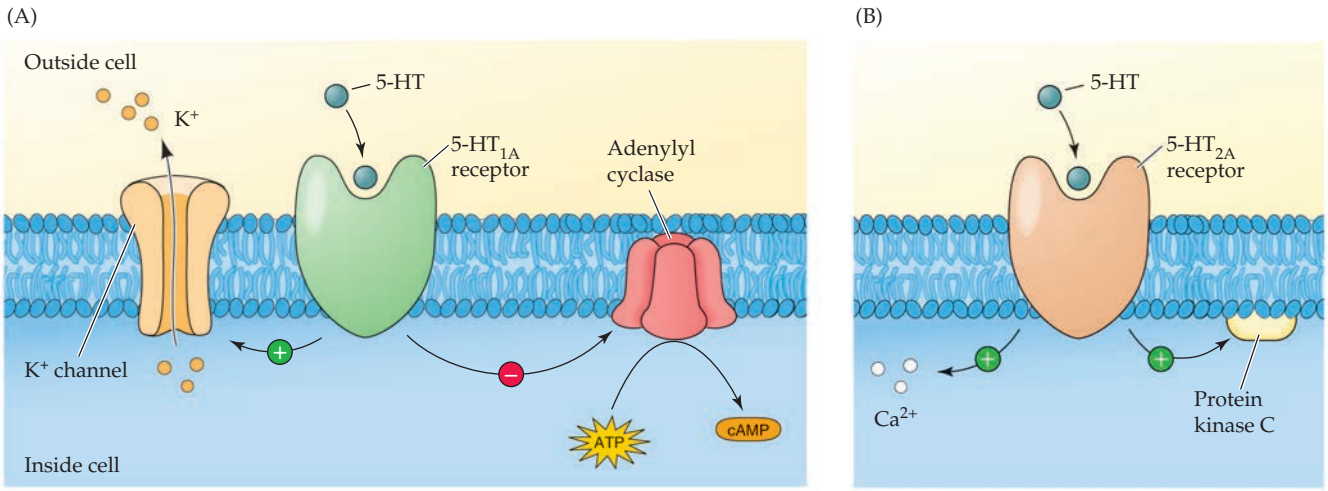


FIGURE 6.8 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors operate through different signaling mechanisms 5-HT$_{1A}$ receptors inhibit cAMP production and activate K^+ channel opening (A), whereas 5-HT$_{2A}$ receptors increase intracellular Ca^{2+} levels and stimulate protein kinase C via the phosphoinositide second-messenger system (B). For purposes of simplification, the G proteins required for coupling the receptors to their signaling pathways are not shown.

of a 5-HT$_{1A}$ agonist has provided important information about the degree to which 5-HT$_{1A}$ receptors (either autoreceptors or postsynaptic receptors) are occupied by endogenous 5-HT under baseline conditions.

5-HT$_{2A}$ RECEPTORS Large numbers of 5-HT$_{2A}$ receptors are present in the cerebral cortex. This receptor subtype is also found in the striatum, in the nucleus accumbens, and in a variety of other brain areas. Similar to α_1-adrenergic receptors, 5-HT$_{2A}$ receptors function mainly by activating the phosphoinositide second-messenger system (**FIGURE 6.8B**). In Chapter 3 you learned that this system increases Ca^{2+} levels within the postsynaptic cell and also activates protein kinase C (PKC). Thus, our discussion of different neurotransmitters and their receptor subtypes has included common mechanisms of transmitter action that occur over and over again. These mechanisms may involve a second messenger such as cAMP or Ca^{2+}, or some type of ion channels such as K^+ channels, which are opened by a wide variety of receptors. **1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane** (**DOI**) is a 5-HT$_{2A}$ agonist that is widely used experimentally, whereas **ketanserin** and **ritanserin** are typical 5-HT$_{2A}$ antagonists.

Giving DOI or another 5-HT$_{2A}$ agonist to rats or mice leads to a characteristic "head-twitch" response (periodic, brief twitches of the head), which is a useful measure of 5-HT$_{2A}$ receptor stimulation in these species. More interesting is the fact that DOI and related drugs are **hallucinogenic** (hallucination producing) in humans. Indeed, the hallucinogenic effects of **lysergic acid diethylamide** (**LSD**) are believed to stem from its ability to stimulate 5-HT$_{2A}$ receptors. LSD and other hallucinogens are discussed in greater detail in Chapter 15.

In recent years, blockade of 5-HT$_{2A}$ receptors has become a major topic of discussion and research with respect to the treatment of schizophrenia. As mentioned in Chapter 5, traditional antischizophrenic drugs that work by blocking D_2 dopamine receptors often produce serious movement-related side effects, some of which can resemble Parkinson's disease. Such side effects are much less severe, however, with later-generation drugs such as **clozapine** (**Clozaril**) and **risperidone** (**Risperdal**). Both clozapine and risperidone block 5-HT$_{2A}$ receptors, in addition to exerting their effects on the dopamine system. This has led to the hypothesis that a combination of D_2 and 5-HT$_{2A}$ receptor antagonism leads to symptom improvement in patients with schizophrenia while minimizing the side effects associated with previous antischizophrenic drugs that don't affect the 5-HT$_{2A}$ receptor (see Chapter 19).

OTHER RECEPTOR SUBTYPES Widespread expression of other serotonergic receptor subtypes, both within the nervous system and in non-neural tissues, has led to the development of various medications that target these receptors. Two well-known examples pertain to the 5-HT$_{1B/1D}$ and 5-HT$_3$ subtypes. Abnormal dilation of blood vessels within the brain is thought to be one of the key factors contributing to migraine headaches. A class of 5-HT$_{1B/1D}$ agonists known as triptans (e.g., **sumatriptan** [**Imitrex**], **zolmitriptan** [**Zomig**]) causes constriction of the vessels, thereby providing relief from migraine symptoms. Another potential mechanism of action is the blocking of pain signals transmitted by the trigeminal nerve. In 2013, the FDA approved a transdermal (patch) form of sumatriptan (**Zecuity**) for the treatment of migraine (Garnock-Jones, 2013; Vikelis et al., 2015). The patch is designed so that drug molecules

are driven from the patch into the skin, and ultimately into the bloodstream, by a process called **iontophoresis** (**FIGURE 6.9**). This route of administration provides for controlled, continuous drug delivery and avoids some of the problems associated with sumatriptan administration by the oral route (e.g., gastrointestinal reactions) or by injection (e.g., pain and/or aversion to needles).

The 5-HT_3 receptors are located on peripheral terminals of the vagus nerve. One function of the vagus is to transmit sensory information from the viscera, including the gastrointestinal (GI) tract, to the brain. Cancer chemotherapy drugs and radiation treatment have both been found to stimulate the release of 5-HT in the gut, which subsequently stimulates the vagal 5-HT_3 receptors and induces activation of the vomiting center in the brainstem (Babic and Browning, 2014). Consequently, 5-HT_3 antagonists such as **ondansetron** (**Zofran**), **granisetron** (**Kytril**), and **palonosetron** (**Aloxi**) are prescribed to counteract the nausea and vomiting associated with cancer treatments. More information on the role of gut 5-HT and the potential therapeutic benefits of drugs that target it can be found at the end of the chapter.

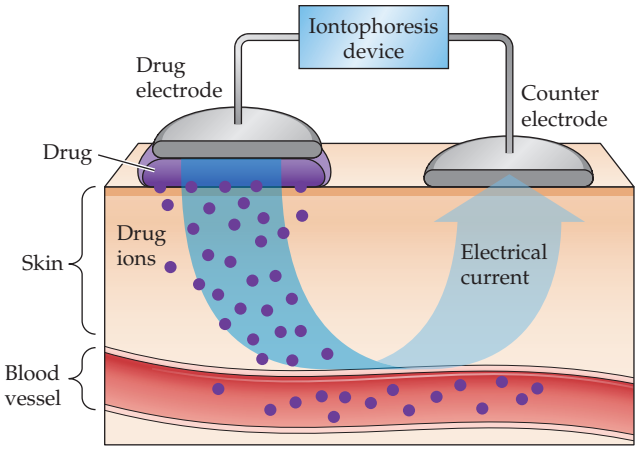


FIGURE 6.9 Drug molecules can be delivered through the skin into the bloodstream by a process called iontophoresis This process uses a skin patch containing an iontophoresis device, two electrodes, and a reservoir containing the drug. The iontophoresis device includes a battery that delivers a small electrical current that drives the drug molecules into the skin, where they can enter the bloodstream. Note that the drug must be ionized (i.e., possess a positive or negative charge) for the system to work. (After Naik et al., 2000.)

Multiple approaches have identified several behavioral and physiological functions of serotonin

It is clear from numerous genetic, neurochemical, and pharmacological studies in both animals and humans that the serotonergic system helps regulate many behavioral and physiological functions. In human clinical research, serotonergic function has been investigated using several different methodological approaches, including the following:

- Determining the association between levels of cerebrospinal fluid (CSF) 5-HIAA or postmortem regional brain 5-HT and 5-HIAA concentrations and behavioral traits or, in some cases, the occurrence of neuropsychiatric disorders
- Assessing behavioral, subjective, and physiological responses to pharmacological challenges with SSRIs or with receptor agonists or antagonists
- Identifying associations between psychiatric disorders and polymorphisms in the genes for SERT or for various serotonergic receptors

Some findings relating SERT polymorphisms with risk for major depression will be presented later, in the chapter on affective disorders (Chapter 18). With respect to CSF and brain concentrations of 5-HIAA and 5-HT, various findings have related the serotonergic system to aggressive behavior, particularly impulsive aggression. These and other results linking 5-HT to aggression are discussed below. Preclinical (animal model) studies of serotonergic function have relied on pharmacological approaches, direct neurochemical assessment of 5-HT and/or 5-HIAA concentrations in specific brain regions, and genetically engineered elimination of 5-HT in the brain or periphery.

Numerous pharmacological tools are available for probing the serotonergic system, ranging from serotonergic neurotoxins to drug challenges with selective receptor agonists and antagonists, as done in human research studies (**TABLE 6.1**). Serotonergic neurotoxins include the previously mentioned compounds *para*-chloroamphetamine and MDMA. Another compound called **5,7-dihydroxytryptamine** (**5,7-DHT**) has also been widely used to produce serotonergic lesions in experimental animals, although one limitation of using 5,7-DHT is that it must be given directly into the brain because it doesn't cross the blood–brain barrier. 5,7-DHT causes massive damage to serotonergic axons and nerve terminals in the forebrain, yet cell bodies in the raphe nuclei are usually spared. Due to space limitations, we cannot review all of the behavioral effects produced by lesioning the serotonergic system; however, various studies have reported changes in hunger and eating behavior, anxiety, pain sensitivity, and learning and memory. Indeed, the involvement of 5-HT in a multitude of behavioral and physiological functions has led to the development of serotonergic receptor agonist and antagonist compounds for the treatment of many clinical disorders. Some of these disorders, the putative role of 5-HT, and the identities of either currently licensed or investigational compounds targeting the relevant serotonergic receptors

are discussed in the subsections below. But first we will describe the profound behavioral and physiological consequences of genetically engineering animals so that they lack brain 5-HT throughout life, from embryonic development onward.

LIFE WITHOUT SEROTONIN[2] Much important information has been obtained about serotonergic system function by pharmacologically manipulating 5-HT levels, activating or blocking specific serotonergic receptors with agonists and antagonists, and using SSRIs to prevent 5-HT reuptake following release. However, there are several common limitations to these pharmacological approaches: drug effects are temporary; the targeted receptor, transporter, or enzyme may only be partially affected by the drug (e.g., incomplete receptor blockade, residual 5-HT synthesis following attempted depletion); and the chosen drug may not be fully selective for its intended target at the administered dose. Are there any alternatives that avoid these limitations? The answer leads us to genetic engineering approaches, specifically mice engineered to lack 5-HT from embryonic development onward. There are two distinct ways to achieve this goal: knocking out the gene for TPH, which leaves the serotonergic cells intact but doesn't permit them to synthesize 5-HT, or preventing the normal differentiation of cells that were destined to become serotonergic neurons. Because the first method involving TPH knockout mice has been used much more widely by researchers, we will primarily discuss the results of those studies.

[2]The title of this section is adapted from a recent review by Mosienko and colleagues (2015) entitled "Life without brain serotonin: Reevaluation of serotonin function with mice deficient in brain serotonin synthesis."

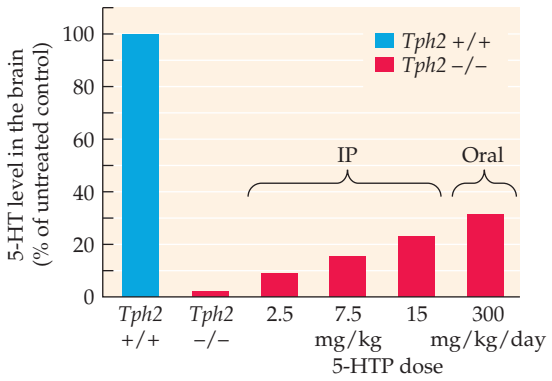


FIGURE 6.10 ***Tph2*-knockout mice show a near complete loss of brain 5-HT** Whole-brain 5-HT concentrations were determined in wild-type mice (*Tph2*+/+) and knockout mice with a homozygous loss of *Tph2* gene expression (*Tph2*–/–) (left two bars). Partial restoration of 5-HT concentration could be produced either by intraperitoneal (IP) injection of 5-HTP (measured 1 hour after the indicated dose) or by 5 days of oral 5-HTP administration at a dose of 300 mg/kg/day (right four bars). (After Mosienko et al., 2015.)

Most TPH knockout studies specifically eliminate TPH2, the form of the enzyme expressed by serotonergic neurons, without influencing TPH1, since the latter is coded for by a different gene. Knockout of the *Tph2* gene causes a virtually complete loss of 5-HT in brain (**FIGURE 6.10**) but a preservation of 5-HT levels in the bloodstream (where it's stored in blood platelets) and in peripheral organs like the intestines, liver, spleen, and pineal gland (Mosienko et al., 2015). This finding confirms the independent expression of *Tph2* and *Tph1* in neuronal compared to non-neuronal tissues. Interestingly, at least one study of *Tph2*-knockout mice found alterations in

Table 6.1 Drugs That Affect the Serotonergic System

Drug	Action
para-Chlorophenylalanine	Depletes 5-HT by inhibiting tryptophan hydroxylase
Reserpine	Depletes 5-HT by inhibiting vesicular uptake
para-Chloroamphetamine, fenfluramine, and MDMA	Release 5-HT from nerve terminals (MDMA and *para*-chloroamphetamine also have neurotoxic effects)
Fluoxetine, paroxetine	Inhibit 5-HT reuptake
5,7-Dihydroxytryptamine	5-HT neurotoxin
Buspirone, ipsapirone, and 8-OH-DPAT	Stimulate 5-HT_{1A} receptors (agonists)
WAY 100635	Blocks 5-HT_{1A} receptors (antagonist)
Sumatriptan and zolmitriptan	Stimulate $5\text{-HT}_{1B/1D}$ receptors (agonists)
DOI	Stimulates 5-HT_{2A} receptors (agonist)
Ketanserin and ritanserin	Block 5-HT_{2A} receptors (antagonists)
Locaserin	Stimulates 5-HT_{2C} receptors (agonist)
Ondansetron, granisetron, palonosetron, and alosetron	Block 5-HT_{3} receptors (antagonists)

the circuitry that developed from the raphe neurons and that would normally have contained 5-HT (Migliarini et al., 2013). Some areas, like the suprachiasmatic nucleus of the hypothalamus (which contains the master biological clock) show a reduced innervation, whereas some other areas, like the nucleus accumbens and hippocampus, are hyperinnervated by fibers from the raphe. These results suggest that 5-HT helps regulate the development of its own fiber system; in the absence of central 5-HT, the system develops abnormally.

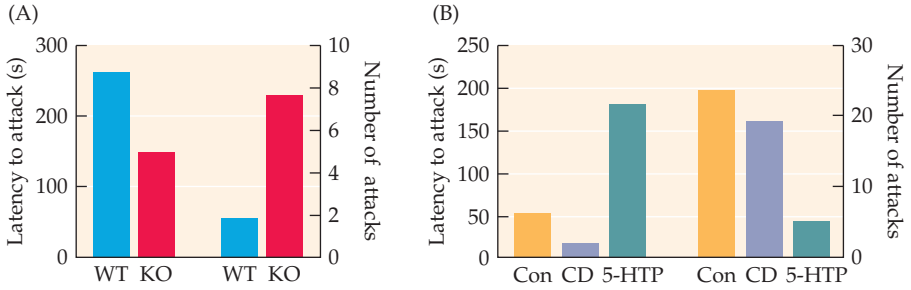


FIGURE 6.11 Impulsive aggression in male *Tph2*-knockout mice
Adult male *Tph2*-knockout (KO) and wild-type (WT) mice were tested for impulsive aggression using the resident–intruder paradigm. (A) Compared with WT controls, the resident KO mice showed a reduced latency to attack (left bars) and an increased number of attacks (right bars) directed toward the intruder. (B) In KO mice, administration of 5-HTP plus carbidopa (5-HTP group) but not carbidopa alone (CD group) increased the latency to attack and reduced the number of attacks, compared with the control group given drug vehicle alone (Con). (After Angoa-Pérez et al., 2012.)

Brain 5-HT in *Tph2*-knockout mice can be restored in a dose-dependent manner by injecting the animals with 5-HTP (see Figure 6.10). This method works because 5-HTP is the *product* of the reaction catalyzed by TPH and, therefore, bypasses the missing step in the biosynthetic pathway (refer back to Figure 6.1). However, recovery of 5-HT levels is only temporary because the newly synthesized transmitter is gradually metabolized by MAO-A and cannot be replenished in the mutant mice. Also worth noting is the fact that AADC, the enzyme that converts 5-HTP to 5-HT, is additionally present in catecholamine neurons (see Chapter 5). Thus, animals given 5-HTP make 5-HT not only within the serotonergic neurons, where the transmitter is normally present, but also within dopaminergic and noradrenergic neurons, where 5-HT should *not* be present.

In Chapter 5, you learned that mice with a complete deletion of tyrosine hydroxylase from embryonic development onward died either in utero or soon after birth. The same is not true for TPH2, indicating that 5-HT is not required for the animal to survive gestation or the immediate postnatal period. However, over time *Tph2*-knockout mice do show reduced body mass and fat stores, and they also have an increased rate of mortality (Mosienko et al., 2015). The severity of postnatal growth deficits and increased mortality depends on which genetic strain of mouse and which genetic methods are used to create the knockout animals.

Extensive behavioral studies have been conducted on *Tph2*-knockout mice (reviewed in Aragi and Lesch, 2013; Fernandez and Gaspar, 2012; Lesch et al., 2012; Mosienko et al., 2015). The most consistently observed effect of eliminating brain 5-HT is a large increase in aggressive behavior, which is typically studied in male mice but also occurs in females. Aggression in male mice is usually measured by the **resident–intruder test**. In this procedure, an adult male mouse (the "resident") is housed alone in a plastic tub for several weeks. In essence, the tub becomes the male's territory, which he will aggressively defend against intruding males. To test this aggressiveness, an unfamiliar male is introduced into the tub and the behavioral interactions between the resident and intruder are observed for the next several minutes. The level of aggressiveness by the resident is measured by the latency to attack the intruder (shorter latencies reflect greater aggressiveness) and the total number of attacks during the testing period. **FIGURE 6.11A** illustrates the heightened aggressiveness of male *Tph2*-knockout mice compared with wild-type males (Angoa-Pérez et al., 2012). In this particular study, even female *Tph2*-knockout mice were more aggressive than wild-type females (data not shown). Additional experiments demonstrated that injection of 5-HTP along with a drug called **carbidopa**, which is an AADC inhibitor that doesn't cross the blood–brain barrier, reverses the high level of aggressive behavior in *Tph2*-knockout males (**FIGURE 6.11B**). Because there is substantial AADC activity in some peripheral tissues (e.g., kidneys), carbidopa was given to block peripheral conversion of 5-HTP to 5-HT. This method permits more 5-HTP to be available to the brain and also prevents adverse consequences of elevated peripheral 5-HT levels such as gastrointestinal distress and diarrhea. Our discussion of the role of 5-HT in aggressive behavior is continued in **BOX 6.2**.

Genetic deletion of TPH2 leads to additional changes in behavioral control, mood-related and motivated behaviors, and social communication. Compared with wild-type animals, *Tph2*-knockout mice are reported to be more impulsive (Aragi and Lesch, 2013), more compulsive (Angoa-Pérez et al., 2012; Kane et al., 2012), and less anxious, at least in some tests of anxiety-like behavior (Mosienko et al., 2015). These animals also show poor social communication and social behaviors, including deficient maternal behavior severe enough to cause increased offspring mortality (Angoa-Pérez et al., 2014;

BOX 6.2 The Cutting Edge

Serotonin and Aggression

What is aggression, and how do we define and conceptualize aggressive behaviors? In the case of human aggression, the intent of the actor is important. Thus, a common definition of aggression in human studies is "behavior that is intended to cause physical or psychological harm or pain to the victim." Human aggression is often classified as either premeditated (instrumental) or impulsive (reactive) (Siever, 2008). Premeditated aggression is planned, rather than provoked by immediate frustration or threat. In contrast, impulsive aggression is a suddenly provoked behavior that occurs in response to conditions of frustration, threat, or stress. It is typically accompanied by feelings of anger and/or fear and by activation of the sympathetic nervous system.

Whereas studies of human aggression can benefit from self-reports of intentionality and emotional state, animal studies must operationalize the concept of aggression. This difference in approach has led to the development of several formal typologies of aggressive behavior. For example, Moyer (1968) proposed that aggression in animals could be categorized as predatory, intermale, fear-induced, irritable, territorial, maternal, or instrumental aggression. A later review by Nelson and Chiavegatto (2001) lists a variety of different types of aggression that can be observed in animals under naturalistic and/or specific experimental conditions (see **Table**).

Some researchers have attempted to develop a classification system that applies to both animal and human aggression. One such system was proposed by Weinshenker and Siegel (2002), who argued that most instances of aggression can be characterized as either affective defense or predatory attack. In this system, affective defense in both animals and humans comprises aggressive acts triggered by a real or perceived threat and is further characterized by strong emotional reactions (particularly fear) and sympathetic nervous system activation. In contrast, predatory attack is purposeful and goal directed, and it lacks the impulsive/reactive qualities and the strong emotional and sympathetic arousal present in affective defense. We can see that Weinshenker and Siegel's affective defense corresponds closely to the impulsive aggression category of human aggression proposed by many investigators, including Siever (2008). The difference, however, is that Weinshenker and Siegel also place most forms of animal aggression (e.g., intermale, fear-induced, irritable, territorial, and maternal) into this same category. Moreover, their category of predatory attack incorporates both Moyer's (1968) predatory animal aggression and premeditated/instrumental human aggression. With these two distinct categories, Weinshenker and Siegel argue for the unification of the animal and human research literature in our studies of aggressive behavior.

The neural circuit underlying aggressive behavior in humans has been elucidated using a combination of approaches, including brain imaging studies and examination of behavioral changes in patients with lesions of various brain areas (produced by stroke, tumor, head trauma, or other forms of brain injury). These approaches have implicated a circuit that

(*Continued*)

Types of Aggression

Category of aggression	Function and/or experimental conditions
Predatory	Attack of a predator on a prey animal
Antipredatory	Attack of a prey animal against its predator
Sex-related	Aggression that occurs in the context of a sexual interaction between two animals
Dominance	Aggression (usually between males) involved in the establishment or maintenance of rank within their group's dominance hierarchy
Maternal	Attack by a mother in the defense of her offspring
Territorial	Aggression that occurs in the context of defending one's territory, commonly modeled in the laboratory by using the resident–intruder test in which a strange male is introduced into the home cage of a resident male
Defensive	Attack elicited by a fear-inducing stimulus
Irritable	Attack elicited by extreme frustration or stress, sometimes modeled in the laboratory by the application of a brief electric shock

Source: After Nelson and Chiavegatto, 2001.

BOX 6.2 The Cutting Edge (*continued*)

includes several key cortical areas (orbitofrontal cortex, temporal cortex, and cingulate cortex), the striatum, and a group of subcortical limbic system structures (amygdala, hypothalamus, and hippocampus) (Siever, 2008; Rosell and Siever, 2015). The neural circuit involved in aggression in rodents includes many of the same areas but also incorporates the bed nucleus of the stria terminalis (BNST; a brain area sometimes considered part of an "extended" amygdala) and the periaqueductal grey (Nelson and Trainor, 2007). In both cases, all of the brain areas mentioned receive substantial serotonergic innervation and express several different 5-HT receptor subtypes.

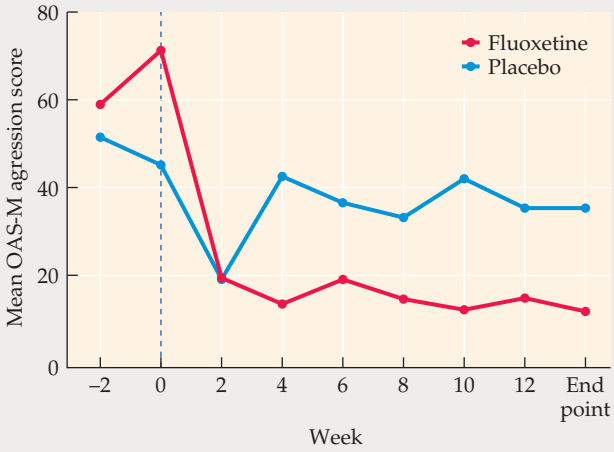
Historically, the first studies linking 5-HT to aggression appeared in the 1970s and 1980s (reviewed in Rosell and Siever, 2015). Particularly noteworthy were reports that levels of 5-HIAA in the CSF, used as an indicator of brain serotonergic activity, were inversely related to aggressiveness in people with personality disorders such as borderline personality disorder. Combined with other findings, these results gave rise to the **serotonin deficiency hypothesis of aggression**, which proposes that low CNS serotonergic activity is associated with hyperaggressiveness (Umukoro et al., 2013). Some research findings have continued to support this general hypothesis. For example, a meta-analysis of the relationship between CSF 5-HIAA levels and antisocial behavior (defined as "a pervasive pattern of a violation of the rights of others through violence against persons or objects that are grounds for arrest [e.g., arson] or involve physical fights, assaults, and/or murder" [p. 304]) found significantly lower levels in the antisocial group than in the non-antisocial controls (Moore et al., 2002). This difference was particularly strong for individuals younger than 30 years of age. On the other hand, a more exhaustive examination of the human literature on 5-HT and aggressiveness found only a very weak relationship between these variables (Duke et al., 2013). The different conclusions from these two reviews may stem, at least in part, from the fact that the analysis of Duke and coworkers included results from aggression-related questionnaires, whereas the analysis of Moore et al. was restricted to individuals who had actually committed serious antisocial acts.

Animal studies on the possible global role of 5-HT in aggressive behavior have benefited from the variety of pharmacological and genetic tools available to preclinical researchers. For example, increasing extracellular 5-HT (thereby enhancing serotonergic transmission) can be achieved by systemic administration of an SSRI or by deletion of the SERT gene. In contrast, serotonergic transmission can be decreased by administration of a 5-HT neurotoxin, by pharmacological inhibition of TPH, or by knocking out the TPH gene. Taken together, these studies have revealed that relatively high serotonergic levels/activity are associated with reduced aggressive behavior, whereas relatively low serotonergic levels/activity are associated with increased aggressive behavior (Carrillo et al., 2009; Ferrari et al., 2005; Holmes et al., 2002; Lesch et al., 2012; Mosienko et al., 2015; Siever, 2008).

A major challenge to the serotonin deficiency hypothesis is the finding that administration of either a 5-HT_{1A} or a 5-HT_{1B} receptor agonist leads to a reduction in aggressive behavior, whereas mutant male mice lacking the 5-HT_{1B} receptor exhibit increased aggression (de Boer and Koolhaas, 2005; Nelson and Chiavegatto, 2001; Nelson and Trainor, 2007). As described earlier in the chapter, both of these receptor subtypes have dual roles as both postsynaptic receptors and autoreceptors (somatodendritic autoreceptor in the case of the 5-HT_{1A} subtype and terminal autoreceptor in the case of the 5-HT_{1B} subtype). Therefore, the aggression-reducing action of these receptors could be due either to an enhancement (postsynaptic site of drug action) or to an inhibition (autoreceptor site of drug action) of serotonergic transmission. Studies by de Boer and Koolhaas (2005) suggest that the antiaggressive effects of 5-HT_{1A} and 5-HT_{1B} agonists are mediated by autoreceptors that acutely inhibit serotonergic transmission. It is not yet clear how best to reconcile these findings, which imply that 5-HT can stimulate aggressive behavior, with the serotonin deficiency hypothesis. However, some researchers like de Boer and Koolhaas (2005) have proposed that this hypothesis does not account for all kinds of aggressive behavior but rather pertains specifically to individuals predisposed to impulsive, pathological, and violent forms of aggressiveness.

Because some personality disorders are associated with violent behaviors, clinicians have sought to find drugs that can quell such behaviors (such drugs are called **serenics**, derived from the Latin word *serenus* meaning "calm" or "tranquil") (Coccaro et al. 2015). Based on the serotonin deficiency syndrome, several studies have tested SSRIs because of their ability to enhance serotonergic transmission. The **Figure** shows the results of one such study comparing fluoxetine with placebo treatment in patients diagnosed with personality disorders that include symptoms of impulsive aggressiveness and irritability. Within several weeks, the fluoxetine-treated group began to show significantly less aggressiveness than the placebo-treated group, and this effect continued through the 12-week drug trial (Coccaro and Kavoussi, 1997). Although these results are encouraging, not all

BOX 6.2 The Cutting Edge (*continued*)



Effect of chronic fluoxetine on impulsive aggression in patients with personality disorder Patients diagnosed with a personality disorder and who exhibited symptoms of impulsive aggression were treated for 12 weeks with either fluoxetine (20–60 mg/day orally) or placebo. Treatments began at week 0 on the graph. Beginning 2 weeks prior to treatment onset and during the 12-week treatment period, impulsive aggression was rated weekly using the aggression subscale of the overt aggression scale–modified for outpatients (OAS-M). By week 4, the fluoxetine group began to exhibit reduced aggression compared with the placebo group, and this difference persisted for the remainder of the study. (After Coccaro and Kavoussi, 1997.)

patients responded to treatment, and clinicians are additionally aware that SSRIs can exert adverse side effects when given chronically (see Chapter 18).

In summary, 5-HT plays a significant role in certain kinds of aggressiveness, especially impulsive and irritable aggression. Unfortunately, our society is currently plagued with high levels of this behavioral pathology. Whereas treatment with serotonergic drugs may be warranted in patients who have been diagnosed with psychiatric conditions that manifest impulsive and irritable aggressiveness, few would argue that we should forcibly medicate everyone who shows signs of violent behavior. It behooves us, therefore, to learn more about the early origins of pathological aggression so that it can be prevented before it develops.

Beis et al., 2015; Kane et al., 2012). The lack of normal social communication in *Tph2*-knockout mice is consistent with the hypothesis that abnormal development of the serotonergic system may underlie some cases of autism spectrum disorder (Muller et al., 2016).

Tph2-knockout mice not only are abnormal behaviorally, but also suffer from several physiological deficits. Two such deficits are (1) poor thermoregulation leading to inadequate maintenance of core body temperature when mice are placed into a cold environment, and (2) abnormal respiration (Alenina et al., 2009). These same deficits have been observed in a different kind of mutant mouse engineered so that the animals actually develop without most of their brain serotonergic neurons (Hodges and Richerson, 2010). Respiratory problems in these mice are particularly severe in the early postnatal period, during which time pups exhibit frequent, long-lasting episodes of **apnea** (cessation of breathing). One of the key characteristics of the abnormal respiratory function in mice lacking serotonergic neurons is a failure to respond adequately to elevated levels of CO_2. This deficit has been related to a lack of 5-HT_{2A} receptor activation in the mutant mice, because the animals respond more strongly to a CO_2 challenge after treatment with a 5-HT_{2A} agonist such as DOI (Buchanan et al., 2015). Failure to respond to apnea-induced **hypercapnia** (elevated blood CO_2 levels) has been proposed as a cause of death in sudden infant death syndrome (SIDS) as well as sudden unexpected death in epilepsy (SUDEP). SUDEP is an unusual condition in which a patient who has chronic epilepsy but is otherwise healthy is found dead, with or without evidence of a recent seizure. Based, in part, on research with serotonergic neuron-deficient knockout mice, some researchers have proposed that abnormalities in the serotonergic system play an important role in both SIDS and SUDEP (Richerson, 2013; Sowers et al., 2013).

HUNGER AND EATING BEHAVIOR Serotonin has long been known to influence hunger and eating behavior. Pharmacological studies have shown that 5-HT_{1B} or 5-HT_{2C} receptor agonists, as well as 5-HT_6 antagonists, produce **hypophagia** (reduced food intake) and weight loss in rodent models (Voigt and Fink, 2015). Serotonergic projections to the hypothalamus participate in a complex circuit involving several other neurotransmitters and neuropeptides that together regulate appetite and energy metabolism (Burke and Heisler, 2015).

Clinically, the use of serotonergic drugs to treat obesity went through an early dark period with unfortunate results. In the early 1990s, physicians began prescribing the 5-HT–releasing drug fenfluramine (Pondimin) or its D-isomer (dexfenfluramine; Redux) to obese patients for weight loss. Although the drugs proved modestly successful in helping patients lose weight, they also led to a significantly elevated risk for two potentially fatal

disorders: heart valve abnormalities and pulmonary hypertension (elevated blood pressure in the arteries between the heart and lungs). Consequently, both compounds were withdrawn from clinical use in 1997. It subsequently became clear that the cardiac side effects of fenfluramine and dexfenfluramine were caused by activation of 5-HT_{2B} receptors in the heart valve tissue, whereas the **anorectic** (appetite-reducing) effect of the drugs could be traced, at least in part, to activation of hypothalamic 5-HT_{2C} receptors. These discoveries led to the development and licensing of **lorcaserin** (trade name **Belviq**), a selective 5-HT_{2C} agonist, for the treatment of obesity (Nigro et al., 2013). As with other weight loss medications, patients must increase their physical exercise and modify their diet appropriately in order to achieve maximum benefit.

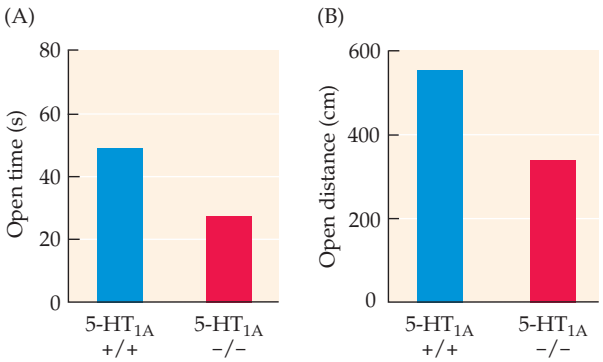


FIGURE 6.12 Genetic deletion of the 5-HT_{1A} receptor increases anxiety-like behavior in the elevated zero-maze Wild-type (*5-HT1A*+/+) and *5-HT1A*-knockout (*5-HT1A*–/–) mice were tested in the elevated zero-maze, which is a circular apparatus with open and closed areas like the elevated plus-maze. Compared with wild-type mice, knockout animals showed increased anxiety, as indicated by less time spent (A) and less distance traveled (B) in open areas of the maze. (After Heisler et al., 1998.)

ANXIETY A large body of research has established a key role for 5-HT in the regulation of anxiety. As we shall see in Chapter 17, SSRIs are among the drugs used most commonly in the treatment of several kinds of anxiety disorders. One of the key receptor subtypes thought to be involved in the anxiolytic (antianxiety) effects of these compounds is the 5-HT_{1A} receptor, and indeed the 5-HT_{1A} partial agonist **buspirone** (**BuSpar**) is licensed as an antianxiety medication. **Vilazodone** (**Viibryd**) is a newer compound that is both an SSRI and a 5-HT_{1A} partial agonist (Sahli et al., 2016). Although vilazodone was developed mainly for the treatment of major depression, it additionally has anxiolytic efficacy and may be particularly useful in patients who suffer from depression-related anxiety (Thase et al., 2014).

Experimental animal studies have been important for studying the role of the 5-HT_{1A} receptor in modulating anxiety-like behaviors. Several strains of *5-HT1A*-knockout mice have been generated, all of which show increased anxiety-like behavior in standard tests such as the elevated plus-maze or a similar test known as the elevated zero-maze (Akimova et al., 2009) (**FIGURE 6.12**). Interestingly, an elegant series of experiments by Gross and colleagues (2002) demonstrated that this behavioral phenotype of enhanced anxiety-like behavior is caused by the lack of 5-HT_{1A} receptors in forebrain areas during early postnatal development and is not due to the absence of the receptor in the adult animals at the time of behavioral testing. Put another way, the presence or absence of this receptor subtype during development alters the neural circuitry regulating the baseline expression of anxiety-like behavior of the mice once they have reached adulthood. This finding raises the intriguing possibility that deficient 5-HT_{1A} receptor activity during a critical developmental period could give rise to later onset of anxiety disorders in humans.

Studies with rats and mice have also implicated the 5-HT_{2A}, 5-HT_{2C}, and 5-HT_6 receptor subtypes in the regulation of anxiety-like behaviors. Administration of the 5-$HT_{2A/2C}$ receptor agonist *meta*-chlorophenylpiperazine (mCPP) increases anxiety-like behavior, whereas genetic deletion of either the 5-HT_{2A} or 5-HT_{2C} receptor has an anxiolytic effect in mice (Heisler et al., 2007; Weisstaub et al., 2006). With respect to the 5-HT_{2A} subtype, normal anxiety-like behavior could be reinstated by selective restoration of these receptors just in the cerebral cortex (Weisstaub et al., 2006). This finding suggests that 5-HT_{2A} receptor modulation of anxiety is mediated by receptors located in one or more neocortical areas, which may include the prefrontal cortex, as has been shown in other research (Etkin, 2009). The experimental drug EMD 386088, a partial agonist at the 5-HT_6 receptor, has anxiolytic effects in rats after either systemic administration or infusion into the hippocampus (Jastrzebska-Wiesek et al., 2014; Nikiforuk et al., 2011). It remains to be seen whether this or similar compounds eventually obtain FDA approval for the treatment of clinical anxiety disorders.

PAIN In addition to the use of serotonergic drugs in the treatment of migraine headaches, 5-HT has also been implicated in the processing of pain signals at the level of the spinal cord. Dorsal root sensory neurons carry pain information from peripheral sites, such as the skin, and synapse within the dorsal horn of the spinal cord. A descending pain modulatory system originates in the periaqueductal gray area of the caudal midbrain, synapses on the raphe nuclei of the rostral medulla (particularly the raphe magnus) and nearby parts of the medullary reticular formation, and then descends farther to the dorsal horn. Of particular interest here are the serotonergic neurons of the raphe magnus, which have been shown to influence the transmission of incoming pain information

to ascending systems that carry this information to higher processing centers (see Figure 11.15).

In experimental animal studies, the influence of this descending serotonergic pathway on pain processing within the spinal cord depends on the state of the animal (reviewed by Viguier et al., 2013). In subjects not previously exposed to painful stimuli, 5-HT generally inhibits pain signaling. But if the animal has been previously sensitized, for example by central or peripheral nervous system damage, then 5-HT has mixed effects and can produce either reduced or enhanced pain signaling. Nerve tissue damage can lead to a form of chronic pain called **neuropathic pain**, a condition that is difficult to treat effectively and can be debilitating to the patient. Neuropathic pain is often associated with **hyperalgesia**, which refers to increased sensitivity to stimuli that are usually painful, and **allodynia**, which refers to pain that is felt in response to a stimulus (e.g., a touch on the skin) that is *not* usually painful (Jensen and Finnerup, 2014). Studies using animal models of neuropathic pain have found that pain could be reduced by treatment with agonists at the 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2C} receptor subtypes, whereas pain was exacerbated by 5-HT_{2A} and 5-HT_4 agonists (Pańczyk et al., 2015; Viguier et al., 2013). A recently emerging target for pain medication development is the 5-HT_7 receptor. Activation of this receptor has a hypoalgesic (pain-reducing) effect in models of neuropathic pain, although this beneficial effect may be mediated somewhere in the brain rather than within the spinal cord. A number of different 5-HT_7 agonists are being developed for potential therapeutic use in treating neuropathic pain as well as several other conditions (Di Pilato et al., 2014).

LEARNING AND MEMORY Learning and memory processes can be powerfully influenced by the administration of various serotonergic receptor agonists and antagonists. Because space considerations do not permit us to consider all of the receptors that have been investigated in this regard, we will focus here on the 5-HT_{1A}, 5-HT_4, and 5-HT_6 subtypes. It is important to keep in mind that the effects of receptor agonists and antagonists on learning and memory may depend on the dose of the drug, the specific task being studied, and, in some cases, the species being tested.

The importance of considering task specificity is shown by the results of manipulating 5-HT_{1A} receptor stimulation. For example, 5-HT_{1A} receptor activation improves performance in spatial learning and memory tasks (Glikmann-Johnston et al., 2015). This effect has been demonstrated not only by the memory-enhancing effects of 5-HT_{1A} receptor activation but also by the impairment on spatial learning tasks (e.g., Morris water maze; see Chapter 4) exhibited by *5-HT1A*-knockout mice. The influence of 5-HT_{1A} receptors on performance in such tasks is believed to be related to the high density of these receptors in the hippocampus, a key brain area for spatial learning. In contrast, activation of hippocampal 5-HT_{1A} receptors has been shown to impair memory encoding in a contextual fear-conditioning task (Ögren et al., 2008). In this task, rats or mice learn in a single trial to associate a novel environment (i.e., test chamber) with a fear-inducing stimulus, namely an electric foot shock. It is not completely clear how we can reconcile these disparate findings; however, we may speculate that the 5-HT_{1A} receptors have different roles in the neural circuits that mediate the two distinct kinds of memory (spatial versus contextual fear).

The 5-HT_4 receptors are expressed most highly in the basal ganglia and the hippocampus. Somewhat lower levels of expression are found in the prefrontal cortex, septal area, and amygdala. Studies in rodents and monkeys have demonstrated enhancement of learning and memory in an assortment of different tasks following administration of 5-HT_4 partial agonists (King et al., 2008; Ramirez et al., 2014). Such enhancement is thought to depend, in part, on facilitation of cholinergic transmission in the cortex and the hippocampus (see Chapter 7 for a discussion of the role of acetylcholine in cognitive function). Moreover, chronic 5-HT_4 partial agonist administration retards the development of Alzheimer's disease–related neuropathology in transgenic mouse models of this disorder (Claeysen et al., 2015; Ramirez et al., 2014). Consequently, there is much current interest in the development of 5-HT_4 receptor compounds for the treatment of Alzheimer's disease. However, it's important to recognize that at the time of this writing, virtually all of the published research in this area has been based on animal model studies. It remains to be seen whether any of these compounds will be equally efficacious in controlled clinical trials with patients with Alzheimer's disease.

The 5-HT_6 receptor is another serotonergic receptor subtype that has been extensively studied with respect to learning and memory. The highest levels of 5-HT_6 receptors are found in the striatum, nucleus accumbens, and olfactory tubercles. Intermediate levels are present in the hippocampus, cortex, amygdala, and hypothalamus. In contrast to the findings with 5-HT_4 receptor compounds, cognition is improved when animals are treated with a 5-HT_6 receptor *antagonist*. Of particular interest are studies showing reversal of age-related cognitive deficits by 5-HT_6 receptor antagonists (Quiedeville et al., 2014). At least one such antagonist, namely **idalopirdine**, has progressed sufficiently in the drug development pipeline to have been tested clinically in patients with Alzheimer's disease (Wilkinson et al., 2014). For this study, idalopirdine was tested as an adjunct therapy when added to donepezil, a standard drug used for Alzheimer's disease treatment (see Chapter 20). **FIGURE 6.13** shows that overall cognitive

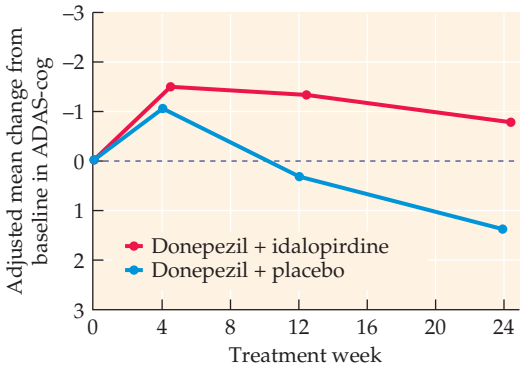


FIGURE 6.13 Improved cognitive function in patients with Alzheimer's disease given idalopirdine plus donepezil, compared with donepezil plus placebo Patients diagnosed with moderate Alzheimer's disease were given 24 weeks of treatment either with the standard medication donepezil (10 mg/day) plus placebo or with donepezil supplemented with idalopirdine (90 mg/day) in a double-blind clinical trial. Cognitive function was assessed at weeks 4, 12, and 24 by means of the cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-cog). The graph shows that the patients supplemented with idalopirdine exhibited better cognitive function than the donepezil-plus-placebo group at weeks 12 and 24. (After Wilkinson et al., 2014.)

function in the donepezil-plus-idalopirdine group was significantly better than in the donepezil-only group. Although these results are promising, there are important limitations of the study that are discussed in an accompanying editorial (Scheider, 2014). In summary, 5-HT_6 receptor antagonists have therapeutic potential for treating Alzheimer's disease (Benhamú et al., 2014; Claeysen et al., 2015; Ramirez et al., 2014); however, as with 5-HT_4 agonists discussed above, we need to remain cautious until additional clinical trials have been performed and shown to be successful.

SEROTONIN IN THE GUT It may surprise you to learn that 90% to 95% of the total bodily 5-HT content is located in the gut, not the brain (McLean et al., 2006). Some of this 5-HT can be found in neurons of the **enteric nervous system**, a large system of ganglia situated within the muscle walls of the intestines. Activity of the enteric nervous system is stimulated by entry of food into the GI tract; this stimulation leads to increased peristalsis (contractions that are necessary for moving food through the GI tract) and release of fluid and hormones by secretory cells of the gut. Even more gut 5-HT, however, is synthesized by **enterochromaffin cells** using the enzyme TPH1, the non-neuronal form of TPH. Enterochromaffin 5-HT is released onto sensory nerve endings within the wall of the gut (**FIGURE 6.14**), after which it is cleared from the extracellular space by SERT. 5-HT taken up by SERT in the membrane of enterocytes (epithelial cells

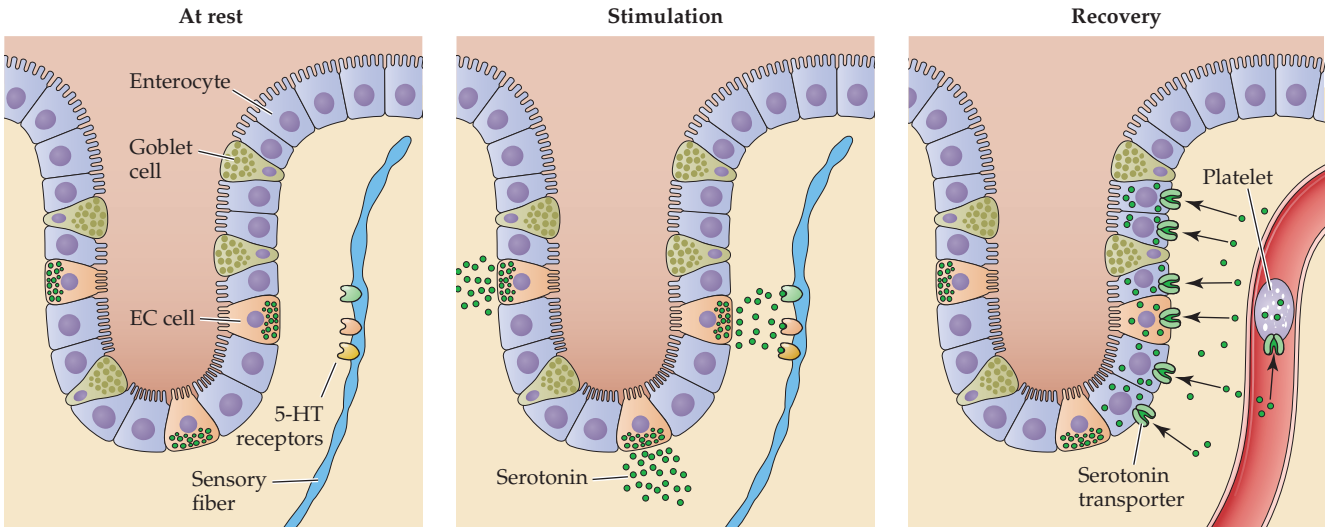


FIGURE 6.14 Release and uptake of 5-HT in the intestine Serotonin is synthesized by specialized secretory cells in the intestinal wall called enterochromaffin (EC) cells. The other cell types depicted in the figure are enterocytes, which are epithelial cells that mediate absorption of nutrients from the gut, and goblet cells, which secrete a layer of protective mucus that lines the intestinal wall. From left to right, the figure first depicts a resting state characterized by minimal 5-HT release, a stimulated state during which 5-HT released by EC cells activates serotonergic receptors on intestinal sensory fibers, and a recovery state in which the released 5-HT is cleared by uptake either into cells of the intestinal wall or into blood platelets in the case of 5-HT molecules that have diffused into the bloodstream. Such uptake is mediated by the serotonin transporter, which is expressed by the EC cells, enterocytes, and platelets. (After Mawe and Hoffman, 2013.)

lining the wall of the intestines) is simply metabolized and excreted, whereas other 5-HT molecules reach the bloodstream and are taken up and stored by blood platelets for later release.[3]

When food is ingested, 5-HT is released both by the serotonergic neurons of the enteric nervous system and the enterochromaffin cells. The resulting increase in 5-HT levels promotes gut peristalsis and secretory activity (McLean et al., 2006). Because of the high concentration of 5-HT in the enterochromaffin cells, many researchers thought that those cells played the most important role in mediating the serotonergic effects on gut function. Surprisingly, however, those effects were eliminated in *Tph2*- but not *Tph1*-knockout mice, demonstrating that 5-HT modulation of gut resides primarily in the serotonergic neurons of the enteric nervous system (Amireault et al., 2013; Gershon, 2013).

Understanding the involvement of 5-HT in the GI tract has significant clinical ramifications. About 10% to 20% of adults suffer from a distressing disorder known as **irritable bowel syndrome** (**IBS**), the symptoms of which include abdominal pain, gas and bloating, frequent abnormal bowel movements (diarrhea, constipation, or an alternation between the two), and mucus in the stool. IBS is more frequent in women than in men for reasons that are not yet known. There are two subtypes of IBS: diarrhea-predominant (IBS-D) and constipation-predominant (IBS-C). Altered serotonergic activity within the gut may be one of the factors contributing to IBS, which has led to interest in treating IBS symptoms with serotonergic agents (Stasi et al., 2014).

The serotonergic receptor subtype studied most extensively with respect to IBS treatment is the 5-HT_3 receptor. 5-HT_3 antagonists have been shown to reduce the rate of food transit through the gut and also relieve abdominal pain in patients with IBS-D (Moore et al., 2013). In fact, the only gut-acting serotonergic medication currently approved for IBS is the 5-HT_3 antagonist **alosetron** (**Lotronex**), which specifically targets IBS-D. Importantly, alosetron has been linked to rare but potentially dangerous side effects, as a result of which the medication can only be prescribed for women with severe IBS-D that has not responded to other kinds of treatment. Alosetron is a full antagonist at the 5-HT_3 receptor, and thus its effects on gut motility are quite strong. However, it might be possible to more subtly influence GI activity using a relatively weak partial agonist at the receptor. As discussed in Chapter 1, partial agonists at a receptor exert lower peak cellular responses than full agonists, but at the same time they block the ability of the full agonist (the neurotransmitter itself) from exerting its peak response. If we hypothesize that IBS-D is due to serotonergic hyperactivity and that IBS-C is due to serotonergic hypoactivity, then a 5-HT_3 partial agonist might be successful in treating the symptoms of both disorders (Moore et al., 2013). This idea, which is depicted in **FIGURE 6.15**, could yield significantly better medications for IBS if successfully implemented.

[3]Stored 5-HT is released locally by platelets during blood clotting at the site of an injury. This 5-HT facilitates the clotting process, though it is not required for clotting to occur.

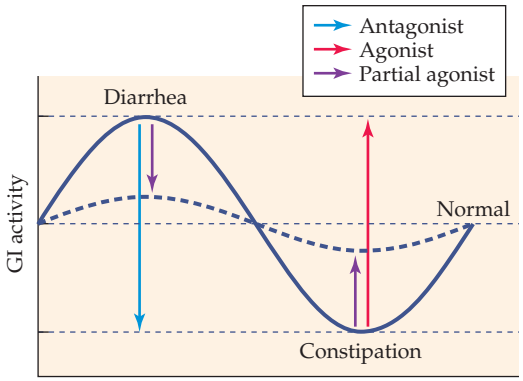


FIGURE 6.15 Hypothesized role of 5-HT in IBS
Some evidence suggests that hyperactivity of the serotonergic system is responsible for IBS-D and that hypoactivity of this system is responsible for IBS-C. Given that the 5-HT_3 receptor is one of the serotonergic receptor subtypes that mediate the effects of this neurotransmitter on GI activity, drugs acting on this subtype could normalize such activity. As shown in the figure, normalization of gut serotonergic activity might be achieved most readily with a 5-HT_3 partial agonist instead of either a full receptor agonist or antagonist. (After Moore et al., 2013.)

Two other receptor subtypes targeted in IBS medications development are the 5-HT_4 and the 5-HT_7 receptors. 5-HT_4 agonists accelerate the movement of food through the GI tract, which makes such compounds useful for reducing constipation in patients with IBS-C (Stasi et al., 2014). Indeed, at the time of this writing, the 5-HT_4 agonist **prucalopride** (**Resolor**) is approved for the treatment of IBS-C in Canada, Europe, and Israel, but the drug is not yet approved by the FDA. Finally, activation of 5-HT_7 receptors causes relaxation of intestinal muscles and, therefore, slows peristaltic activity and transit of food through the gut. Compounds acting on this receptor subtype are being considered not only for IBS but also for the treatment of other GI tract disorders such as inflammatory bowel disease (Kim and Khan, 2014).

Section Summary

- Most of the serotonergic neurons in the CNS are associated with the raphe nuclei of the brainstem. Together, the cells of the dorsal and median raphe nuclei send 5-HT–containing fibers to virtually all forebrain areas.
- Dorsal raphe neuronal firing varies with behavioral state and in response to rewards and punishments. Tonic firing is slow and regular in the

awake state, becomes even slower in slow-wave sleep, and completely ceases during REM sleep. Phasic bursts of activity occur in response to rewarding and punishing stimuli. These phasic bursts are controlled by many different synaptic inputs, including excitatory inputs using the neurotransmitters glutamate and acetylcholine and inhibitory inputs using the neurotransmitter GABA.

- At least 14 different 5-HT receptor subtypes have been identified. Some of these fall within groups, such as the 5-HT_1 family (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F}) and a smaller group of three 5-HT_2 receptors (5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}). The remaining 5-HT receptors are designated 5-HT_3, 5-HT_4, 5-HT_{5A}, 5-HT_{5B}, 5-HT_6, and 5-HT_7. All of the 5-HT receptors are metabotropic, except for the 5-HT_3 receptor, which is an excitatory ionotropic receptor.
- Two of the best-characterized 5-HT receptor subtypes are the 5-HT_{1A} and 5-HT_{2A} receptors. High levels of 5-HT_{1A} receptors have been found in the hippocampus, the septum, parts of the amygdala, and the dorsal raphe nucleus. In the raphe nuclei, including the dorsal raphe, these receptors are mainly somatodendritic autoreceptors on the serotonergic neurons themselves. In other brain areas, 5-HT_{1A} receptors are found on postsynaptic neurons that receive serotonergic input. 5-HT_{1A} receptors function by inhibiting cAMP formation and by enhancing the opening of K^+ channels within the cell membrane. Among a variety of compounds that act on the 5-HT_{1A} receptor subtype are the agonist 8-OH-DPAT and the antagonist WAY 100635.
- There are 5-HT_{2A} receptors in the neocortex, striatum, and nucleus accumbens, as well as in other brain regions. This receptor subtype activates the phosphoinositide second-messenger system, which increases the amount of free Ca^{2+} within the cell and stimulates protein kinase C. When given to rodents, 5-HT_{2A} receptor agonists such as DOI trigger a head-twitch response. In humans, such drugs (which include LSD) produce hallucinations. Certain drugs used in the treatment of schizophrenia can block 5-HT_{2A} receptors, and some researchers hypothesize that such blockade may reduce certain harmful side effects usually associated with antischizophrenic medications.
- Other 5-HT receptor subtypes are currently targeted for specific medical conditions. Thus, 5-$HT_{1B/1D}$ agonists such as sumatriptan and zolmitriptan are used for the treatment of migraine headaches, whereas 5-HT_3 antagonists such as ondansetron, granisetron, and palonosetron offer relief from the nausea and vomiting that can be produced by cancer chemotherapy and radiation treatments.
- Mutant mice in which the *Tph2* gene has been knocked out lack the capacity to synthesize 5-HT in the nervous system. Such mice suffer postnatal growth retardation and increased mortality. They exhibit heightened aggressiveness, impulsive and compulsive behaviors, and impaired social communication, but less anxiety (at least under some conditions) than wild-type mice. Physiologically, *Tph2*-knockout mice suffer from poor thermoregulation and abnormal respiration, manifested in part by episodes of apnea during early postnatal development. Serotonergic neurons are believed to be important in the brain's response to hypercapnia, which has led to the hypothesis that abnormalities in the serotonergic system may be involved in SIDS and SUDEP.
- Pharmacological approaches have elucidated a key role for various 5-HT receptors in the regulation of many functions, including eating behavior (5-HT_{1B}, 5-HT_{2C}, and 5-HT_6), anxiety (5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, and 5-HT_6), neuropathic pain (5-HT_{1A}, 5-HT_{1B}, 5-HT_{2C}, and 5-HT_7), learning and memory (5-HT_{1A}, 5-HT_4, and 5-HT_6), and GI activity (5-HT_3, 5-HT_4, and 5-HT_7). Serotonergic compounds either currently licensed or being clinically tested for the treatment of disorders related to these functions include the following: lorcaserin (5-HT_{2C} agonist; obesity), buspirone (5-HT_{1A} partial agonist; anxiety disorders), vilazodone (combined SSRI and 5-HT_{1A} partial agonist; anxiety disorders), idalopirdine (5-HT_6 antagonist; Alzheimer's disease), alosetron (5-HT_3 antagonist; IBS-D), and prucalopride (5-HT_4 agonist; IBS-C).

STUDY QUESTIONS

1. List the steps involved in 5-HT synthesis, including the name of the enzyme catalyzing each step. Which is the rate-limiting step in the synthetic pathway?
2. Describe the pharmacological and dietary methods used either to increase or to decrease brain 5-HT levels.
3. Discuss the effects of increasing or decreasing brain 5-HT on mood and cognition in humans.
4. Describe the processes involved in 5-HT storage, release, and inactivation. Name the drugs mentioned in the text that influence these processes, including the effect of each drug on serotonergic transmission.
5. What is the name given to the group of cells that synthesize 5-HT in the brain? Name the two specific cell groups that are responsible for most of the serotonergic projections to the forebrain, and list the major forebrain areas that receive these projections.
6. Discuss how the firing of dorsal raphe serotonergic neurons varies with behavioral state and in response to rewards and punishments.
7. List the names of all the serotonergic receptor subtypes. Which of these receptors are metabotropic, and which are ionotropic?
8. Describe the signaling mechanisms of the 5-HT_{1A} and 5-HT_{2A} receptor subtypes.
9. List the drugs mentioned in the text that act on 5-HT_{1A}, $5\text{-HT}_{1B/1D}$, 5-HT_{2A}, and 5-HT_{3} receptors, and describe the behavioral effects of each drug in rodents or humans.
10. Some of the important functions mediated by brain 5-HT have been studied using *Tph2*-knockout mice along with mice that have developed without central serotonergic neurons. Discuss the various ways in which these mice differ from normal mice behaviorally and physiologically.
11. Several medications used clinically to treat anxiety disorders exert their primary actions on the serotonergic system. Which serotonergic receptor subtypes have been implicated in the control of anxiety and anxiety-related behaviors? Provide relevant experimental findings to support your answer.
12. Discuss the involvement of 5-HT in pain regulation and the use of serotonergic medications to treat pain-related disorders.
13. 5-HT_{1A}, 5-HT_{4}, and 5-HT_{6} receptors have all been implicated in processes of learning and memory. Describe the experimental findings obtained from laboratory animals that implicate each receptor subtype, including the brain area(s) of receptor expression thought to be important for each subtype.
14. What are the sources of 5-HT in the GI tract, and why are pharmacologists interested in 5-HT from these sources? Include in your answer a discussion of the clinical relevance of gut 5-HT, including serotonergic medications that have been developed to treat GI disorders.
15. Discuss the pharmacology of MDMA. Include in your answer the history of its discovery and its use as both a recreational drug and as an adjunct to psychotherapy for treatment-resistant PTSD. What is the current status of research concerning MDMA's putative neurotoxic effects on the serotonergic system?
16. Aggressive behaviors both in humans and in animals are often subject to some type of classification system. Which of the classification systems described in Box 6.2 do you think is best suited to help us understand human aggression? Would you choose the same classification system to apply to aggression in animals?
17. What is meant by the "serotonin deficiency" hypothesis of aggression? Discuss experimental evidence that either supports or contradicts this hypothesis. Which kinds of aggressive behavior appear to be most clearly related to the serotonergic system?

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CHAPTER 7

Curare, a toxin that blocks acetylcholine receptors at the neuromuscular junction, has long been used as an arrow poison by various South American tribesmen. (© Jim Clare/Minden Pictures.)

Acetylcholine

IF YOU WERE A SCIENTIFIC RESEARCHER, how much discomfort would you be willing to endure as a participant in your own experiments? For example, would you be willing to take a drug that would cause complete paralysis, thus preventing you from breathing, while you might still be fully conscious and aware of the sensations of asphyxia? Remarkably, that is exactly what was done independently by two researchers, Frederick Prescott and Scott Smith, in the mid-1940s (Prescott et al., 1946; Smith et al., 1947). Both were experimenting with curare, a toxin present in the bark of several South American plants that was discovered centuries ago by native tribes, who used the substance to tip their poison arrows for hunting and fighting. According to Mann (2000), some tribes calibrated the potency of their curare-containing plant extracts based on the effects of the material on prey animals. For example, a highly potent preparation was called "one-tree" curare, because a monkey hit with an arrow containing this preparation only had time to leap to one nearby tree in its attempts to escape before it died.

Accounts of the effects of curare were first brought back to Europe by Sir Walter Raleigh at the end of the sixteenth century, but as late as the 1940s some pharmacologists were still unsure whether curare caused unconsciousness and/or analgesia, in addition to paralysis. This was important information since curare was still occasionally being administered during medical (including surgical) procedures without the accompaniment of a known anesthetic agent. Answering this question was the primary rationale offered by Smith and colleagues when they published the account of his experience under curare alone. Prescott and coworkers focused instead on determining the optimal dose and method of administration as an adjunct (i.e., additional medication) to a regular anesthetic, but their paper also provides a graphic account of Prescott's reactions to receiving curare by itself. On the basis of self-reports given by both Prescott and Smith after recovery from the drug, the results were conclusive—curare does *not* cause anesthesia. Indeed, both men were fully awake as their growing paralysis prevented them from breathing, and

they began to become asphyxiated. Both teams provided artificial respiration to their "subjects" during the experiments; however, the method used by Prescott's group was less effective, which resulted in a subjective experience described in the paper as "terrifying."[1]

How does curare work to induce muscular paralysis? And why doesn't it alter conscious experience when given to an individual? As we shall see in this chapter, the toxic agent in curare blocks a particular kind of receptor for the neurotransmitter **acetylcholine** (**ACh**; adjective form, **cholinergic**). Acetylcholine is a particularly fascinating transmitter—a molecule that is life sustaining in its function but that is also the target of some of the most deadly known toxins, both naturally occurring and synthetic. ■

Acetylcholine Synthesis, Release, and Inactivation

Acetylcholine is a key neurotransmitter at many synapses in the peripheral nervous system (PNS), including the type of synapse that is between motor neurons and muscles (called the **neuromuscular junction**) and specific synapses within the sympathetic and parasympathetic divisions of the autonomic nervous system. In contrast, this transmitter is synthesized by only a small number of neurons in the brain; yet, those neurons play an important role in several important behavioral functions. The following sections examine the processes through which this vital transmitter is produced, released, and inactivated. Since both too much and too little ACh is dangerous or even deadly, we also take a look at the various agents that act to either increase or limit the level of ACh in the body.

Acetylcholine synthesis is catalyzed by the enzyme choline acetyltransferase

In contrast to the multiple steps required to synthesize the catecholamine transmitters, ACh is formed in a single step from two precursors: **choline** and **acetyl coenzyme A** (**acetyl CoA**) (**FIGURE 7.1**). The choline comes mainly from fat in our diet (choline-containing lipids), although it is also produced in the liver. Acetyl CoA is generated within all cells by the metabolism of sugars and fats. The synthesis of ACh is catalyzed by the enzyme **choline acetyltransferase** (**ChAT**), which does just what its name implies: it transfers the acetyl group (–$COCH_3$) from acetyl CoA to choline to form ACh. Choline acetyltransferase is present in the cytoplasm of the cell, and this enzyme is found only in neurons that use ACh as their transmitter. This specificity allows us to identify cholinergic neurons by staining for ChAT.

The rate of ACh synthesis is controlled by several factors, including the availability of its precursors inside the cell, as well as the rate of cell firing. Thus, cholinergic neurons make more ACh when more choline and/or acetyl CoA is available and when the neurons are stimulated to fire at a higher rate. Although knowledge of these regulatory processes has helped researchers understand how the cholinergic system functions, it has not yet led to the development of useful pharmacological agents. For example, it has been difficult to find highly selective inhibitors of ChAT, and even if such drugs are eventually isolated, it is not clear that inhibiting ACh synthesis will have any obvious clinical usefulness. At one time, it was thought that boosting brain ACh levels by administering large doses of choline might be beneficial for patients with Alzheimer's disease, since damage to the cholinergic system is one of the factors contributing to the cognitive deficits seen in that disorder. However, not only did choline treatment fail to produce symptom improvement, peripheral metabolism of this compound unfortunately caused the patients to give off a strong fishy odor!

Many different drugs and toxins can alter acetylcholine storage and release

The axon terminals of cholinergic neurons contain many small synaptic vesicles that store ACh for release when the nerve cell is active. It is estimated that a few thousand molecules of transmitter are present in each vesicle. Vesicles are loaded with ACh by a transport protein in the vesicle membrane called, appropriately, the **vesicular ACh transporter** (**VAChT**) (**FIGURE 7.2**). This protein can be blocked by a drug called **vesamicol**. What effect would you expect vesamicol to have on cholinergic neurons? Would you predict any drug-induced change in the distribution of ACh between the cytoplasm and the synaptic vesicles within the cholinergic nerve terminals? Furthermore, if redistribution of ACh occurred, what effect would this have on ACh release by the cells? If you predicted that

[1]Readers interested in learning more about the history of curare, including how Prescott and Smith came to perform their bold experiments, are referred to an excellent account by Anderson (2010).

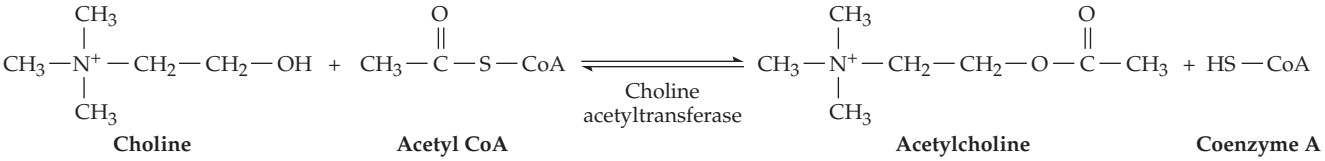


FIGURE 7.1 Synthesis of acetylcholine (ACh) by choline acetyltransferase

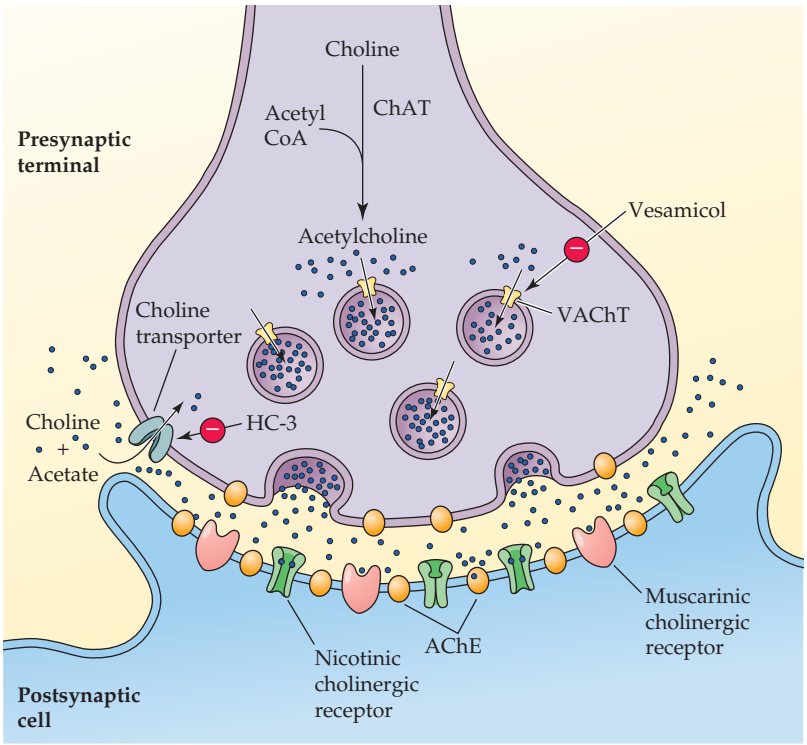


FIGURE 7.2 A cholinergic synapse
The processes of ACh synthesis and metabolism, presynaptic choline uptake, and vesicular ACh uptake and release occur at the synapse. Postsynaptic nicotinic and muscarinic ACh receptors are also shown. AChE, acetylcholinesterase; ChAT, choline acetyltransferase; HC-3, the drug hemicholinium-3; VAChT, vesicular ACh transporter.

vesamicol treatment would decrease vesicular ACh but increase the level of ACh in the cytoplasm, you were correct. This is because vesamicol doesn't affect the rate of ACh synthesis; therefore, ACh molecules that normally would have been transported into the vesicles remain in the cytoplasm of the terminal. Moreover, because synaptic vesicles are the main source of ACh release when the cholinergic neurons fire, such release is reduced in the presence of vesamicol. We can imagine that the cholinergic vesicles are still present and are continuing to undergo exocytosis when the neurons are activated, but the amount of ACh available within the vesicles to be released is abnormally low.

The release of ACh is dramatically affected by various animal and bacterial toxins. For example, a toxin found in the venom of the black widow spider, *Latrodectus mactans*, leads to a massive release of ACh at synapses in the PNS. Overactivity of the cholinergic system causes numerous symptoms, including muscle pain in the abdomen or chest, tremors, nausea and vomiting, salivation, and copious sweating. Ounce for ounce, black widow spider venom is 15 times more toxic than prairie rattlesnake venom, but a single spider bite is rarely fatal in healthy young adults because of the small amount of venom injected into the victim. In contrast to the effects of black widow spider venom, toxins that cause botulism poisoning potently inhibit ACh release, which can have deadly consequences due to muscular paralysis. On the other hand, careful localized administration of the same toxin has several medical and cosmetic applications (**BOX 7.1**).

Acetylcholinesterase is responsible for acetylcholine breakdown

Levels of ACh are carefully controlled by an enzyme called **acetylcholinesterase** (**AChE**), which breaks down the transmitter into choline and acetic acid (**FIGURE 7.3**). Within the cell, AChE is present at several strategic locations. One form of the enzyme is found inside the presynaptic cell, where it can metabolize excess ACh that may have been synthesized. Another form of AChE is present on the membrane of the postsynaptic cell to break down molecules of ACh after their release into the synaptic cleft. Finally, a unique type of AChE is found at neuromuscular junctions, the specialized synapses between neurons and muscle cells where ACh is released by motor neurons to stimulate muscular contraction. Muscle cells actually secrete AChE into the space between themselves and the cholinergic nerve endings, and enzyme molecules become immobilized there by attaching to other proteins within the neuromuscular junction. This unique location helps the transmission process function very precisely at neuromuscular junctions. Immediately after a squirt of ACh causes a particular muscle to contract, the transmitter

FIGURE 7.3 Breakdown of ACh by acetylcholinesterase

BOX 7.1 Pharmacology In Action

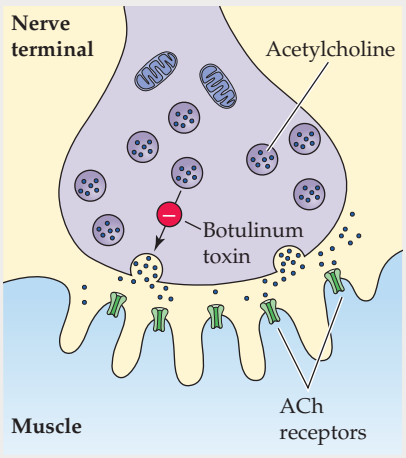
Botulinum Toxin—Deadly Poison, Therapeutic Remedy, and Cosmetic Aid

The bacterium *Clostridium botulinum*, which is responsible for botulism poisoning, produces what is perhaps the most potent toxin known to pharmacologists. The estimated lethal dose of botulinum toxin in humans is 0.3 μg; in other words, 1 gram (equivalent to the weight of three aspirin tablets) is enough to kill more than 3 million individuals. *Clostridium botulinum* does not grow in the presence of oxygen; however, it can thrive in an anaerobic (oxygen-free) environment such as a sealed food can that has not been properly heated to kill the bacteria.

Botulinum toxin actually consists of a mixture of seven related proteins known as botulinum toxins A through G. These proteins are taken up selectively by peripheral cholinergic nerve terminals, including those located at the neuromuscular junction, autonomic ganglia, and postganglionic parasympathetic fibers (e.g., cholinergic fibers innervating smooth muscle and secretory glands). The toxin molecules interfere with the process of ACh release at the terminals, thereby blocking neurotransmission (see **Figure**). When cholinergic motor neurons have been affected by botulinum toxin, the result is muscle weakness and possible paralysis. Other symptoms of botulism poisoning include blurred vision, difficulty speaking and swallowing, and gastrointestinal distress. Most victims recover, although a small percentage die as the result of severe muscle paralysis and eventual asphyxiation.

Once the mechanism of action of botulinum toxin became known, researchers began to consider that this substance, if used carefully and at very low doses, might be helpful in treating clinical disorders characterized by involuntary muscle contractions. After appropriate testing, in 1989 the U.S. Food and Drug Administration approved the use of purified botulinum toxin A for the treatment of strabismus (crossed eyes), blepharospasm (spasm of the eyelid), and hemifacial spasm (muscle spasms on just one side of the face). Since that time, this substance has been administered for a variety of other disorders, including spastic cerebral palsy, stroke-related spasticity, dystonias (prolonged muscle contractions, sometimes seen as repeated jerking movements), muscle-specific orofacial pain, axillary hyperhidrosis (extreme underarm sweating), chronic migraine, overactive bladder, and achalasia (failure of sphincter muscles to relax when appropriate). You should be able to discern that some of these treatments are aimed at relaxation of specific skeletal muscles, whereas others are aimed at reduction of smooth muscle or secretory activity.

Even if you're not familiar with the abovementioned medical applications of botulinum toxin, you may have heard of its use (under the trade name Botox) by dermatologists for cosmetic purposes in patients with excessive frown lines, worry lines, or crow's-feet around the eyes. Such "dynamic wrinkles," as they are called, result from chronic contraction of specific facial muscles. When injected locally into a particular muscle or surrounding area, Botox causes paralysis of that muscle as the result of blockade of ACh release from incoming motor nerve fibers. This leads to a reduction in the offending lines or wrinkles, although each treatment remains effective for only a few months, after which it must be repeated. Despite this limitation, many dermatologists and their patients have chosen Botox treatment over plastic surgery for improving facial appearance (Loyo and Kontis, 2013). Moreover, recent reviews point to the potential use of Botox for treating dental problems that have a neuromuscular origin (Nayyar et al., 2014; Azam et al., 2015). It seems safe to say that both the medical and cosmetic uses of botulinum toxin will continue to grow in the coming years.



Mechanism of action of botulinum toxin at the neuromuscular junction Botulinum toxin blocks ACh release at the neuromuscular junction by preventing fusion of synaptic vesicles with the nerve terminal membrane.

is metabolized extremely rapidly, and the muscle can relax until the next command arrives to squirt out some more ACh and contract that muscle once again.

Once ACh has been broken down within the synaptic cleft, a significant portion of the liberated choline is taken back up into the cholinergic nerve terminal by a **choline transporter** in the membrane of the terminal (see Figure 7.2). If the choline transporter is blocked by means of the drug **hemicholinium-3** (**HC-3**), the rate of ACh production declines. Furthermore, mutant mice in which the choline transporter has been knocked out die within about 1 hour after birth (Ferguson et al., 2004). The cause of death in these animals is lack of normal breathing due to failure to sustain ACh synthesis and release at the neuromuscular junction. These findings tell us that utilization of recycled choline plays a critical role in maintaining ongoing ACh synthesis.

Drugs that block AChE prevent inactivation of ACh, thereby increasing the postsynaptic effects of the transmitter. Several AChE inhibitors occur naturally, but many others are synthetic compounds. Inhibition of ACh breakdown can be beneficial for neurological disorders in which cholinergic transmission is deficient, but such inhibition can also be highly toxic, even fatal (reviewed by Čolović et al., 2013). Besides the obvious factor of drug dose, whether a particular compound is likely to be used as a medicine or as a poison depends on whether it crosses the blood–brain barrier and whether it blocks AChE reversibly or irreversibly.

The most widespread use of AChE inhibitors is for the treatment of mild to moderate **Alzheimer's disease**. Currently licensed drugs of this class are **donepezil** (**Aricept**), **rivastigmine** (**Exelon**), and **galantamine** (**Reminyl**), all of which are synthetic compounds that enter the brain to exert their therapeutic action. The rationale for the use of AChE inhibitors in patients with Alzheimer's disease is the significant loss of forebrain cholinergic neurons in this disorder and the evidence that such loss contributes to the profound cognitive deficits suffered by these patients. Thus, increasing the availability of the remaining ACh offers some cognitive benefit, although the effects are modest and the drugs do not prevent progression of the disease. Much more information about Alzheimer's disease, including symptoms, neuropathology, and current treatments, is provided in Chapter 20.

One of the best-characterized natural AChE inhibitors is **physostigmine**, also known as **eserine**, a compound isolated from Calabar beans (the seeds of a woody plant found in the Calabar region of Nigeria). When applied directly to the eyes, physostigmine can help treat glaucoma because of its ability to increase drainage of the excess fluid. But when given systemically, the drug can cause serious symptoms both peripherally and centrally because of its ability to cross the blood–brain barrier. Symptoms of physostigmine poisoning include slurred speech, mental confusion, hallucinations, loss of reflexes, convulsions, and even coma and death. Nineteenth-century missionaries to West Africa discovered societies that used extracts of the Calabar bean to determine the guilt of accused prisoners (Davis, 1985). The defendant was considered innocent if he was fortunate enough to regurgitate the poison or, alternatively, if he could manage to walk 10 feet under the influence of the toxin (in which case vomiting was induced). Needless to say, however, most prisoners did neither and were consequently judged guilty and were permitted to die a horrible death.

Neostigmine (**Prostigmin**) and **pyridostigmine** (**Mestinon**) are synthetic analogs of physostigmine that do not cross the blood–brain barrier. These drugs are beneficial in the treatment of a rare but serious neuromuscular disorder called **myasthenia gravis**. Myasthenia gravis is an example of an **autoimmune disorder**, a condition in which a part of the body is attacked by one's own immune system. In this case, antibodies are formed against the skeletal muscle cholinergic receptors, first blocking the receptors, and eventually causing them to be broken down by the muscle cells (**FIGURE 7.4**). Loss of receptor function

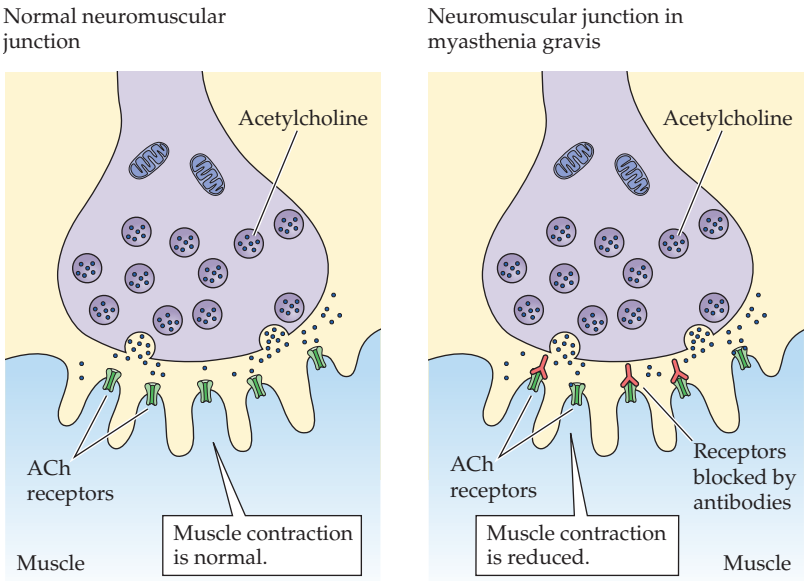


FIGURE 7.4 Myasthenia gravis, an autoimmune disorder In myasthenia gravis, antibodies interfere with cholinergic transmission at the neuromuscular junction by binding to and blocking the muscle ACh receptors.

causes the patient's muscles to be less sensitive to ACh, which, in turn, leads to severe weakness and fatigue (Trouth et al., 2012). By inhibiting AChE, neostigmine and pyridostigmine reduce the rate of ACh breakdown at the neuromuscular junction, which causes increased stimulation of the remaining undamaged cholinergic receptors. Unfortunately, whereas the drugs help alleviate the symptoms of myasthenia gravis, they do not prevent progression of the disease.

Physostigmine and its analogs, along with the drugs used to treat Alzheimer's disease, are all reversible AChE inhibitors. This means that drug molecules bind temporarily to the enzyme protein to inhibit its action, after which the drug dissociates from the enzyme and ACh breakdown is restored. However, certain other chemicals, which are called **organophosphorus compounds** due to their chemical structure, produce a nearly irreversible inhibition of AChE activity. This can occur either because dissociation of the compound from the enzyme is extremely slow or because the compound actually forms a covalent chemical bond with the enzyme. Weaker versions of these irreversible inhibitors are widely used as insecticides, since preventing ACh breakdown is just as harmful to ants and wasps as it is to humans. On the other hand, it's important to be aware that acute organophosphate poisoning is a significant health problem in developing countries as well as in agricultural workers in the developed world (Eddleston et al., 2008; Chowdhary et al., 2014; Suratman et al., 2015). In addition to cases of accidental poisoning, some developing countries are plagued by intentional consumption of the poison for suicidal purposes. Finally, long-term exposure to organophosphorus compounds in the environment poses a worldwide health risk both to humans and to other organisms, with a special emphasis on the adverse consequences of developmental exposures (Burns et al., 2013; Muñoz-Quezada et al., 2013; González-Alzaga et al., 2014). See the Companion Website for a discussion of environmental neurotoxicants and endocrine disruptors.

Tragically, even more-toxic varieties of irreversible AChE inhibitors have been developed as "nerve gases" for use in chemical warfare. These agents go by names such as **sarin** and **soman**. They are designed to be dispersed as a vapor cloud or spray, which allows their entry into the body through skin contact or by inhalation. In either case, the drug quickly penetrates into the bloodstream and is distributed to all organs, including the brain. Symptoms of nerve gas poisoning, which include profuse sweating and salivation, vomiting, loss of bladder and bowel control, and convulsions, are due to rapid ACh accumulation and overstimulation of cholinergic synapses throughout both the CNS and the PNS. Death occurs through asphyxiation due to paralysis of the muscles of the diaphragm. Sarin and other chemical warfare agents were reportedly used by Iraqi forces during the Iran–Iraq war of the 1980s (Haines and Fox, 2014). More recently, an investigation conducted by the United Nations concluded that in 2013, the Syrian government was responsible for a sarin gas attack during the course of that country's civil war (Sellström et al., 2013).

When an enemy possesses a stockpile of nerve agents and has demonstrated a willingness to use them, the opponent must take measures to counteract the threat. During the Persian Gulf War of 1990–1991, as well as the subsequent conquest of Iraq, Allied forces were very concerned about possible use of sarin by the Iraqi army. Consequently, tablets of pyridostigmine bromide (PB) were widely distributed to Allied troops for use as a nerve gas antidote. How can a reversible AChE inhibitor be an antidote against sarin or soman? It appears that the temporary interaction of PB with the enzyme protects AChE from permanent inactivation by the nerve gas. This protective effect, however, requires that the antidote be administered ahead of time—before exposure to the toxic agent. Therefore, soldiers were instructed to take three PB pills daily at times when they were thought to be at risk for nerve gas attack. However, some soldiers took the drug much more frequently, thus leading to heavy exposure. Review of scientific findings by the Research Advisory Committee on Gulf War Veterans' Illnesses (2008; 2014) led the committee to conclude that such exposure may have contributed significantly to the development of "Gulf War illness," a complex multisymptom disorder currently thought to afflict at least 25% of U.S. veterans who served in that combat theater.

Section Summary

- ACh is not only found in the brain, it is also a key transmitter at the neuromuscular junction and at specific synapses within the sympathetic and parasympathetic branches of the autonomic nervous system.
- ACh is synthesized from choline and acetyl CoA in a single reaction catalyzed by the enzyme choline acetyltransferase. The rate of ACh synthesis is controlled by precursor availability and is increased by cell firing.
- ACh is loaded into synaptic vesicles by the specific vesicular transporter VAChT.
- A variety of animal and bacterial toxins influence the cholinergic system either by stimulating or inhibiting ACh release. Local administration of the paralytic toxin botulinum toxin A has both medical and cosmetic uses.
- Following its release into the synapse or neuromuscular junction, ACh is rapidly degraded by the enzyme AChE.

- Much of the choline liberated from ACh breakdown is taken back up into the cholinergic nerve terminal by a choline transporter that plays a critical role in maintaining ongoing ACh synthesis.
- Drugs that block AChE cause prolongation of ACh action at postsynaptic or muscular cholinergic receptors.
- Several reversible AChE antagonists that enter the brain are currently used to treat mild to moderate Alzheimer's disease. Other reversible antagonists that do not cross the blood–brain barrier help alleviate symptoms of the neuromuscular disorder myasthenia gravis.
- Organophosphorus compounds that block AChE irreversibly are the active ingredients of some insecticides, and similar but more potent inhibitors are the main components of dreaded nerve gases.

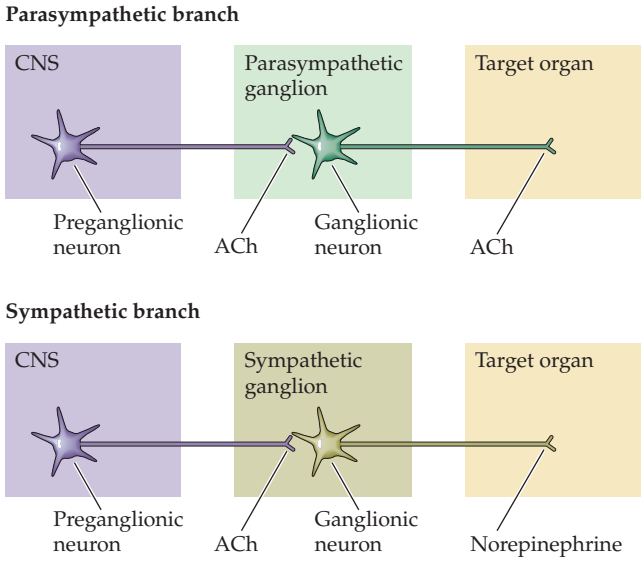


FIGURE 7.5 Cholinergic synapses in the parasympathetic and sympathetic branches of the autonomic nervous system

Organization and Function of the Cholinergic System

We have already noted the presence of ACh as a transmitter at the neuromuscular junction and within the autonomic nervous system. Indeed, this substance was first identified as a neurotransmitter in the PNS. In the following sections we will describe the location of cholinergic cell bodies and synapses in both the PNS and CNS, the receptor subtypes for ACh, and some of the key behavioral and physiological functions of this neurotransmitter.

Cholinergic neurons play a key role in the functioning of both the peripheral and central nervous systems

Before we examine the function of cholinergic neurons, let's briefly review the organization of the autonomic nervous system. Both the sympathetic and parasympathetic branches consist of preganglionic neurons, which are cells located within the CNS that send their axons to the autonomic ganglia, as well as ganglionic neurons located within the ganglia that innervate various target organs throughout the body. The preganglionic neurons of both branches are cholinergic, as are the ganglionic neurons of the parasympathetic system (we saw in Chapter 5 that norepinephrine is the transmitter of the sympathetic ganglionic neurons). **FIGURE 7.5** illustrates the chemical coding of these cells and the synapses they make. Widespread involvement of ACh in both the neuromuscular and autonomic systems explains why drugs that interfere with this transmitter exert such powerful physiological effects and sometimes are highly toxic.

Within the brain, the cell bodies of cholinergic neurons are clustered within just a few areas (**FIGURE 7.6**). Some of these nerve cells, such as the ones found within the striatum, are interneurons. In Chapter 5, we saw that dopaminergic input to the striatum plays a critical role in the regulation of movement. This regulation depends in part on the balance between ACh and dopamine (DA); that is, when DA is low, as in Parkinson's disease, the resulting neurotransmitter imbalance contributes to motor symptoms of the disorder. Consequently, anticholinergic drugs such as **orphenadrine** (**Norflex**), **benztropine mesylate** (**Cogentin**), and **trihexyphenidyl** (**Artane**) are sometimes prescribed instead of L-DOPA in the early stages of Parkinson's disease.

Other cholinergic neurons send their axons longer distances to innervate many different brain areas. For example, a diffuse collection of cholinergic nerve cells called the **basal forebrain cholinergic system** (**BFCS**) comprises neurons interspersed among several anatomical areas, including the nucleus basalis, substantia innominata, medial septal nucleus, and diagonal band nuclei. The BFCS is the origin of a dense cholinergic innervation of the cerebral cortex, as well as the hippocampus and other limbic system structures. As discussed in **BOX 7.2**, this system plays a significant role in cognitive function. Recent findings suggest an additional involvement of the BFCS, specifically the cholinergic neurons within the diagonal band nuclei, in the regulation of appetite and feeding behavior (Herman et al., 2016).

Researchers have identified another group of important cholinergic cells in the dorsolateral pons within the **laterodorsal** and **pedunculopontine tegmental nuclei** (abbreviated **LDTg** and **PPTg**, respectively). One pathway from these cell groups projects to midbrain

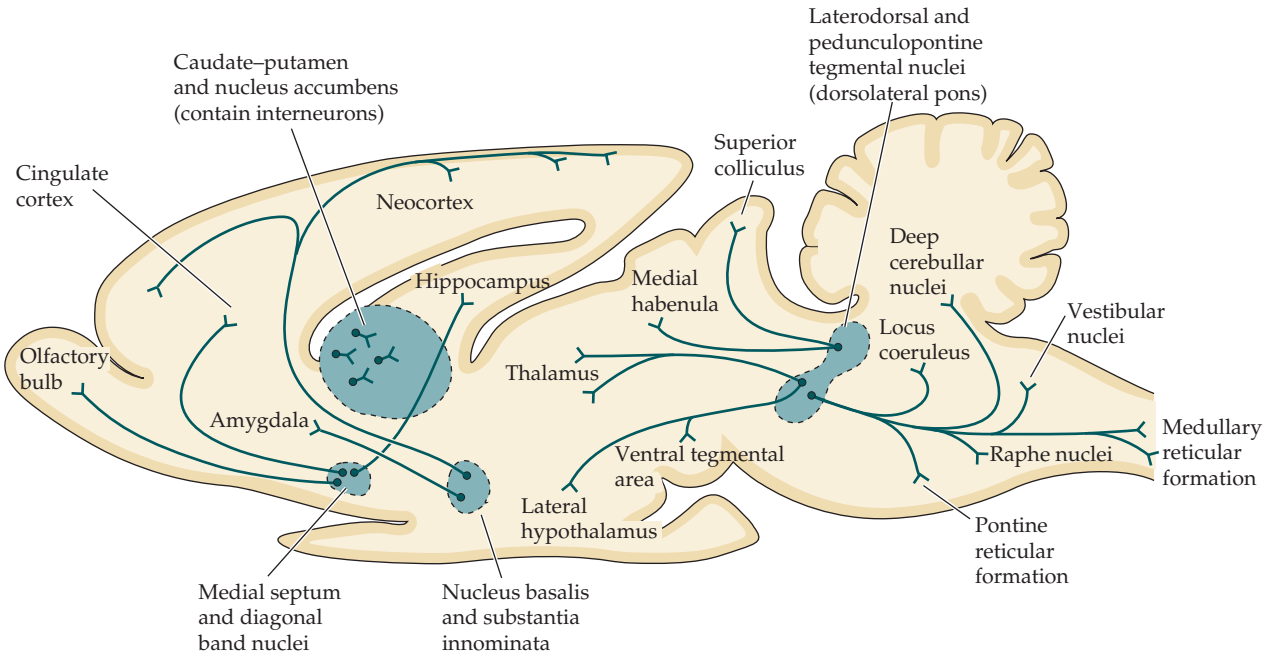


FIGURE 7.6 Anatomy of cholinergic pathways in the brain The cell bodies of cholinergic neurons are located primarily in the caudate–putamen, nucleus accumbens, nucleus basalis, medial septum, diagonal band nuclei, substantia innominata, pedunculopontine tegmental nucleus, and dorsolateral tegmental nucleus. Note that the basal forebrain cholinergic cell groups send fibers to all areas of the cortex, as well as to limbic system structures such as the hippocampus and amygdala. Cell groups of the pedunculopontine tegmental and dorsolateral tegmental nuclei primarily innervate subcortical structures that do not receive input from the basal forebrain system, caudate–putamen, and nucleus accumbens.

dopaminergic cells —the LDTg to the ventral tegmental area, and the PPTg to the substantia nigra. ACh released from this pathway exerts a powerful excitatory influence on dopamine neuron firing, which is mediated, in part, by activation of postsynaptic **nicotinic cholinergic receptors** (Maskos, 2008). Indeed, these nicotinic receptors are intimately involved in the reinforcing effects of nicotine in experimental animals (see Chapter 13). Among the other pathways arising from the LDTg and the PPTg are excitatory projections to brainstem and thalamic areas that play important roles in behavioral arousal, sensory processing, and initiation of rapid-eye-movement sleep (Kobayashi and Isa, 2002; McCarley, 2007; Grace and Horner, 2015).

BOX 7.2 The Cutting Edge

Acetylcholine and Cognitive Function

Cognition is an amazingly complex process that requires attending to external and internal sensory stimuli; processing this stimulus information and relating it to past experience; forging connections between multiple stimuli, between stimuli and responses, and between responses and rewards (i.e., "learning"); encoding newly learned information into short-term memory; consolidating short-term memories into long-term memory stores; and finally recalling the learned information when needed. Research over many years has led to the view that ACh plays an important role in many features of cognitive functioning. Indeed, as we have already seen, AChE inhibitors, which boost synaptic availability of ACh, are among the first-line treatments for Alzheimer's disease–related cognitive deficits.

The earliest evidence for involvement of ACh in cognition came from observations that the general antimuscarinic agents atropine and scopolamine can produce amnesic effects in humans. Indeed, during the first half of the twentieth century, it was common for obstetricians to administer morphine and scopolamine to women during labor. The resulting state, often called "twilight sleep," enabled patients to avoid both the pain and the memory of labor; however, the procedure unfortunately had adverse effects on the newborn infants and therefore is rarely used in modern obstetrics. Behavioral pharmacologists later

BOX 7.2 The Cutting Edge (*continued*)

began to administer scopolamine or occasionally atropine to experimental animals, particularly rats and mice, and found that these compounds produced deficits in the acquisition and maintenance of many different kinds of learning tasks. The initial interpretation of these findings was that the drugs interfered with memory consolidation, which is consistent with the reported amnesic effects of scopolamine in humans. Indeed, some researchers have argued that ACh in the hippocampus plays an important role in the encoding of episodic memories (i.e., memories of experiences, events, and situations) (Hasselmo and Sarter, 2011). In the remainder of this discussion, we will consider several lines of research using other experimental approaches that have attempted to pin down what ACh is doing to facilitate cognition and where in the brain it might be acting.

We have known for many years that the cholinergic cell bodies most involved in cognition are located in the basal forebrain cholinergic system (BFCS), which has strong projections to the prefrontal cortex and hippocampus, two areas that have crucial roles in cognitive function. Key evidence for this notion came from the introduction of a selective cholinergic neurotoxin called **192 IgG–saporin**. This odd-sounding substance contains a monoclonal antibody, 192 IgG, which binds specifically to a cell surface protein expressed by basal forebrain cholinergic neurons. The other part of the molecule, saporin, is a cellular toxin obtained from the soapwort plant, *Saponaria officinalis*. When 192 IgG–saporin is injected into the region of the BFCS, cholinergic neurons take up the toxin because of binding of the antibody part of the molecule. As a result, those neurons are destroyed, while neighboring noncholinergic cells are spared. Although some investigators have observed learning deficits in animals subjected to 192 IgG–saporin lesions of the BFCS (Wrenn and Wiley, 1998), other studies suggest that lesion-induced impairments stem from deficits in sensory attention (Baxter, 2001).

Recent findings from Sarter and his colleagues support a key role for ACh in sensory attention. First, the researchers demonstrated the existence of phasic ACh release in the prefrontal cortex of rats and mice when the animals detect a sensory stimulus (i.e., a cue) during performance of a sustained-attention task (reviewed in Sarter et al., 2016). Because such results are only correlative in nature, a subsequent study used the technique of optogenetics (see Chapter 4) to test whether these brief pulses of ACh are *causally* related to the ability of animals to detect and respond accurately to a cue (Gritton et al., 2016). In this study, mice were first trained on a task requiring them to detect whether a brief flash of light, ranging from 25 ms to 500 ms in duration, had been presented. Both cued (light presented) and noncued (no light presented) trials were given, and the animal reported whether it detected a cue or not by making one of two different nose-poke responses. Correct responses on both kinds of trials were reinforced. Of particular interest in sustained-attention studies are "hits," which are trials in which the subject correctly reports detection of the cue, and "false alarms," which are trials in which the subject makes an error by reporting detection of the cue when no cue is actually presented.

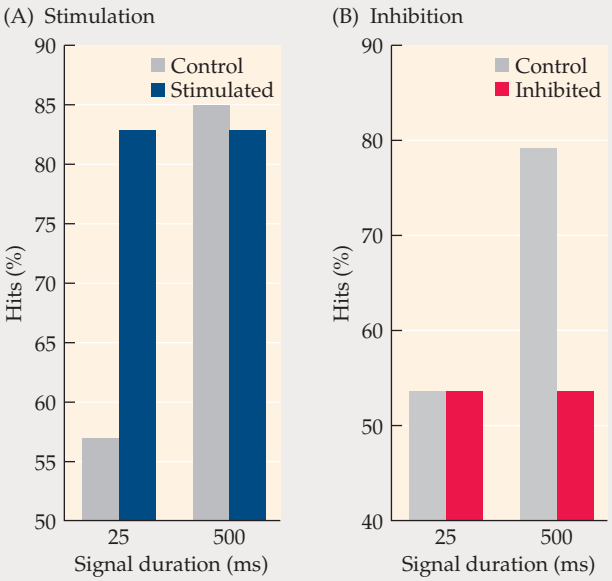
To experimentally control activity of the BFCS neurons, the researchers transfected some mice with channelrhodopsin-2 (ChR2), a light-sensitive protein that excites neurons when activated by a laser, whereas other mice were transfected with halorhodopsin (NpHR), which inhibits neuronal activity when activated by laser light of a different wavelength. Key results of the study are shown in the figures. **Figure A** depicts the effects on hit rate of strongly stimulating ChR2 in the BFCS neurons under conditions of long and short cue duration. With a long-duration cue, which is easy for the mice to detect, the mean control hit rate (left) was approximately 85%, and this rate was unaffected by BFCS neuronal stimulation (right). On the other hand, with a short-duration cue, which is much more difficult for the mice to detect, the mean control hit rate was much lower (just over 55%) but the hit rate was increased to that of the long-duration cue when the cholinergic neurons were stimulated. In contrast, **Figure B** shows that the hit rate with the long-duration cue was reduced to the hit rate of the short-duration cue when firing of the BFCS neurons was inhibited using NpHR. Not shown are other data demonstrating that the false alarm rate (subjects reporting detection of the cue when the cue was not present) was significantly increased under conditions of ChR2 activation. Together, these results strongly support the hypothesis that bursts of ACh release from the BFCS play a key role in detecting and responding to learned sensory cues. Furthermore, the same hypothesis may be applicable to human sensory attention, as performance in a selective visual attention task was enhanced by the AChE inhibitor physostigmine and reduced by the muscarinic antagonist scopolamine (Furey et al., 2008).

Over 30 years ago, Raymond Bartus and colleagues proposed that the cognitive decline that often occurs with aging is due, at least in part, to dysfunction of the BFCS (Bartus et al., 1982). This spurred tremendous interest in the BFCS, not only with respect to normal aging but also regarding a

(*Continued*)

BOX 7.2 Cutting Edge (*continued*)

possible role in the age-related disorder Alzheimer's disease. Indeed, the cholinergic hypothesis of Alzheimer's dementia subsequently led to the development of AChE inhibitors for treating this disorder (see above and Chapter 20). Although damage to the BFCS almost certainly contributes to the cognitive deficits seen in patients with Alzheimer's, it is only part of the problem, because widespread loss of other cells and synaptic connections throughout the cerebral cortex and hippocampus also occurs. Nevertheless, there is continued interest in developing novel cholinergic agents targeting either muscarinic or nicotinic receptors for treating not only Alzheimer's dementia but also the cognitive deficits associated with schizophrenia and certain other neuropsychiatric disorders (Bertrand et al., 2015; Terry Jr. et al., 2015; Fuenzalida et al., 2016). One novel approach worth mentioning in this regard is the application of **allosteric modulators**, defined as compounds that (1) bind to a receptor site distinct from the main agonist binding site, (2) may or may not have an effect on the receptor when administered alone, and (3) either enhance (in the case of a positive allosteric modulator) or reduce (in the case of a negative allosteric modulator) the effectiveness of an agonist (e.g., ACh) on the receptor. Allosteric modulators, especially those that exert no agonist activity in isolation, may be preferable to regular agonists for therapeutic use because such compounds have the potential to tune receptor activation more subtly and with fewer adverse side effects. Researchers are currently seeking to develop positive allosteric modulators for both muscarinic (Bubser et al., 2012) and nicotinic (Uteshev, 2016) receptors for use as cognitive enhancers in neuropsychiatric disorders.



Effects of optogenetic stimulation or inhibition of BFCS cholinergic neurons on visual cue detection (A) Mice exposed to a short duration (25 ms) or long duration (500 ms) visual cue (light flash) reported whether they detected the cue by performing a nose-poke response. The gray bars show control performance that consisted of a low hit rate associated with the short cue and a high hit rate associated with the long cue. The blue bars show that optogenetic stimulation of the BFCS cholinergic neurons increased the hit rate to the short cue but had no effect on the hit rate to the long cue. (B) The gray bars show results from the same control conditions as in (A). The red bars show performance associated with optogenetic inhibition of the cholinergic neurons. In this case, inhibition of the cells diminished the hit rate to the long cue but had no effect on the hit rate to the short cue. (After Gritton et al., 2016.)

There are two acetylcholine receptor subtypes: nicotinic and muscarinic

Like dopamine and norepinephrine, ACh has many different kinds of receptors. The story can be simplified a little by recognizing that the various cholinergic receptors belong to one of two families: **nicotinic receptors** and **muscarinic receptors**. Nicotinic receptors were so named because they respond selectively to the agonist nicotine, an alkaloid found in the leaves of the tobacco plant.[2] The pharmacology of nicotine is discussed in Chapter 13. Muscarinic receptors are selectively stimulated by muscarine, another alkaloid, which was first isolated in 1869 from the fly agaric mushroom, *Amanita muscaria*. **TABLE 7.1** presents some of the drugs that affect the cholinergic system, including nicotinic and muscarinic receptor agonists and antagonists. A discussion of these receptors and the mechanisms of action of the drugs listed in the table are presented in the sections below.

NICOTINIC RECEPTORS Nicotinic receptors are highly concentrated on muscle cells at neuromuscular junctions, on ganglionic neurons of both the sympathetic and parasympathetic systems, and on some neurons within the brain. They are ionotropic receptors, which, you will recall from Chapter 3, means that they possess an ion channel as an integral part of the

[2]Alkaloids are nitrogen-containing compounds, usually bitter tasting, that are often found in plants.

TABLE 7.1 Drugs and Toxins That Affect the Cholinergic System

Drug	Action
Vesamicol	Depletes ACh by inhibiting vesicular uptake
Black widow spider venom	Stimulates ACh release
Botulinum toxin	Inhibits ACh release
Hemicholinium-3	Depletes ACh by inhibiting choline uptake by the nerve terminal
Physostigmine	Centrally acting reversible AChE inhibitor that increases ACh levels
Donepezil, rivastigmine, and galantamine	AChE inhibitors used in the treatment of Alzheimer's disease
Neostigmine and pyridostigmine	Peripherally acting reversible AChE inhibitors used in the treatment of myasthenia gravis
Sarin and soman	Irreversible AChE inhibitors used as nerve gas agents
Nicotine	Stimulates nicotinic receptors (agonist)
Succinylcholine	Nicotinic receptor agonist that causes depolarization block
Mecamylamine and D-tubocurarine	Block nicotinic receptors (antagonists)
Muscarine, pilocarpine, and arecoline	Stimulate muscarinic receptors (agonists)
Atropine and scopolamine	Block muscarinic receptors (antagonists)

receptor complex (**FIGURE 7.7**). When ACh binds to a nicotinic receptor, the channel opens very rapidly, and sodium (Na^+) and calcium (Ca^{2+}) ions enter the neuron or muscle cell. This flow of ions causes depolarization of the cell membrane, thereby increasing the excitability of the cell. If the responding cell is a neuron, its likelihood of firing is increased. If it is a muscle cell, it responds by contracting. In this manner, nicotinic receptors mediate fast excitatory responses in both the CNS and the PNS. Another important function of nicotinic receptors within the brain is to enhance the release of neurotransmitters from nerve terminals. In this case, the nicotinic receptors are located presynaptically, right on the terminals. Thus, activation of nicotinic receptors by ACh can stimulate cell firing if the receptors are located postsynaptically on dendrites or cell bodies, or the receptors can stimulate neurotransmitter release without affecting the firing rate of the cell if they are located presynaptically on nerve endings.

The structure of the nicotinic receptor has been known for many years. Each receptor is a complex that contains five **subunits**, which are proteins that are assembled and then inserted into the cell membrane. These subunits are identified by the Greek letters α, β, γ, δ, and ε. However, their complexity is increased by the existence of 10 different kinds of α subunits (designated α1 through α10) and 4 different kinds of β subunits (designated β1 through β4). Moreover, the subunit composition of the nicotinic receptors found in muscle cells differs from what is found in neurons. Specifically, each muscle nicotinic receptor contains two α1 subunits, one β1, one γ, and either a δ or an ε subunit

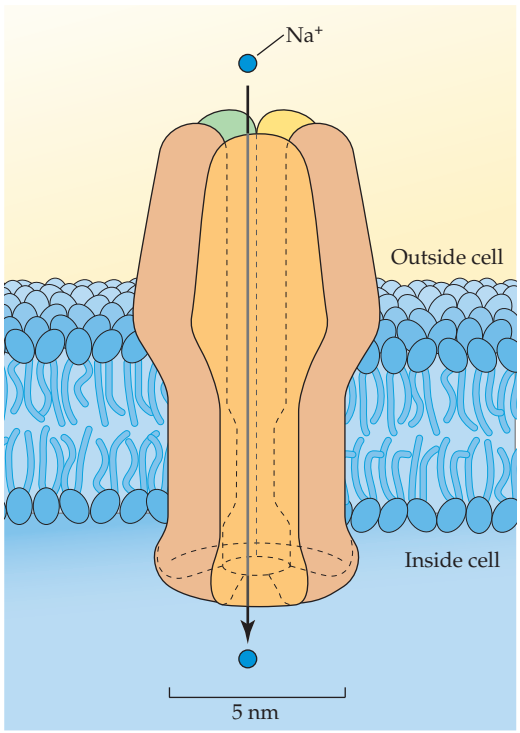


FIGURE 7.7 Structure of the nicotinic ACh receptor
The receptor comprises five protein subunits that are arranged to form a central pore or channel that allows Na^+ (and to some extent Ca^{2+}) ions to flow into the cell when the receptor is activated.

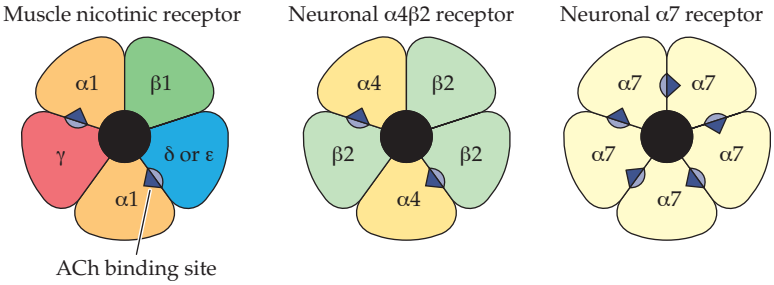


FIGURE 7.8 Three common types of nicotinic ACh receptors The muscle nicotinic receptor shown on the left is designated as $(\alpha1)_2(\beta1)(\gamma)(\delta/\varepsilon)$, which means that each receptor complex contains two α1 subunits, one β1 subunit, one γ subunit, and one δ or ε subunit. The γ, δ, and ε subunits each have only one form, which is why no numbering is needed in those cases. Nicotinic receptors in neurons are more complex because of the variety of α and β subunit combinations that have been detected in these cells. For simplicity, we are depicting here just two important kinds of neuronal nicotinic receptors found in the brain: $(\alpha4)_2(\beta2)_3$ and $(\alpha7)_5$. Note that the muscle and the $(\alpha4)_2(\beta2)_3$ receptors have two ACh binding sites, whereas the $(\alpha7)_5$ receptor has five ACh binding sites. Whether two or five such sites are present, all must be occupied by ACh or another agonist such as nicotine for the receptor channel to open.

(**FIGURE 7.8**). Two ACh binding sites are present, and both must be occupied for the receptor channel to open. In contrast, neuronal nicotinic receptors have no γ, δ, or ε subunits but instead come in three main forms with the following subunits: (1) two α and three β subunits [designated $(\alpha)_2(\beta)_3$], (2) three α and two β subunits [designated $(\alpha)_3(\beta)_2$], or (3) five α subunits [designated $(\alpha)_5$]. Figure 7.8 illustrates two particularly important types of neuronal nicotinic receptors—$(\alpha4)_2(\beta2)_3$ and $(\alpha7)_5$—both of which have been implicated in the cognitive effects of ACh and are current targets of medications under development (see Box 7.2). Receptors with other subunit compositions have also been identified, and their distribution varies with brain area (Zoli et al., 2015; **FIGURE 7.9**). To understand the nicotinic receptor numbering system, be aware that the number *within* the parentheses shown after the Greek letter represents which member of a family of subunits (e.g., 1–10 in the case of the α subunit) is present, whereas the subscript number shown just *after* the parentheses represents how many of that subunit (e.g., two, three, or five αs) help make up the receptor complex.

Ionotropic receptors, indeed all ion channels, can exist in three different states: **closed**, **open**, and **desensitized**. In the "closed" state of an ionotropic receptor, the neurotransmitter (in this case ACh) is not bound to the receptor and the channel pore is closed. When a "closed" nicotinic receptor is activated by the binding of an agonist such as ACh or nicotine, the receptor converts to the "open" state in which the pore opens and ions can flow across the membrane. The third state, "desensitized," is unlike either of the other two states in that the pore is closed and *cannot* be opened while in the desensitized state. Nicotinic receptors and other ionotropic receptors can spontaneously convert from one state to the other, depending, in part, on agonist association or dissociation from the ligand binding site (**FIGURE 7.10**). But it is important to note that *prolonged* exposure to an agonist enhances the rate of conversion to the desensitized state. Only after a desensitized nicotinic receptor spontaneously undergoes **resensitization** is it capable of responding to an agonist. In Chapter 13 we will discuss the relevance of this phenomenon for nicotine addiction. Even if cells are continuously exposed to nicotinic stimulation, not all of the receptors are desensitized. Those that remain active produce a persistent depolarization of the cell membrane. If this continues for very long, a process called **depolarization block** occurs, in which the resting potential of the membrane is lost, and the cell cannot be excited until the agonist is removed and the membrane repolarized. A chemical relative of ACh called **succinylcholine**, or alternatively **suxamethonium**, is a powerful muscle relaxant that is used medically to induce brief paralysis for insertion

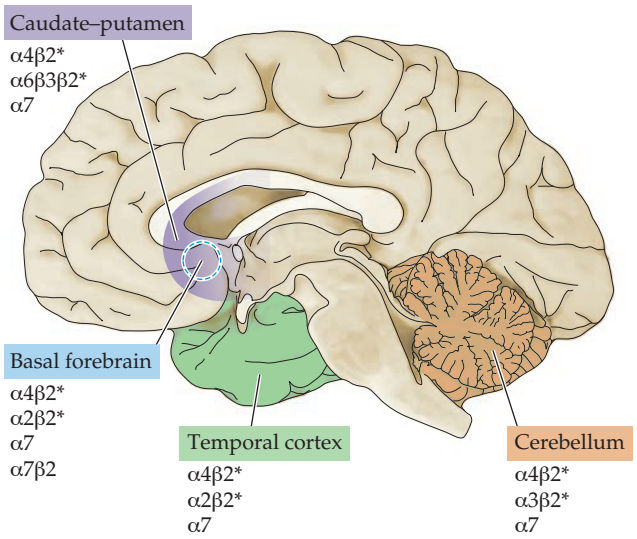


FIGURE 7.9 Composition of nicotinic receptor subtypes identified in selected areas of the human brain Multiple receptor subtypes are found in each of the brain regions shown in the figure. All of the receptors shown with an asterisk (*) contain two α and two β subunits [e.g., $(\alpha4)_2(\beta2)_2$ in the caudate–putamen] that form the two agonist binding sites. The fifth subunit is either an α or a β that does not participate in agonist binding. The α7β2 receptor shown in the basal forebrain is a rare subtype that is thought to be expressed only in this brain area. (After Zoli et al., 2015.)

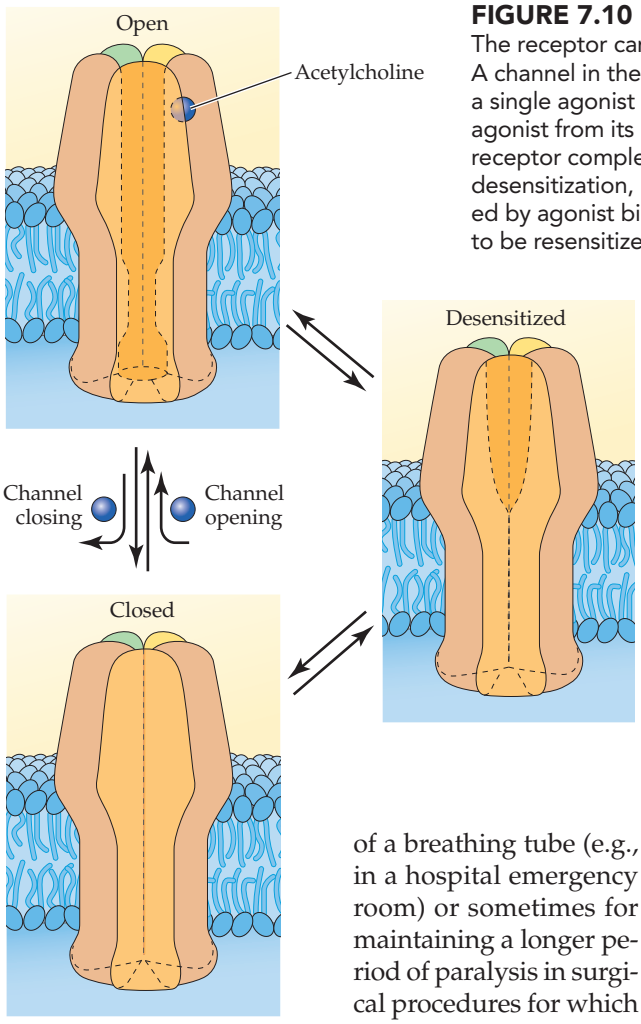


FIGURE 7.10 Functional states of the nicotinic ACh receptor
The receptor can exist in three different states: open, closed, and desensitized. A channel in the closed state can be opened by agonist binding (note that only a single agonist binding site is shown for the sake of simplicity). Dissociation of agonist from its binding site can return the receptor to the closed state. However, receptor complexes can also move between open or closed states to a state of desensitization, in which the receptor neither permits ion flow nor can be activated by agonist binding. The only way a desensitized receptor can be activated is to be resensitized into either a closed or open state. (After Wu et al., 2015.)

of a breathing tube (e.g., in a hospital emergency room) or sometimes for maintaining a longer period of paralysis in surgical procedures for which anesthesia alone may not provide sufficient relaxation. Unlike ACh, succinylcholine is resistant to breakdown by AChE; thus it continuously stimulates the nicotinic receptors and induces a depolarization block of the muscle cells. It is important to note that one of the paralyzed muscles is the diaphragm, which is the large muscle responsible for inflating and deflating the lungs. Consequently, the patient must be maintained on a ventilator until the effect wears off as succinylcholine is metabolized by nonspecific cholinesterases present in the blood.

Mecamylamine (**Inversine**) is an antagonist at neuronal nicotinic receptors that blocks these receptors both centrally and in autonomic ganglia. The ganglionic blocking effect of mecamylamine led to its development many years ago as an antihypertensive agent, although in modern medicine it has been largely supplanted by other medications with fewer side effects. A structurally different compound, **D-tubocurarine**, is a well-known antagonist of muscle nicotinic receptors. This substance is the main active ingredient of curare, the paralytic agent discussed in the chapter opener. D-Tubocurarine has relatively little effect on central cholinergic transmission, not only because of its selectivity for muscle nicotinic receptors, but also because it exhibits low penetrance across the blood–brain barrier.

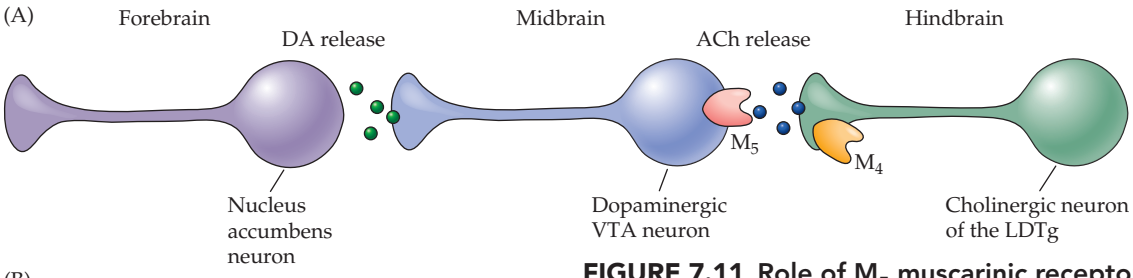
MUSCARINIC RECEPTORS As mentioned earlier, muscarinic receptors represent the other family of ACh receptors. Like the receptors for dopamine and norepinephrine, muscarinic receptors are all metabotropic. Five different types of muscarinic receptors (designated M_1 through M_5) have been identified, each with specific pharmacological characteristics and coded for by a different gene. Muscarinic receptors operate through several different second-messenger systems. Some activate the phosphoinositide second-messenger system, while others inhibit the formation of cyclic adenosine monophosphate (cAMP). Another important mechanism of muscarinic receptor action is the stimulation of potassium (K^+) channel opening. As mentioned in previous chapters, this leads to hyperpolarization of the cell membrane and a reduction in cell firing.

Muscarinic receptors are widely distributed in the brain. Some areas containing high levels of muscarinic receptors are the neocortex, hippocampus, thalamus, striatum, and basal forebrain. Receptors in the neocortex and hippocampus play an important role in the cognitive effects of ACh described earlier in this chapter, whereas those in the striatum are involved in motor function. Researchers have investigated the functional roles of these receptors not only by using selective agonists and antagonists but also by generating knockout mice for each receptor. Results of these genetic studies are helping pharmacologists design medications targeting a variety of neuropsychiatric and physiological disorders (Kruse et al., 2014). Among the interesting findings are those pertaining to the M_5 muscarinic receptor, which is expressed in the brain primarily in the hippocampus, hypothalamus, and midbrain DA areas (i.e., the substantia nigra pars compacta and the ventral tegmental area, or VTA). Indeed, M_5 is the only muscarinic receptor subtype expressed in those midbrain dopaminergic neurons. We mentioned earlier that cholinergic neurons in the LDTg exert an excitatory effect on VTA dopaminergic neurons mediated by nicotinic receptors on the postsynaptic cells. As shown in **FIGURE 7.11A**, M_5 receptors on the DA cells also contribute significantly to this excitatory effect (Foster et al., 2014). Interestingly, these receptors

appear to be involved in the rewarding and dependence-producing effects of abused drugs that are studied using animal models. As reviewed by Wess and colleagues (2007), M_5 receptor knockout mice exhibit deficits in both morphine and cocaine reward in studies using place conditioning and/or drug self-administration procedures. **FIGURE 7.11B** shows that in the place conditioning paradigm, morphine doses that produced a robust place preference in normal animals had no effect on knockout mice. Loss of M_5 receptor function also reduced withdrawal symptoms in mice that were made dependent on morphine, but it had no effect on morphine-induced analgesia (Basile et al., 2002). Although transgenic manipulation eliminated M_5 muscarinic receptors throughout the entire brain, it is likely that receptors located in the VTA play a key role in this modulation of drug reward and dependence through the known involvement of DA in these processes (Steidl et al., 2011; see Chapter 9 for more information).

Outside of the brain, muscarinic receptors are found at high densities in the cardiac muscle of the heart, in the smooth muscle associated with many organs (e.g., bronchioles, stomach, intestines, bladder, urogenital organs), and in secretory glands, including salivary glands, sweat glands, and the β-cells of the pancreas. These peripheral muscarinic receptors are activated by ACh released from postganglionic fibers of the parasympathetic nervous system. Stimulation of the parasympathetic system has two effects on the heart: a slowing of heart rate and a decrease in the strength of contraction, both of which are mediated by M_2 muscarinic receptors in cardiac muscle. In contrast, smooth muscle cells and secretory glands express M_3 receptors and are typically excited by receptor activation, thus causing muscle contraction and increased secretory activity. Unfortunately, many of the drugs used to treat depression, schizophrenia, and other major psychiatric disorders produce serious side effects because of their blockade of peripheral muscarinic receptors, especially the receptors associated with secretory glands. Consequently, patients taking such medications often complain about the so-called **dry mouth effect** (technically referred to as xerostomia), which reflects the reduced production of saliva that results from muscarinic receptor blockade. For some, the dry mouth effect is severe enough to cause the patient to stop taking his or her medication. If the medication is continued, the chronic lack of salivation can lead to mouth sores, increased tooth decay, and difficulty in chewing and swallowing food. Later in the book, we will see that pharmaceutical companies have worked to develop newer medications that react less with muscarinic receptors and therefore do not produce the dry mouth effect.

Researchers have recently focused on the role of pancreatic muscarinic receptors in regulating glucose and insulin homeostasis. The β-cells of the pancreas secrete insulin in response to carbohydrate consumption. The resulting increase in circulating insulin facilitates the uptake of glucose into cells throughout most of the body, thereby helping (in conjunction with other hormones such as glucagon) to maintain blood glucose levels within a healthy range. How are muscarinic receptors involved in this homeostatic process? Consumption of food triggers activation of the parasympathetic



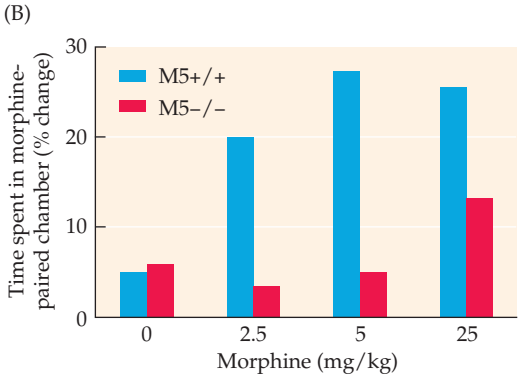


FIGURE 7.11 Role of M_5 muscarinic receptors in midbrain dopaminergic cell activity and in morphine reward
(A) ACh released by LDTg cholinergic neurons acts on M_5 muscarinic receptors expressed by VTA DA neurons to stimulate these cells and enhance dopamine release in the nucleus accumbens. M_4 receptors shown on the terminals of the cholinergic neurons are thought to be autoreceptors inhibiting ACh release from the terminals. (B) M_5 receptors likewise are implicated in the rewarding effects of morphine, as shown by a place conditioning study comparing genetically normal mice (also known as wild type; shown here as $M_5^{+/+}$) with M_5 knockout mice (mutant mice lacking the M_5 receptor; shown here as $M_5^{-/-}$). Morphine reward in the wild-type animals was demonstrated by increased time spent in the drug-paired chamber compared with controls given saline injections (0 mg/kg dose group). In the knockout animals, no rewarding effect of morphine was noted, except for a partial effect at the highest drug dose. (B after Basile et al., 2002.)

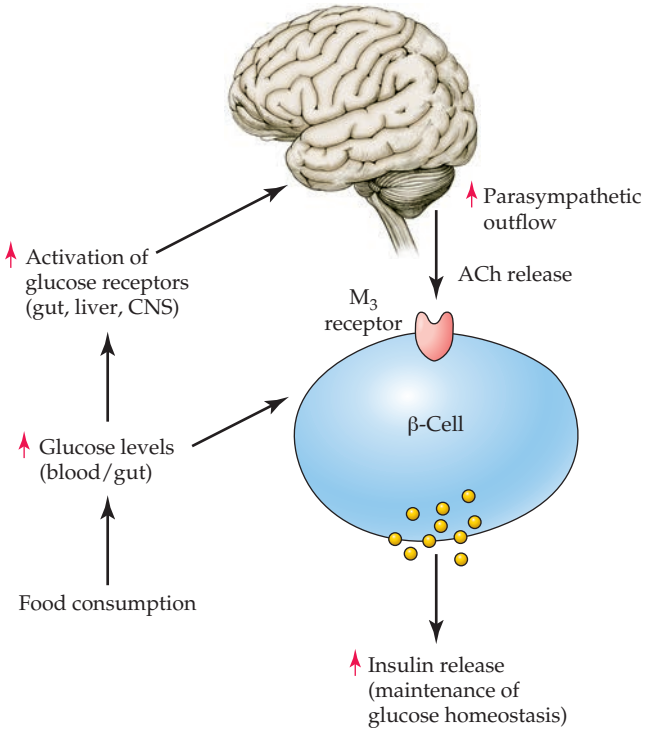


FIGURE 7.12 M_3 muscarinic receptors regulate insulin release by pancreatic β-cells Elevated levels of glucose caused by food consumption stimulate glucoreceptors in the gut, liver, and CNS. This stimulation leads to enhanced release of ACh by the vagus nerve of the parasympathetic system and activation of M_3 receptors on the β-cells. Insulin produced by these cells assists in uptake and utilization of glucose by peripheral tissues such as the liver and skeletal muscles. (After Ruiz de Azua et al., 2011.)

nervous system, ultimately leading to ACh release onto β-cell M_3 receptors. The resulting activation of these receptors stimulates the secretion of insulin by the β-cells (Nakajima et al., 2013) (**FIGURE 7.12**). Obesity in adulthood can give rise to a form of **type 2 diabetes** in which the ability of insulin to promote glucose uptake becomes compromised. This condition, which is called insulin resistance, leads to chronically elevated levels of both insulin (hyperinsulinemia) and glucose (hyperglycemia) in the bloodstream. Several antipsychotic drugs (i.e., drugs used to treat schizophrenia and bipolar disorder), particularly clozapine and olanzapine, have the major side effect of weight gain and elevated risk of developing insulin resistance when given chronically (Weston-Green et al., 2013). Despite the fact that antipsychotic drugs were not designed to target muscarinic receptors, the compounds that exert these side effects all possess potent M_3 receptor–blocking activity. It is not clear why *blocking* these receptors, which *inhibits* insulin secretion when the drugs are given acutely, has the opposite effect when the drugs are administered chronically; however, one contributing factor may be a drug-induced compensatory up-regulation of receptor expression (Miranda et al., 2014). In any case, information about the muscarinic control of β-cell insulin secretion has led not only to increasing study of the mechanisms underlying this dangerous side effect of some antipsychotic drugs, but also to an interest in possibly targeting the muscarinic cholinergic system for the treatment of a different kind of type 2 diabetes characterized by insulin insufficiency instead of insulin resistance (Ruiz de Azua et al., 2011).

Several muscarinic receptor agonists occur in nature, including muscarine, from *Amanita muscaria*; **pilocarpine**, from the leaves of the South American shrub *Pilocarpus jaborandi*; and **arecoline**, which is found in the seeds of the betel nut palm *Areca catechu*. These substances are sometimes referred to as **parasympathomimetic agents** because their ingestion mimics many of the effects of parasympathetic activation. Thus, poisoning due to accidental ingestion of *Amanita* or the two plants mentioned leads to exaggerated parasympathetic responses, including lacrimation (tearing), salivation, sweating, pinpoint pupils related to constriction of the iris, severe abdominal pain, strong contractions of the smooth muscles of the viscera, and painful diarrhea. High doses can even cause cardiovascular collapse, convulsions, coma, and death.

Given the autonomic effects of muscarinic agonists, it is understandable that antagonists of these receptors would inhibit the actions of the parasympathetic system. Such compounds, therefore, are called **parasympatholytic agents**. The major naturally occurring muscarinic antagonists are **atropine** (also sometimes called hyoscyamine) and the closely related drug **scopolamine** (hyoscine). These alkaloids are found in a group of plants that includes henbane (*Hyoscyamus niger*) and deadly nightshade (*Atropa belladonna*) (**FIGURE 7.13**). Extracts of these plants are toxic when taken systemically—a fact that was exploited during the Middle Ages, when deadly nightshade was used as a lethal agent to settle many political and family intrigues. On the other hand, a cosmetic use of the plant also evolved, in which women instilled the juice of the berries into their eyes to cause pupillary dilation by blocking the muscarinic receptors

FIGURE 7.13 Deadly nightshade *Atropa belladonna* is a natural source of the muscarinic antagonist atropine. (Bob Gibbons/Alamy Stock Photo.)

on the constrictor muscles of the iris. The effect was considered to make the user more attractive to men. Indeed, the name *Atropa belladonna* reflects these two facets of the plant, since *bella donna* means "beautiful woman" in Italian, whereas Atropos was a character in Greek mythology whose duty it was to cut the thread of life at the appropriate time.

Muscarinic antagonists have several current medical applications. Modern ophthalmologists use atropine just as women of the Middle Ages did, except in this case they are dilating the patient's pupils to obtain a better view of the interior of the eye. Another use is in human and veterinary surgery, where the drug reduces secretions that could clog the patient's airways. Atropine is also occasionally needed to counteract the effects of poisoning with a cholinergic agonist. Scopolamine in therapeutic doses produces drowsiness, euphoria, amnesia, fatigue, and dreamless sleep. As mentioned in Box 7.2, this drug was historically used along with narcotics as a preanesthetic medication before surgery, or alone prior to childbirth to produce "twilight sleep," a condition characterized by drowsiness and amnesia for events that occur during the duration of drug use.

Despite their therapeutic uses, muscarinic antagonists can themselves be toxic when taken systemically at high doses. CNS effects of atropine poisoning include restlessness, irritability, disorientation, hallucinations, and delirium. Even higher doses can lead to CNS depression, coma, and eventually death by respiratory paralysis. As in the case of nicotinic drugs, these toxic effects point to the delicate balance of cholinergic activity in both the CNS and the PNS that is necessary for normal physiological functioning.

Section Summary

- Acetylcholine is an important neurotransmitter in the PNS, where it is released by motor neurons innervating skeletal muscles, by preganglionic neurons of both the parasympathetic and sympathetic branches of the autonomic nervous system, and by ganglionic parasympathetic neurons.
- In the brain, cholinergic neurons include a group of interneurons within the striatum, a diffuse system of projection neurons that constitute the basal forebrain cholinergic system (BFCS), and a group of brainstem neurons in the laterodorsal and pedunculopontine tegmental (LDTg and PPTg) nuclei.
- The BFCS plays an important role in cognitive functioning, whereas the LDTg and the PPTg exert multiple roles, including stimulation of midbrain dopamine neurons, behavioral arousal, sensory processing, and initiation of rapid-eye-movement sleep.
- Cholinergic receptors are divided into two major families: nicotinic and muscarinic receptors.
- Nicotinic receptors are ionotropic receptors comprising five subunits. When the receptor channel opens, it produces a fast excitatory response resulting from an influx of Na^+ and Ca^{2+} ions across the cell membrane. In the brain, these receptors are located both postsynaptically, where they stimulate cell firing, and presynaptically, where they directly enhance neurotransmitter release from nerve terminals.
- Nicotinic receptors in neurons and muscles possess somewhat different subunits, and this leads to significant pharmacological differences between the two types of receptors. Some of the cognitive functions of ACh have been ascribed to activation of $(\alpha4)_2(\beta2)_3$ or $(\alpha7)_5$ nicotinic receptors.
- Ionotropic receptors, including nicotinic receptors, can exist in a closed, open, or desensitized state. Prolonged exposure to a nicotinic receptor agonist such as nicotine itself promotes conversion of the receptor to a desensitized state in which the channel will not open despite the presence of the agonist. In such a case, the receptor must be resensitized before it can be activated again. Nicotinic receptors can also cause a process of depolarization block involving temporary loss of the cell's resting potential and an inability of the cell to generate action potentials. This is the basis for certain muscle relaxants used in medicine.
- There are five kinds of muscarinic receptors, designated M_1 through M_5, all of which are metabotropic receptors. Muscarinic receptors function through several different signaling mechanisms, including activation of the phosphoinositide second-messenger system, inhibition of cAMP synthesis, and stimulation of K^+ channel opening.
- Muscarinic receptors are widely distributed in the brain, with particularly high densities in various forebrain structures. M_5 receptors in the VTA are believed to modulate processes of drug reward and dependence.
- Outside of the brain, muscarinic receptors are found in targets of the parasympathetic system, including the heart, secretory glands, and smooth muscle found in many internal organs. Consequently, general muscarinic agonists are called parasympathomimetic agents, whereas antagonists are considered parasympatholytic in their actions.
- M_2 receptors mediate the effects of the parasympathetic system on the heart, whereas M_3 receptors stimulate secretory responses of the sweat and salivary glands and also the insulin-secreting β-cells of the pancreas. Blockade of muscarinic receptors in the salivary glands leads to the dry mouth effect, which is a serious side effect of many drugs used to treat various psychiatric disorders.

STUDY QUESTIONS

1. What are the chemical reactions involved in ACh synthesis and breakdown? Include the names of the enzymes catalyzing each reaction. Which of these enzymes is used as a biochemical marker for cholinergic neurons?
2. Briefly discuss the factors that regulate the rate of ACh synthesis. Are there any current therapeutic interventions targeting the process of ACh synthesis?
3. By what mechanism is ACh taken up and stored in synaptic vesicles? Name a drug that interferes with vesicular ACh uptake, and describe the effects of this drug on cholinergic transmission.
4. List and discuss the effects of toxins that either stimulate or inhibit ACh release. Include in your answer the therapeutic applications of toxins that inhibit the release of this neurotransmitter.
5. Unlike the monoamine transmitters discussed in previous chapters, there is no reuptake system for ACh itself. Describe the alternative mechanism that has evolved to help cholinergic neurons recycle/reutilize the transmitter. How do we know that this recycling process plays an important role in normal functioning of the nervous system?
6. List and discuss the various drugs that function as AChE inhibitors. In general, what is the effect of these drugs on cholinergic transmission? Include in your answer a consideration of the different consequences of peripheral versus central AChE inhibition and also the different consequences of reversible versus irreversible inhibition of the enzyme.
7. Describe the localization and functions of ACh in the peripheral nervous system.
8. Name and indicate the location of the principle cholinergic cell groups in the brain. Discuss the role of the BFCS in cognitive function, including relevant experimental findings.
9. Describe the molecular structure and signaling mechanism of nicotinic cholinergic receptors.
10. A nicotinic receptor complex can be in one of three different states: open, closed, and desensitized. Describe the properties of each state, including whether agonist is bound or not as well as the state of the receptor channel.
11. The text discusses three different drugs that act on nicotinic receptors: succinylcholine, mecamylamine, and D-tubocurarine. For each compound, indicate whether it is an agonist or an antagonist, and briefly discuss the effects of administering the drug (including any current medical use).
12. Discuss the molecular structure, signaling mechanisms, and localization of muscarinic cholinergic receptors in the brain.
13. Discuss the role of M_5 muscarinic receptors in the control of dopaminergic cell firing and the rewarding and reinforcing effects of abused drugs. Include relevant experimental findings in your answer.
14. Describe the locations and functional roles of muscarinic receptors expressed by peripheral organs and glands. Include in your answer a discussion of the so-called dry mouth effect.
15. Discuss the role of pancreatic M_3 muscarinic receptors in the control of insulin secretion and the involvement of this receptor subtype in the development of insulin resistance that accompanies the administration of certain antipsychotic drugs.
16. Discuss the underlying rationale for the idea of using nicotinic or muscarinic allosteric modulators in the treatment of Alzheimer's disease or other neuropsychiatric disorders involving deficits in cognitive functioning.
17. What is meant by the terms *parasympathomimetic* and *parasympatholytic agents*? List examples of drugs belonging to these categories along with their physiological effects and medicinal or other uses.

Go to the *Psychopharmacology* Companion Website at oup-arc.com/access/meyer-3e for animations, web boxes, flashcards, and other study aids.

CHAPTER 8

Some day we may be able to purchase "smart pills" that will enable us to generate "pearls of wisdom" much more easily than we do now. (© Kush Fine Arts Limited 2017.)

Glutamate and GABA

THE IDEA OF A "SMART PILL" THAT WOULD ENHANCE YOUR INTELLIGENCE without requiring effort on your part is appealing to many. Indeed, an informal survey conducted by Marilyn vos Savant (author of the "Ask Marilyn" column in the popular Sunday newspaper magazine *Parade*) found that if given a choice, a large majority of respondents would prefer raising their intelligence to improving their physical appearance. Pills that turn ordinary people into astounding geniuses have been a topic of science fiction novels and movies, one example being the 2011 movie *Limitless*, which spawned a 2015–2016 TV series of the same name that featured an average guy who could temporarily achieve super intelligence by consuming a pill called NZT.

No genius pills are yet in sight, despite claims by internet purveyors of the miraculous effects of substances they are selling. Nevertheless, researchers have been hard at work to find drugs that improve cognitive function. Most of this effort is directed toward ameliorating the cognitive deficits associated with Alzheimer's disease, schizophrenia, and various other neuropsychiatric disorders. However, research has shown that cognition can be enhanced above baseline in healthy individuals, a prospect that raises not only practical but also ethical and health issues (Lynch et al., 2014; Urban and Gao, 2014; Frati et al., 2015; Whetstine, 2015). Some cognitive-enhancing compounds, which are called **nootropics**,[1] act on the cholinergic system (see Chapters 7 and 13). Others, such as amphetamine and methylphenidate, work primarily through catecholaminergic activation (Chapters 5 and 12). Still others influence the amino acid neurotransmitter glutamate, which is the subject of the first part of the present chapter. You will learn about the basic neurochemistry of glutamate, its role in learning and memory, and the development of novel glutamate-related drugs that show promise in treating cognitive impairment. The second part of the chapter covers **γ-aminobutyric acid** (**GABA**), another important amino acid neurotransmitter that has a very different role in brain and behavioral functioning. ■

[1]The term *nootropic* comes from two Greek words: *noos*, which means "mind," and *tropein*, which means "toward."

Glutamate

Glutamate is the term we use for the ionized (i.e., electrically charged) form of the amino acid glutamic acid. Since most of the glutamic acid in our bodies is in this ionized state, we will refer to it as glutamate throughout the text. Like other common amino acids, glutamate is used by all of our cells to help make new proteins. But glutamate also has numerous other biochemical functions (e.g., in energy metabolism); this is reflected in the fact that it is the most abundant amino acid in the brain. Glutamate and **aspartate** (the name for the ionized form of aspartic acid) are the two principal members of a small family of **excitatory amino acid neurotransmitters**. These transmitters are so named because they cause a powerful excitatory response when applied to most neurons in the brain or spinal cord. We will focus on glutamate, which is the principal excitatory transmitter in the brain.

Glutamate Synthesis, Release, and Inactivation

When a nerve cell synthesizes a molecule of dopamine (DA), acetylcholine (ACh), or serotonin (5-HT), it is almost always done for the purpose of neurotransmission. Moreover, in the brain, these substances are localized specifically within the cells that use them as transmitters. However, we must recognize that the situation is different for glutamate because of its roles in protein synthesis and general cellular metabolism. First, all neurons and glial cells contain significant amounts of glutamate, although neurons that use glutamate as a transmitter (called **glutamatergic neurons**) possess even greater concentrations than other cells in the brain. Second, glutamatergic neurons are thought to segregate the pool of glutamate that they use for transmission from the pool of glutamate used for other cellular functions. These facts complicate both our ability to determine which nerve cells actually are glutamatergic and our understanding of how these cells synthesize, release, and dispose of the transmitter-related glutamate. Nevertheless, researchers have accumulated considerable information, which we summarize in this section.

Neurons generate glutamate from the precursor glutamine

Glutamate can be synthesized by several different chemical reactions. Most molecules of glutamate are derived ultimately from the normal metabolic breakdown of the sugar glucose. The more immediate precursor for much of the transmitter-related glutamate is a related substance known as **glutamine**. Neurons can transform glutamine into glutamate using an enzyme called **glutaminase** (**FIGURE 8.1**). We will see in the next section that the role of glutamine in glutamate synthesis involves a fascinating metabolic partnership between glutamatergic neurons and neighboring glial cells, specifically astrocytes.

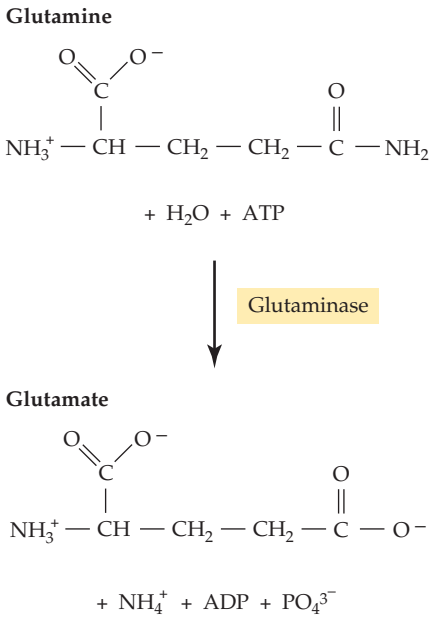


FIGURE 8.1 Glutamate is synthesized from glutamine This reaction, catalyzed by the enzyme glutaminase, requires energy provided by the breakdown of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and phosphate (PO_4^{3-}).

Glutamate packaging into vesicles and uptake after release are mediated by multiple transport systems

GLUTAMATE PACKAGING AND RELEASE Like the other transmitters already discussed, glutamate is packaged in and released from synaptic vesicles. It is technically difficult to measure exactly how many glutamate molecules are present in a single vesicle, but estimates range from 2000 to 9000 (Marx et al., 2015). Researchers assumed that a system must exist to transport glutamate from the cell cytoplasm into the vesicles, but this system eluded discovery for a long time. Then between the years 2000 and 2002, researchers discovered *three* distinct proteins that package glutamate into vesicles: **VGLUT1**, **VGLUT2**, and **VGLUT3** (**VGLUT** standing for **vesicular glutamate transporter**). These proteins serve as good markers for glutamatergic neurons because unlike glutamate itself, they are found only in cells that use glutamate as a neurotransmitter. Most glutamatergic neurons possess either VGLUT1 or VGLUT2 (but usually not both), with VGLUT3 being much less abundant than the other two transporters. *VGLUT1* gene expression occurs primarily in the cortex

and hippocampus, whereas *VGLUT2* gene expression is found mostly in subcortical structures (El Mestikawy et al., 2011; Vigneault et al., 2015). VGLUT2 knockout mice die immediately after birth, signifying the critical importance of glutamate signaling by VGLUT2-expressing neurons for life-sustaining functions (Wallén-Mackenzie et al., 2010). In contrast, VGLUT1 knockout mice survive birth but eventually begin to die during the third week of life, whereas mice lacking VGLUT3 are viable but are completely deaf (Seal et al., 2008). The reason for this unusual defect is that the inner hair cells of the cochlea use glutamate as their neurotransmitter, and the only vesicular transporter they express is VGLUT3. Consequently, when the inner hair cells from VGLUT3 knockout mice are stimulated by sound waves striking the eardrum, they have no glutamate in their vesicles to transmit the stimulus to the auditory nerve.

Remarkably, VGLUT3 and to a lesser extent VGLUT1 and VGLUT2 mRNA and protein are sometimes co-expressed with markers of other classical neurotransmitters, implying that in neurons with such co-expression, glutamate is also stored and released as a co-transmitter. After the discovery of numerous examples of vesicular transporter co-expression, investigators began to ask important questions such as whether a glutamate and another transporter (e.g., VMAT2) are present on the same vesicles in the nerve terminal, or whether neurons exhibiting this kind of transporter co-expression segregate their vesicles into two different types (i.e., one type of vesicle that takes up and releases glutamate and a second type of vesicle that takes up and releases the other transmitter synthesized by that neuron). Evidence suggests that both types of situations can exist (El Mestikawy et al., 2011). Moreover, the possibility exists that some neurons not only segregate their vesicle populations but also segregate their axon terminals so that the two different neurotransmitters are released at separate kinds of terminals. The DA–glutamate co-expressing neurons of the ventral tegmental area may represent an example of this phenomenon. These cells are believed to release DA alone from varicosities that do not have a classical synaptic morphology and to release glutamate alone from terminals that make classical synaptic contacts with postsynaptic elements (Trudeau and Gutiérrez, 2007; Trudeau et al., 2014). Additional research is needed to identify the mechanism involved in vesicle segregation when it occurs and, more importantly, to determine the functional significance of glutamate as a co-transmitter with other classical transmitters such as DA, 5-HT, ACh, GABA, and glycine.

Considerable evidence has accumulated for another novel aspect of glutamate transmission, namely, stimulated release of this transmitter from astrocytes (Araque et al., 2014; Sahlender et al., 2014). As in the case of neuronal transmitter release, astrocytic glutamate release is stimulated by a rise in intracellular Ca^{2+} concentrations. Some astrocytes express vesicular glutamate transporters, and high-resolution electron micrographs of astrocytes reveal the presence of small vesicle-like structures that could be the source of glutamate storage and release. However, there is evidence supporting an alternate hypothesis that glutamate is released from astrocytes through channels in the membrane. Moreover, astrocytes not only store and release glutamate, but they also take up glutamate from the extracellular space (see next section), express glutamate receptors, and respond to activation of those receptors (Martínez-Lozada and Ortega, 2015). Taken together, these findings indicate that **gliotransmission**, as it has been called, must now be taken into consideration in the study of brain function. Indeed, abnormalities in gliotransmission have now been implicated in the brain's response to injury and in several other brain disorders, including epilepsy and Huntington's disease (Harada et al., 2016).

GLUTAMATE UPTAKE AFTER RELEASE After glutamate molecules are released into the extracellular space, they are rapidly removed by glutamate transporters located on cell membranes. Always keep in mind that the plasma membrane transporters that remove neurotransmitters from the extracellular space, including the synaptic cleft, are distinct from the transporters on the vesicle membranes that are responsible for loading the vesicles in preparation for transmitter release. In the case of glutamate, five different plasma membrane transporters have been identified with different cellular localizations (Divito and Underhill, 2014; Robinson and Jackson, 2016). Because these transporters take up aspartate as well as glutamate, they are considered to represent a class of **excitatory amino acid transporters** (**EAATs**). There are five members of this class, designated **EAAT1** to **EAAT5**. Interestingly, astrocytes rather than neurons play the most important role in taking up glutamate after its release. This is particularly true for EAAT2, which is expressed by astrocytes throughout the brain. Some studies have estimated that EAAT2 accounts for about 90% of total glutamate uptake in the brain (Divito and Underhill, 2014). As we will see later, prolonged high levels of glutamate in the extracellular fluid are very dangerous, producing excessive neuronal excitation and even cell death. The importance of EAAT2 in glutamate clearance was shown by Tanaka and coworkers (1997), who found that knockout mice lacking EAAT2 (termed GLT-1 in their paper, which used different terminology) developed spontaneous epileptic seizures, were more susceptible than wild-type mice to experimentally induced seizures and brain

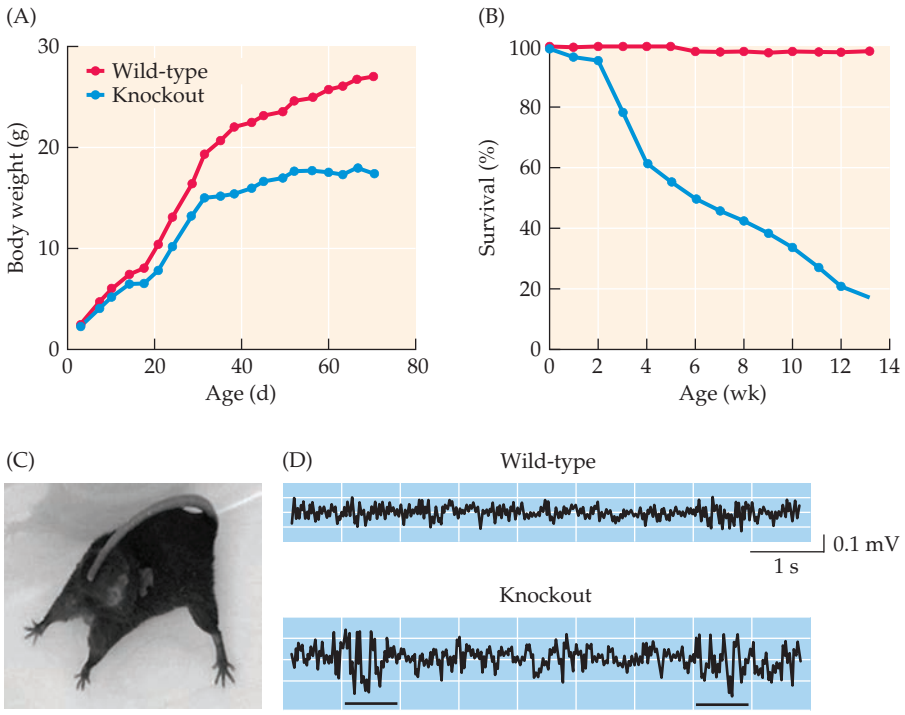


FIGURE 8.2 Phenotypic characteristics of mutant mice lacking EAAT2 (A) EAAT2 knockout mice exhibited a reduction in weight gain compared with genetically normal wild-type mice, particularly once they reached 30 days of age. (B) The knockout mice showed increased mortality beginning at 3 weeks of age. (C) and (D) Mice lacking EAAT2 had increased brain excitability that manifested as spontaneous behavioral seizures (note the abnormal posture in C, which is caused by epileptic activity in the brain) and seizure-like EEG changes (underlined traces in D) when given a subthreshold dose of the convulsant agent pentylenetetrazole. Note that at this drug dose, the wild-type mice showed no such EEG changes. (From Tanaka et al., 1997.)

injury, and had a greatly shortened life span (**FIGURE 8.2**). EAAT1 is another transporter found in astrocytes, with a particularly high expression in specialized astrocytes cells of the cerebellum where it plays an important role in cerebellar function. The major neuronal glutamate transporter is EAAT3, which is thought to have a *postsynaptic* rather than a *presynaptic* localization (Robinson and Jackson, 2016). Finally, EAAT4 is expressed primarily by Purkinje cells in the cerebellum, whereas EAAT5 is present in bipolar cells within the retina.

Besides playing a key role in removing excess glutamate from the extracellular space, astrocyte transporters are also intimately involved in the metabolic partnership between neurons and astrocytes. After astrocytes have taken up glutamate by means of EAAT1 or EAAT2, they convert a major portion of it to glutamine by means of an enzyme called **glutamine synthetase**. Glutamine is then transported out of the astrocytes and picked up by neurons, where it can be converted back into glutamate by glutaminase, as described earlier. This interplay between glutamatergic neurons and neighboring astrocytes is illustrated in **FIGURE 8.3**. We might wonder why such a complex system has evolved: why don't the neurons themselves have the primary responsibility for glutamate reuptake, as we have seen previously for the catecholamine neurotransmitters and for serotonin? Although we aren't certain about the answer to this question, it's worth noting that glutamine does not produce neuronal excitation and therefore is not potentially dangerous, as glutamate is. Hence, glial cell production of glutamine may be the brain's way of storing glutamate in a form that is "safe" but still available for use once the glutamine has been transferred to the neurons and reconverted to glutamate.

In addition to inactivation of glutamate, a second function of glutamine synthetase is to help in the metabolism and removal of ammonia that is either generated metabolically in the brain or taken up from the bloodstream (Cooper, 2012). The toxic effects of eliminating glutamine synthetase can be seen in mice with a knockout of the glutamine synthetase gene and in rare human mutations that result in greatly reduced activity of the enzyme. The knockout mice die before birth, and humans with congenitally reduced glutamine synthetase activity suffer from brain deformities, seizures, and a short life span (Rose et al., 2013).

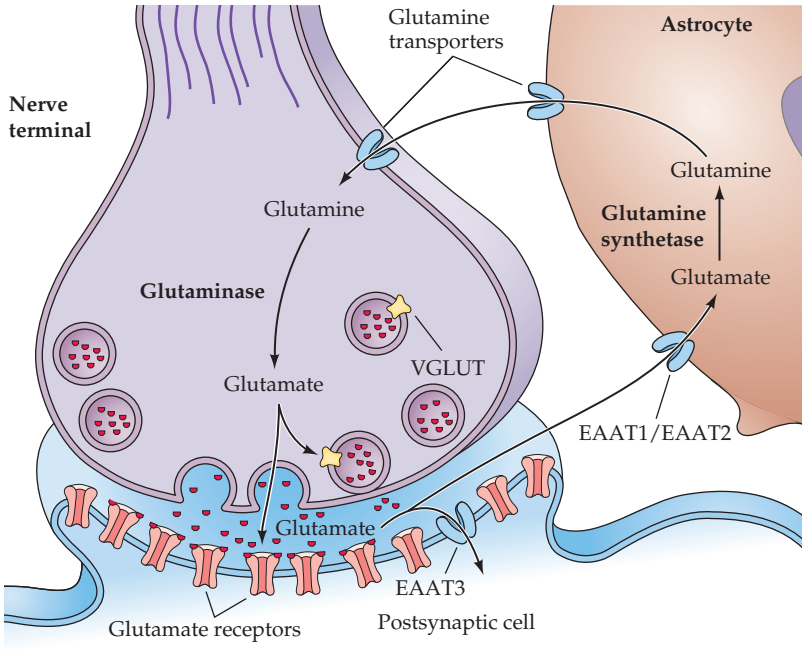


FIGURE 8.3 Cycling of glutamate and glutamine between glutamatergic neurons and astrocytes
After neurons release glutamate, it can be transported into nearby astrocytes by EAAT1 or EAAT2, or less commonly it can be transported into the postsynaptic cell by EAAT3. Inside the astrocyte, glutamate is converted into glutamine by the enzyme glutamine synthetase. The glutamine can be later released by the astrocytes, taken up by neurons, and converted back into glutamate by the enzyme glutaminase.

Section Summary

- Glutamate and aspartate are amino acid neurotransmitters that have potent excitatory effects on neurons throughout the brain and spinal cord. Although glutamate is contained within all cells because of its multiple biochemical functions, glutamatergic neurons are thought to possess higher glutamate concentrations than other cells and to segregate their neurotransmitter pool of this amino acid.
- Many of the glutamate molecules that are released synaptically are synthesized from glutamine in a chemical reaction catalyzed by the enzyme glutaminase.
- Glutamate is packaged into vesicles by the vesicular transporters VGLUT1, VGLUT2, and VGLUT3. In some neurons, these transporters are expressed with markers for other neurotransmitters such as 5-HT, DA, ACh, and GABA, indicating that glutamate is a co-transmitter in these cells. Some evidence suggests that the DA–glutamate co-expressing neurons of the ventral tegmental area release the two transmitters from separate vesicles at different kinds of axon terminals.
- After they are released, glutamate molecules are removed from the extracellular fluid by five different excitatory amino acid transporters, designated EAAT1 to EAAT5. Uptake of glutamate into astrocytes by EAAT2 is especially important for glutamate clearance, as can be seen by the fact that mutant mice lacking this transporter exhibit seizures, neuronal cell death, and a shortened life span. The major neuronal glutamate transporter is EAAT3, which is thought to be located postsynaptically rather than presynaptically.
- Glutamate taken up by astrocytes is converted to glutamine by the enzyme glutamine synthetase.
- This glutamine subsequently can be transported from the astrocytes to the glutamatergic neurons, where it is transformed back into glutamate by the enzyme glutaminase and then reutilized. This constitutes an important metabolic interplay between glutamatergic nerve cells and their neighboring glial cells.

Organization and Function of the Glutamatergic System

Glutamate is the workhorse transmitter for fast excitatory signaling in the nervous system. Not only is it used in many excitatory neuronal pathways, but the most important receptors for glutamate are ionotropic receptors that produce fast postsynaptic responses (see the next section). We will not discuss a large number of glutamatergic pathways here but will simply mention several that have been extensively studied. We will also consider a few important functions of the glutamatergic system in health and in disease.

Glutamate is the neurotransmitter used in many excitatory pathways in the brain

In the cerebral cortex, glutamate is the main neurotransmitter used by the pyramidal neurons. These cells,

which are named on the basis of their pyramid-like shape, are the major output neurons of the cortex. Their axons project to numerous subcortical structures, including the striatum, the thalamus, various limbic system structures, and regions of the brainstem. Glutamate is also used in the numerous parallel fibers of the cerebellar cortex and in several excitatory pathways within the hippocampus.

Because glutamate is found throughout the brain, it is more difficult to assign specific functional roles to this neurotransmitter than it is for some of the other transmitters covered previously. Glutamate is undoubtedly involved in many different behavioral and physiological functions under both normal and abnormal conditions. Among the most important are synaptic plasticity (i.e., changes in the strength of synaptic connections), learning and memory, cell death in some neurological disorders, and possibly also the development of various psychopathological disorders, including drug addiction and schizophrenia. Later in this chapter we will consider the role of glutamate in synaptic plasticity, learning and memory, and neuronal cell death. Current findings regarding the possible involvement of glutamate in drug addiction, schizophrenia, and other types of psychopathology will mainly be taken up in other chapters of this book (with the exception of a few examples presented in the section on metabotropic glutamate receptors).

Both ionotropic and metabotropic receptors mediate the synaptic effects of glutamate

Glutamate receptors are divided into two broad families: a group of ionotropic receptors for fast signaling and a group of slower metabotropic receptors that function by means of second-messenger systems. We will focus first on the ionotropic receptors, since they are most important for understanding the mechanisms of glutamate action in the brain. Note that glutamate receptors are also used by aspartate and possibly by other excitatory amino acid transmitters that may exist. Hence, these receptors are sometimes called excitatory amino acid receptors rather than simply glutamate receptors.

IONOTROPIC GLUTAMATE RECEPTORS Three subtypes of ionotropic glutamate receptors have been identified. Each is named for a relatively selective agonist for that receptor subtype. First is the **AMPA receptor**, which is named for the selective agonist AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid), a synthetic (not naturally occurring) amino acid analog. Most fast excitatory responses to glutamate are mediated by stimulation of AMPA receptors. The second ionotropic receptor subtype is the **kainate receptor**, which is named for the selective agonist kainic acid. Even though kainic acid powerfully stimulates kainate receptors in the mammalian brain, this substance actually comes from a type of seaweed called *Digenea simplex*. The third ionotropic glutamate receptor is the **NMDA receptor**, the agonist of which is obviously NMDA (*N*-methyl-D-aspartate). Like AMPA, NMDA is a synthetic amino acid. Thus, we see that pharmacologists have had to take advantage of several unusual compounds (either synthetic or plant derived) to distinguish between the different ionotropic receptor subtypes, since glutamate itself obviously activates all of these receptors.

Like the nicotinic receptors discussed in Chapter 7, ionotropic glutamate receptors depolarize the membrane of the postsynaptic cell, which leads to an excitatory response. For the AMPA and kainate receptors, this depolarizing effect is produced mainly by the flow of sodium (Na^+) ions into the cell through the receptor channel. In the case of NMDA receptors, the channel conducts not only Na^+ but also significant amounts of calcium (Ca^{2+}). Because Ca^{2+} can function as a second messenger within the postsynaptic cell (see Chapter 3), this is an interesting case in which an ionotropic receptor (the NMDA receptor) directly activates a second-messenger system (**FIGURE 8.4**).

Going back to the nicotinic receptor (see Figure 7.7), recall that the complete receptor contains five separate proteins (subunits) that come together to form the receptor channel. Ionotropic glutamate receptors are similarly formed from multiple subunits, but in this case, there are four subunits comprising each receptor complex. It is interesting to note that each receptor subtype (AMPA, kainate, and NMDA) is composed of a different set of subunits; this explains why the three subtypes differ in their pharmacology. Not only does

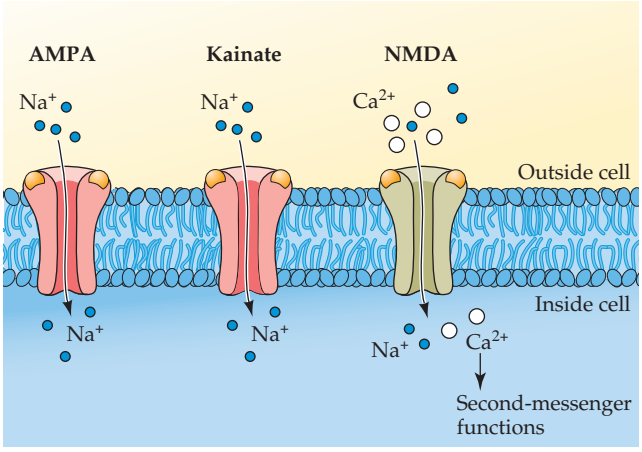


FIGURE 8.4 Ion permeabilities of ionotropic glutamate receptor channels All three ionotropic glutamate receptor channels conduct Na^+ ions into the cell. NMDA receptor channels additionally conduct Ca^{2+} ions, which can activate several important second-messenger functions.

each subtype have its own selective agonist, but various receptor antagonists have also been developed that have helped us understand the behavioral and physiological functions of these receptors.

One widely used antagonist called **NBQX** [6-nitro-7-sulfamoyl-benzo(f)quinoxaline-2,3-dione] can block both AMPA and kainate receptors, although it is somewhat more effective against the former subtype. This compound has no effect on NMDA receptors. Rats and mice treated with high doses of NBQX exhibit sedation, reduced locomotor activity and ataxia (impaired coordination in movement; an example in humans is staggering), poor performance in the rotarod task (another test of coordination), and protection against electrically or chemically induced seizures. These findings indicate a broad role for AMPA (and possibly also kainate) receptors in locomotor activity, coordination, and brain excitability (as shown by the seizure results).

NMDA receptors possess a number of characteristics not found in the other glutamate ionotropic receptors (**FIGURE 8.5**). First, as we've already mentioned, unlike AMPA and kainate receptor channels, the channels for NMDA receptors allow Ca^{2+} ions to flow into the postsynaptic cell, thus triggering Ca^{2+}-dependent second-messenger activities. Second, NMDA receptors are very unusual in that two different neurotransmitters are required to stimulate the receptor and open its ion channel. The first neurotransmitter, of course, is glutamate. But in addition to the binding site for glutamate on the NMDA receptor complex, there is a binding site that recognizes the amino acid **glycine**. **FIGURE 8.6** shows a three-dimensional model of the predicted structure of an NMDA receptor in the cell membrane (Zhu and Paoletti, 2015). This particular receptor is composed of two GluN1 and two GluN2 subunits, which is the most common type of NMDA receptor in the brain (some NMDA receptors contain GluN3 instead of GluN2 subunits). Note how most of the receptor complex sticks out from the membrane into the extracellular space. The right-hand part of the figure presents a cutaway view that shows the locations of the ion channel pore within the cell membrane, one of the glutamate binding sites on a GluN2 subunit, and one of the glycine binding sites on a GluN1 subunit. Because the receptor complex contains two of each type of subunit, we can see that there is a total of four binding sites on the receptor.

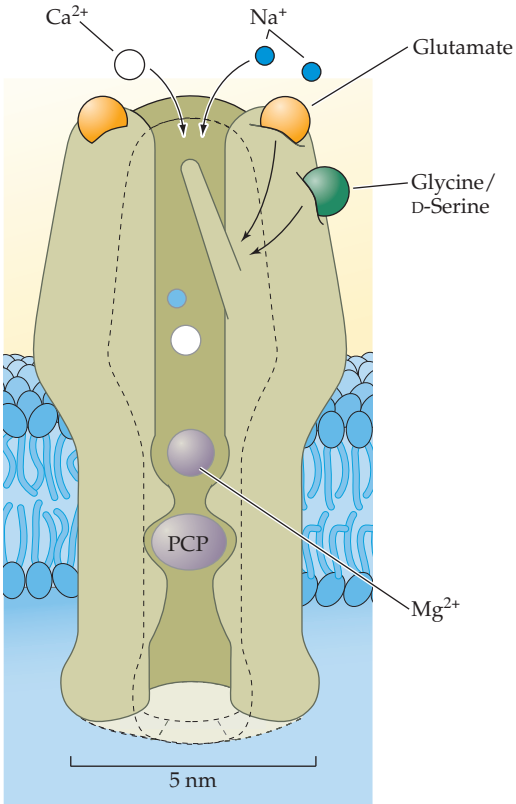


FIGURE 8.5 NMDA receptor properties The NMDA receptor is activated by simultaneous binding of glutamate and a co-agonist, either glycine or D-serine. The receptor channel can be blocked by Mg^{2+} ions under resting conditions and also by the presence of the abused drug phencyclidine (PCP).

Another amino acid, **D-serine**, also binds to and activates the glycine binding site on the NMDA receptor. This unusual substance (amino acids normally exist in the L-conformation) is not a normal constituent of cells and, therefore, must be synthesized by a specific enzyme that is located both in neurons and in astrocytes (Mothet et al., 2015). Because the NMDA receptor channel cannot open unless both the glutamate and glycine binding sites are occupied at the same time, glycine and D-serine are considered to be **co-agonists** with glutamate at the NMDA receptor.

There are two additional binding sites on the NMDA receptor that affect its function. One is a site within the receptor channel that binds magnesium (Mg^{2+}) ions. When the cell membrane is at the resting potential (typically –60 or –70 mV), Mg^{2+} ions are bound to this site relatively tightly. This causes the receptor channel to be blocked, even if glutamate and either glycine or D-serine are present to activate the receptor. However, if the membrane becomes depolarized, then the Mg^{2+} ions dissociate from the receptor and permit the channel to open if glutamate and a co-agonist are present. Consider the implications of this property of NMDA receptors. How does the membrane become depolarized? The answer, of course, is that some other source of excitation (other than NMDA receptors) must have already activated the cell. This other source of excitation could have been glutamate, acting through AMPA or kainate receptors, or a different transmitter such as acetylcholine, acting through nicotinic receptors. The point is that an NMDA receptor is a kind of biological "coincidence detector." That is, the channel only opens

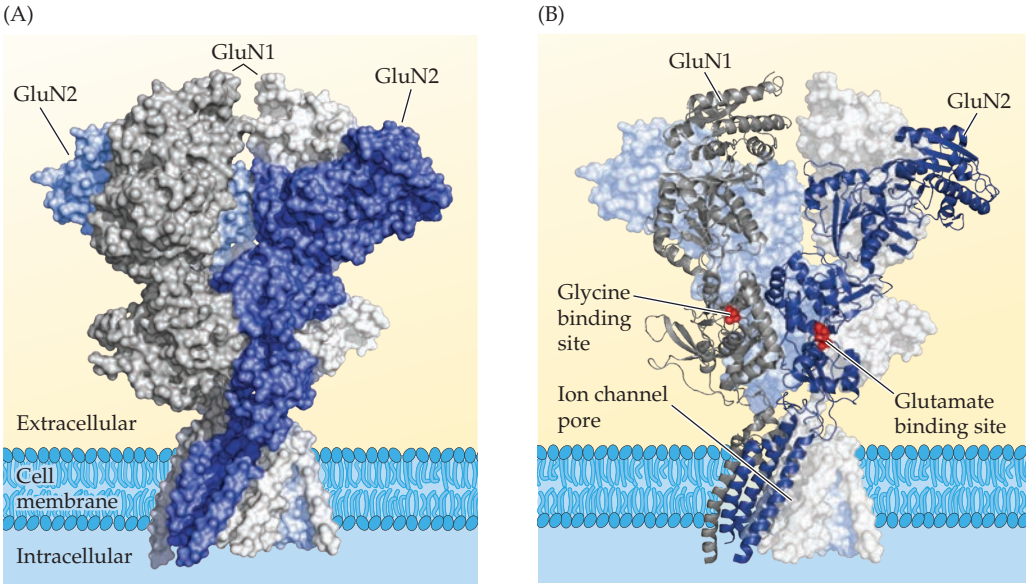


FIGURE 8.6 Three-dimensional depiction of an NMDA receptor in the cell membrane (A) The receptor is composed of two GluN1 subunits (shown in shades of gray) and two GluN2 subunits (shown in shades of blue). (B) A cutaway view of the receptor with a molecule of glycine bound to a GluN1 subunit and a molecule of glutamate bound to a GluN2 subunit. Both agonists are shown in red. This view shows regions of the two subunits that are predicted to have a ribbon structure (wavy lines) and other regions that are predicted to have a helical structure (lines in a corkscrew configuration). Note that the ion channel pore is shown in the closed state. (From Zhu and Paoletti, 2015.)

when two events occur close together in time: (1) glutamate is released onto the NMDA receptor (assuming that a co-agonist is also bound to its site on the receptor complex), and (2) the cell membrane is depolarized by stimulation of a different excitatory receptor.

The second site, which is also located within the receptor channel, recognizes several different drugs that block the channel and thus prevent ion flow. Because these compounds do not interfere with the ability of glutamate to bind to its site on the receptor complex, they are noncompetitive rather than competitive antagonists of the NMDA receptor. One such noncompetitive antagonist called **MK-801**, or **dizocilpine**, is mainly used for research purposes. Other channel blockers include the abused drugs **phencyclidine** (**PCP**) and **ketamine**, as well as the drug **memantine** (**Namenda**), which is used to treat mild to moderate Alzheimer's disease. Memantine and ketamine are believed to bind in a very similar location within the NMDA receptor channel pore (Johnson et al., 2015). Indeed, it seems remarkable that compounds that share this common mode of NMDA channel blockade are used for such different purposes and have such different effects on cognitive function (PCP and ketamine disrupt normal cognition, whereas memantine is a cognitive enhancer in patients with Alzheimer's disease). At present, researchers hypothesize that these differences depend on several factors, including (1) dose (recreational versus medicinal), (2) subtle but significant differences in how each drug influences functioning of the NMDA receptor, and (3) effects of each drug on other pharmacological targets besides the NMDA receptor (Johnson et al., 2015). Chapter 15 describes in more detail the behavioral and psychological effects of PCP and ketamine, whereas Chapter 20 presents more information on the use of memantine in Alzheimer's disease pharmacotherapy.

METABOTROPIC GLUTAMATE RECEPTORS Besides the three ionotropic receptors, there are also eight different metabotropic glutamate receptors. They are designated **mGluR1** to **mGluR8**. The metabotropic glutamate receptors can be divided into three groups based on their amino acid sequences, subcellular localization, and signaling pathways (Golubeva et al., 2015). Group I receptors, which include mGluR1 and mGluR5, are located postsynaptically and mediate excitatory responses by activating the phosphoinositide second-messenger system. Group II receptors, consisting of mGluR2 and mGluR3, and group III receptors, consisting of mGluR4, mGluR6, mGluR7, and mGluR8, signal by inhibiting cyclic adenosine monophosphate (cAMP) formation. Many of these group II and group III receptors are located presynaptically where they function as auto- or heteroreceptors to inhibit release of glutamate (autoreceptors) or other neurotransmitters (heteroreceptors). The novel amino acid **L-AP4** (**L-2-amino-4-phosphonobutyrate**) is a selective agonist at group III glutamate autoreceptors, thereby suppressing glutamatergic synaptic transmission.

Metabotropic glutamate receptors are widely distributed throughout the brain, and they participate in many normal functions, including locomotor activity, motor coordination, cognition, mood, and pain perception. Recognition of this involvement has led to increasing interest in the development of mGluR drugs for the treatment of numerous neuropsychiatric disorders such as depression, anxiety disorders, disorders characterized by cognitive deficits, and drug addiction (Golubeva et al., 2015; Willard and Koochekpour, 2013). **BOX 8.1** describes a congenital disorder known as **fragile X syndrome** for which mGluR5 has been investigated as a potential therapeutic target.

AMPA and NMDA receptors play a key role in learning and memory

Earlier, we mentioned that AMPA and NMDA receptors are two glutamate receptor subtypes that have been strongly implicated in the mechanisms underlying learning and memory. Many neuropsychiatric disorders are associated with cognitive impairment, and dysregulation of glutamate receptors has been found in at least some of these disorders, including intellectual disability (previously called mental retardation), autism spectrum disorders, and schizophrenia (Volk et al., 2015). Consequently, a major ongoing search is under way to identify new compounds that can help patients suffering from cognitive impairment (Livingstone et al., 2015; Solé et al., 2015; Vreeker et al., 2015; Kulshreshtha and Piplani, 2016; Sallustio and Studer, 2016).

COGNITIVE ENHANCEMENT BY AMPA RECEPTOR MODULATORS Several types of proposed cognitive enhancers are positive allosteric modulators of AMPA receptors (Chang et al., 2012; Partin, 2015). In Chapter 7 we mentioned the use of positive allosteric modulators of muscarinic or nicotinic cholinergic receptors as potential cognitive enhancers. Because AMPA receptors are likewise important for various aspects of learning and memory (see below and **Web Box 8.1**), drugs that positively modulate these receptors are also under development for possible clinical use. One such class of cognitive enhancers is a group of compounds termed **ampakines**. As summarized by Arai and Kessler (2007), these compounds positively modulate AMPA receptor activity (i.e., they enhance the action of glutamate) but do not directly activate the receptor themselves. The principal mechanism of action of ampakines (and most other positive AMPA receptor modulators) is to reduce the rate of receptor deactivation (i.e., transition from an active to an inactive state) and/or the rate of receptor desensitization (i.e., transition to a desensitized state; see Chapters 3 and 7 for a discussion of ionotropic receptor desensitization). Both mechanisms increase the time during which the AMPA receptor channel remains open, thereby permitting

BOX 8.1 Clinical Applications

Fragile X Syndrome and Metabotropic Glutamate Receptor Antagonists: A Contemporary Saga of Translational Medicine

This box presents an example of **translational medicine** as applied to neuropharmacology. In this type of translational medicine, researchers determine the mechanisms underlying a disease or disorder, develop animal models of the disorder, use information derived from the model systems to test potential therapeutic approaches (including drugs) for the disorder, and finally conduct clinical trials, which, if successful, will result in new and effective treatments. The National Institutes of Health (NIH) even has a research program called Bench-to-Bedside that funds "research teams seeking to translate basic scientific findings into therapeutic interventions for patients and to increase understanding of important disease processes." (National Institutes of Health, 2017).

The specific disorder discussed here is fragile X syndrome, a congenital disorder that is thought to be the leading cause of intellectual disability and autistic symptoms that can be attributed to a single gene (Gomez-Mancilla et al., 2014; Pop et al., 2014). The core features of the syndrome consist of cognitive deficits (including deficits in memory and language), impaired attention, and socioemotional problems. Affected individuals, particularly males, may additionally exhibit anxiety, irritability, hyperactivity, aggression, and self-injury. Although the brains of patients with fragile X syndrome appear grossly normal upon postmortem inspection, microscopic examination has revealed the presence of many long and thin, immature-looking dendritic spines in the cerebral cortex, and also a higher spine density than in healthy control brains (Irwin et al., 2000).

Fragile X syndrome is caused by mutations in the ***fragile X mental retardation 1* gene** (***FMR1***), which (as its name implies) is located on the X chromosome[1]

[1]Fragile X syndrome was so named because the location of the *FMR1* gene on the X chromosome is susceptible to breakage (hence "fragile") under certain conditions.

(*Continued*)

BOX 8.1 Clinical Applications (*continued*)

where it codes for the **fragile X mental retardation protein** (**FMRP**). Certain mutations in the *FMR1* gene can cause the gene to be underexpressed during embryonic development, and in the most severe cases, the gene is completely silenced and no FMRP is produced at all because of epigenetic hypermethylation of the *FMR1* gene promoter region (see Chapter 2 for a discussion of epigenetic mechanisms of gene regulation). To understand the cellular consequences of losing FMRP expression, we need to review some basic information about the sites of protein synthesis in neurons. According to classical cell biology, mRNA is translated into protein within the body of a cell. In neurons, however, some mRNAs are translocated out of the soma into axons and dendrites, where those mRNAs are translated in a process of local protein synthesis. In turn, local protein synthesis at postsynaptic sites plays an important role in synaptic plasticity and long-term memory consolidation. The major function of FMRP is to bind to many of these mRNAs, thereby not only affecting mRNA transport out of the cell body but also inhibiting local mRNA translation to protein (Fernández et al., 2013). Consequently, loss of FMRP in fragile X syndrome leads to dysregulated local protein synthesis in many parts of the brain, causing abnormal brain development and persistent aberrant synaptic functioning.

Production of an *Fmr1*-knockout mouse was first reported in 1994, and these mutant mice quickly became a model of fragile X syndrome because they reproduced the *FMR1* gene silencing present in patients with fragile X syndrome. Studies of *Fmr1*-knockout mice by different laboratories consistently found dendritic spine abnormalities similar to those observed in patients with fragile X syndrome; however, behavioral studies were considerably more variable with respect to whether the knockout mice exhibited significant autism-like cognitive and social deficits (Kazdoba et al., 2014). One of the key synaptic functions of FMRP is to regulate a form of plasticity in the hippocampus called **long-term depression** (**LTD**). Normal learning and memory require that synapses can be strengthened at certain times but weakened at others. Long-lasting synaptic strengthening is called **long-term potentiation** (**LTP**), which we discuss in detail below and in **Web Box 8.1**. In contrast, LTD is a mechanism for weakening neurotransmission at the affected synapses. Relevant to fragile X syndrome is a specific type of LTD that is activated by the group I metabotropic glutamate receptors, mGluR1 and mGluR5 (Gladding et al., 2009). Research with *Fmr1*-knockout mice found that *too much* LTD was occurring at the group I metabotropic glutamate synapses, because FMRP restricts the LTD process when the protein is present in genetically normal mice (and presumably also in healthy people not suffering from fragile X syndrome) (Sidorov et al., 2013). Together, these findings led Bear and colleagues (2004) to propose a **metabotropic glutamate receptor theory** of fragile X syndrome. This theory hypothesizes that loss of FMRP causes exaggerated group I mGluR-related functions, including dendritic spine abnormalities and elevated LTD, which together lead to the phenotypic characteristics of fragile X syndrome.

A logical extension of the metabotropic glutamate receptor theory is that group I mGluR antagonists might alleviate at least some of the symptoms of fragile X syndrome. Consequently, pharmaceutical companies began drug development programs to test this idea. The primary focus of these programs was mGluR5 rather than mGluR1, because the former has a regional expression pattern in the brain that implicates it more strongly in the symptoms of fragile X syndrome. Leading the race was pharmaceutical giant Novartis, based in Switzerland, which developed a selective mGluR5 antagonist designated AFQ056 during experimental testing and then later renamed **mavoglurant** for clinical use (Gomez-Mancilla et al., 2014). Theoretically, chronic treatment with mavoglurant should help normalize synaptic function, including dendritic structure and rates of LTD, by blocking glutamate activation of mGluR5 (see **Figure**). In fact, either mavoglurant or other mGluR5 antagonists worked as predicted when administered to *Fmr1*-knockout mice (Gomez-Mancilla et al., 2014).

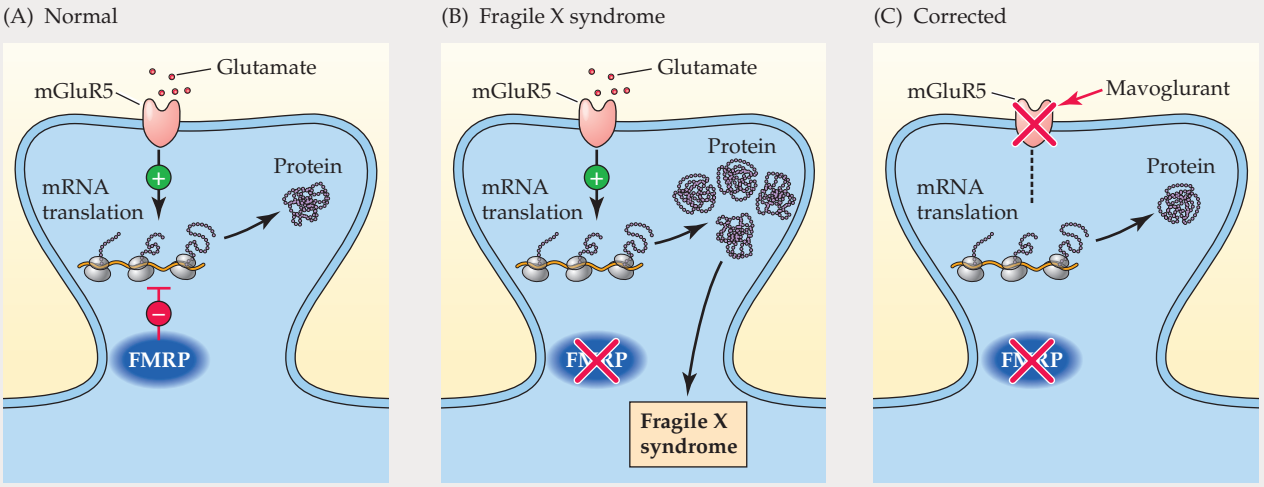
The promising findings using the mouse model prompted Novartis, at great effort and cost, to sponsor two double-blind, placebo-controlled clinical trials with patients with fragile X syndrome, one involving adults and the other involving adolescents. To the great disappointment of the company, the trial participants and their families, and the psychiatric community at large, both trials showed little measurable improvement in the treated cohorts (Bailey Jr. et al., 2016; Berry-Kravis et al. 2016). As a result, the mavoglurant development program at Novartis was canceled. Jeste and Geschwind (2016) published a thoughtful commentary accompanying the Berry-Kravis et al. paper that can help us learn some important lessons from this foray into translational medicine.

Why did the clinical trials fail when the drug seemed so promising? Is the mouse model inadequate or inappropriate for the disorder? Of course this is possible, particularly because the knockout mice did not exhibit the same degree of cognitive impairment that was seen in the most severe cases of fragile X syndrome. Nevertheless, a single-gene-silencing disorder like fragile X should be the most amenable to transgenic

BOX 8.1 Clinical Applications (*continued*)

animal modeling, much more so than, for example, a disorder like schizophrenia for which there is no single genetic cause but rather many different genes that seem to confer increased risk. Was the dose of mavoglurant wrong? Might a higher dose have yielded better results? This is also possible, but one must keep in mind that higher doses would have significantly increased the risk of adverse side effects of the treatment. Was the drug given long enough? Because the clinical trial was only 12 weeks in duration, we cannot rule out the possibility that some improvement would have been seen with a longer period of treatment. Were the best outcome measures selected for the clinical trials? Berry-Kravis et al. (2016) point out that although some cognitive testing was performed, the particular tests used may have missed some possible signs of cognitive improvement that were seen in the mouse model. Continuing on, was the selected treatment population (adults and adolescents) the best choice for assessing drug efficacy? This is quite an important question because it could be the case that mavoglurant would have been more beneficial if administered to affected infants or children, before their brains had endured the deleterious effects of the *FMR1* gene mutation for up to 45 years (the oldest patients in the adult trial). Was the trial compromised by a strong placebo effect? Indeed, the researchers observed a marked placebo effect in caregiver evaluations of the patients, leading Jeste and Geschwind to speculate that this effect may have reflected "the eagerness of caregivers of individuals with developmental disabilities for treatments that will improve symptoms and reduce the burden of disease" (p. 2). Such a placebo effect could have diminished the ability to detect a treatment effect in the mavoglurant group. Finally, we must acknowledge that group I mGluR signaling is only one of many neurochemical pathways regulated by FMRP, and, therefore, it may be naive to think that the major symptoms of fragile X can be treated effectively by targeting this pathway alone.

In conclusion, if the mGluR theory of fragile X syndrome does have some validity, then administration of an mGluR antagonist may yet prove beneficial, if we can find the right drug, administer it at the right dose for a sufficient period of time, select the right outcome measures, and provide the treatment sufficiently early in development. But whether or not that comes to pass, we hope that this story illustrates just how difficult it is to develop new medications for neuropsychiatric disorders and why the pace of progress has been so slow despite the remarkable recent advances in our understanding of the nervous system at a molecular genetic level. Although the story also shows some of the limitations of animal models of human disorders, we emphasize that such models continue to be crucial in our search for new treatments. Indeed, in the absence of animal research, pharmacologists would have no clues about what drugs to even test, since they wouldn't have the underlying knowledge of metabotropic glutamate receptor signaling pathways, mechanisms of LTD, and the detailed molecular effects of FMRP and the consequences of its absence.



mGluR5, FMRP, and synaptic plasticity in health and in fragile X syndrome (A) In a normal healthy brain, the presence of FMRP regulates postsynaptic mRNA translation and synaptic plasticity in response to activation of mGluR5 and other neurotransmitter receptors. (B) Loss of FMRP in fragile X syndrome causes excessive mRNA translation, increased long-term depression, and abnormal dendritic structure. (C) Blockade of mGluR5 by mavoglurant is hypothesized to at least partially reverse the effects of FMRP loss, potentially leading to normalization of synaptic structure and plasticity. (After After Dölen and Bear, 2009, CC-BY.)

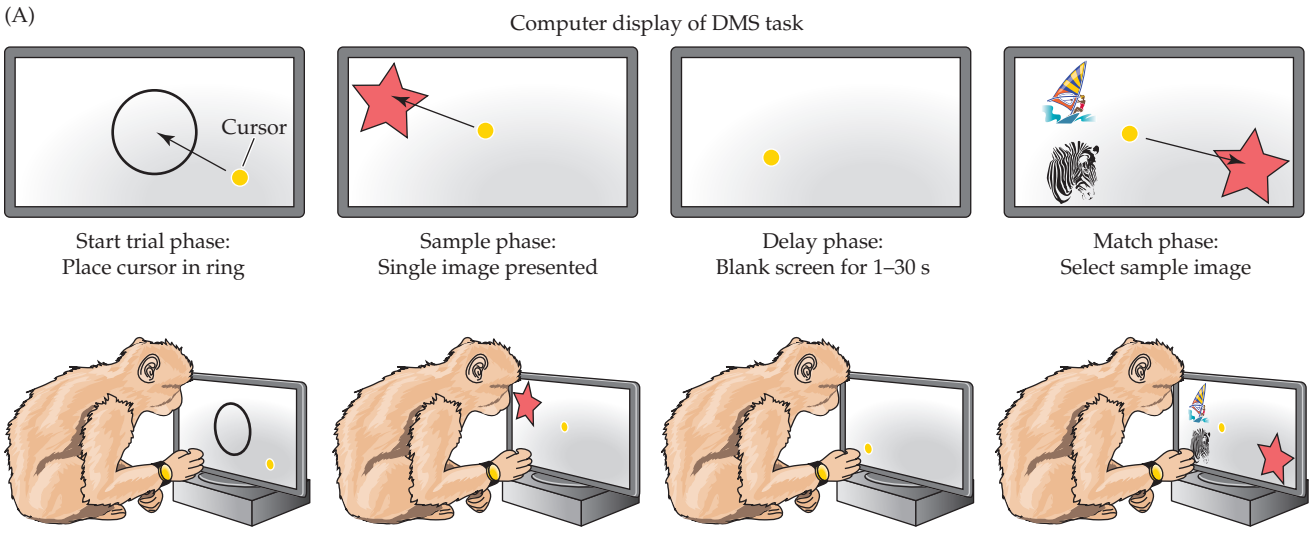


FIGURE 8.7 Ampakine-induced performance improvement on a delayed match-to-sample (DMS) memory task in monkeys (A) Each trial in the DMS task had four phases: (1) in the start trial phase, the monkey placed a hand-controlled cursor into a circular ring displayed on a computer screen; (2) in the sample phase, a single image of a clip art object such as a red star was displayed on the screen, after which the monkey had to move the cursor into the image; (3) movement of the cursor into the sample image triggered a delay phase of 1 to 30 seconds, during which the screen was blank; (4) in the match phase, the sample image was displayed in a random location on the screen, along with one to five additional "nonmatch" images; to make the correct response, the monkey had to move the cursor into the sample image, after which it was rewarded with a sip of fruit juice. (B) The ampakine CX717 administered by IV injection produced a dose-dependent improvement in overall performance on the DMS task. (After Porrino et al., 2005, CC-BY.)

glutamate to exert a more prolonged excitatory effect on the postsynaptic cell.

Ampakines have been demonstrated to enhance cognitive function in several kinds of animal models. One example is shown in **FIGURE 8.7A**, which illustrates a memory task in monkeys called the **delayed match-to-sample** task. The figure shows that the monkey must remember a sample image displayed briefly on a computer screen for a variable length of time (the delay), after which the original image and two other images are presented, and the subject must select the originally displayed image. **FIGURE 8.7B** shows that the ampakine CX717 dose-dependently improved the performance of the monkeys on this task (Porrino et al., 2005). In a more recent study, researchers tested whether chronically administering a different ampakine, CX929, would reverse some of the effects of aging on neuronal structure and function in rats (Lauterborn et al., 2016). Among other changes, aging rats show a shortening of the dendritic branches of pyramidal neurons in the hippocampal CA1 area. Treatment of middle-aged (i.e., 10-month-old) rats with CX929 for 3 months caused their pyramidal neuron dendrites to look similar to the dendrites of young (2.5-month-old) rats (**FIGURE 8.8**). Ampakine treatment also led to a significant increase in dendritic spine density above that seen either in young rats or in middle-aged control rats given the drug vehicle. On a more discouraging note, the few clinical trials that have thus far been published on the use of ampakines to enhance cognition in humans have yielded inconclusive results (Partin, 2015). Nevertheless, these and other positive allosteric AMPA receptor modulators continue to be explored because of the importance of finding drugs that will be effective in reversing cognitive dysfunction in patients with various neuropsychiatric disorders and, perhaps, even in people with typical age-related cognitive decline.

NMDA RECEPTORS IN LEARNING AND MEMORY The NMDA receptor is the other ionotropic glutamate receptor subtype believed to play a key role in learning and memory. It has been well established that release of glutamate from a nerve terminal coupled with strong activation of NMDA receptors in the postsynaptic cell receiving that glutamate input can lead to a strengthening of that synapse. The mechanism underlying this phenomenon, which is called **long-term potentiation** (**LTP**), is described in the next section. But first, we will simply point out that one aspect of this mechanism involves the coincidence detector feature of the NMDA receptor mentioned earlier (i.e., the fact that the

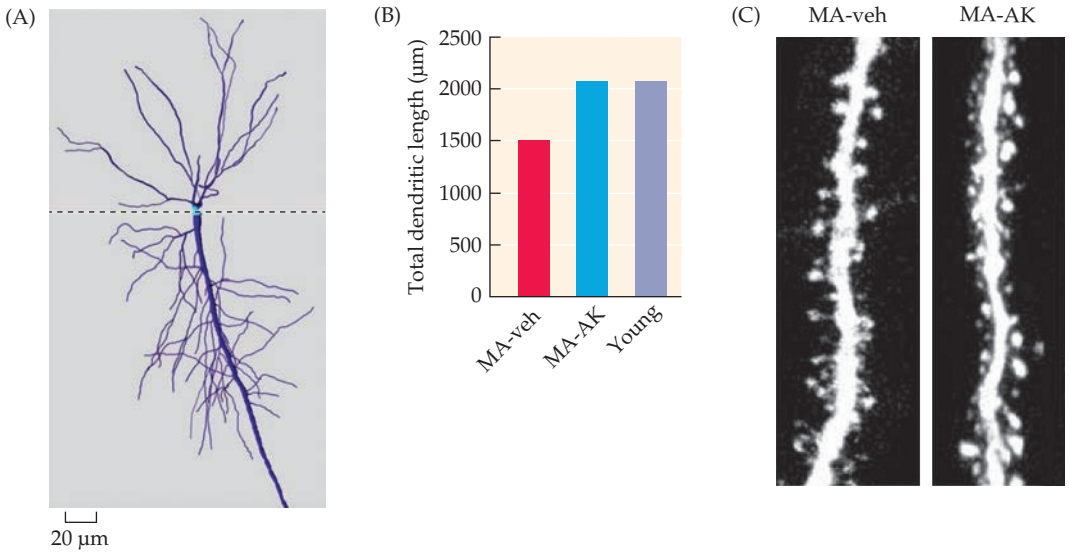


FIGURE 8.8 Chronic ampakine treatment of middle-aged rats enhances dendritic growth of hippocampal CA1 pyramidal neurons Rats at 10 months of age were housed in an enriched environment and given an oral dose of the ampakine CX929 (5 mg/kg) or vehicle 5 days per week for 3 months. Dendritic arbors and spines were examined at the end of the treatment period and compared with measurements obtained from young 2.5-month-old rats. (A) Reconstruction of apical (top) and basal (bottom) dendritic fields of a typical hippocampal CA1 pyramidal neuron from a 2.5-month-old rat. (B) Middle-aged rats given the drug vehicle for 3 months (MA-veh) showed a significantly shorter total dendritic arbor length than young rats. This difference was seen for both apical and basal dendrites (not shown). However, 3 months of treatment with the ampakine (MA-AK) completely prevented the aging-related dendritic retraction. (C) Numbers of spines per unit length of apical or basal dendrite were also increased by ampakine treatment. The photomicrograph shows representative images of apical dendrites from middle-aged rats treated either with vehicle or ampakine. (After Lauterborn et al., 2016.)

postsynaptic cell membrane must be depolarized simultaneously with occupation of agonist binding sites on the receptor). A possible link between this coincidence detection feature and learning can most readily be seen in the case of associative learning, which involves the pairing of two events, such as two different stimuli or a stimulus and a response. All psychology students have encountered a simple kind of associative learning called classical (Pavlovian) conditioning, as exemplified by Pavlov's original experiment with dogs. Like the opening of an NMDA receptor channel, classical conditioning is based on the close timing of two events: the pairing of a conditioned stimulus (the bell in Pavlov's experiment) with an unconditioned stimulus (the meat powder). Figure 8.12 depicts what is thought to be occurring at the synaptic level in this kind of simple associative learning. Neurons that receive input from the unconditioned stimulus (US) start out with strong connections to their postsynaptic partners, while neurons that receive input from the conditioned stimulus (CS) initially have weak connections with the US postsynaptic cells (**FIGURE 8.9A**). However, repeated pairings of the CS and the US (**FIGURE 8.9B**) theoretically strengthen the synaptic connections between the CS input neurons and the postsynaptic cells, because those latter cells (which express NMDA receptors) are being depolarized at the same time that the nerve terminals of the CS neurons are releasing glutamate. Finally, presentation of the CS alone elicits a strong response in the US postsynaptic cells (**FIGURE 8.9C**), which then can trigger the conditioned response.

MECHANISMS OF LTP LTP was discovered by Bliss and Lømo (1973) more than 40 years ago, and this discovery had such a powerful impact on neuroscience that it has given rise to thousands of subsequent experiments aimed at elucidating its underlying mechanisms and behavioral functions. When researchers refer to LTP, they generally mean a persistent (at least 1 hour) increase in synaptic strength produced by a burst of activity in the presynaptic neuron. This burst of firing is usually produced experimentally by a single brief train of electrical stimuli (e.g., 100 stimuli over a period of 1 second) called a **tetanic stimulus** (or simply a **tetanus**). The synaptic enhancement produced by the tetanus is measured by changes in the excitatory postsynaptic potential (EPSP) recorded in the postsynaptic cell. Several different forms of LTP can be elicited, depending on the stimulation parameters and the brain area where the process is being studied. Varieties of LTP may differ in the time period of potentiation (i.e., how long it lasts), whether the potentiation is pre- or postsynaptic, whether the synapses undergo structural in addition to biochemical modification, and the underlying cellular

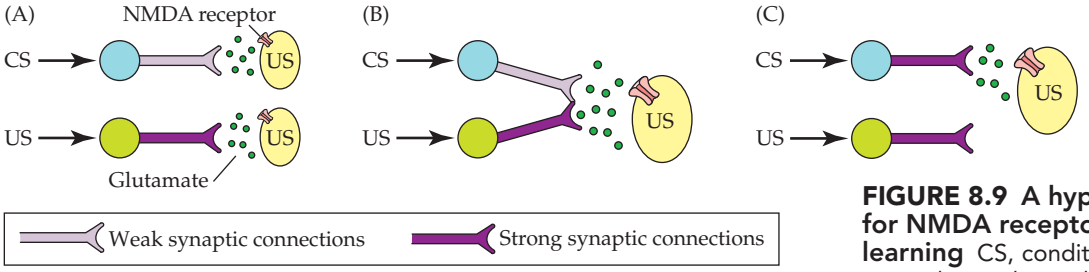


FIGURE 8.9 A hypothesized role for NMDA receptors in associative learning CS, conditioned stimulus; US, unconditioned stimulus (After Rudy, 2008.)

mechanisms (e.g., which neurotransmitter receptors are involved and which signaling pathways are activated by those receptors). LTP that is relatively short-lived, meaning a few hours at most, has been termed **early LTP** (**E-LTP**) and is usually studied in a brain slice. In contrast, **late LTP** (**L-LTP**) can last for days or even months when produced in an animal instead of a slice. We will focus most of our discussion on E-LTP, which was discovered first and provides the conditions required for L-LTP. Later we will mention some of the additional conditions needed to produce L-LTP.

Although LTP occurs in many brain areas, it was first discovered in the hippocampus and has been studied most extensively in that structure. The hippocampus from a rat or a mouse is cut into slices about 200 μm (0.2 mm) thick. These slices are then placed in a dish where the neurons can be maintained in a healthy state for many hours while the investigator stimulates them and records their electrophysiological responses. The important cellular anatomy of a hippocampal slice is illustrated in **FIGURE 8.10**. Without going into great detail, it is sufficient to know that all of the pathways shown in the diagram use glutamate as their transmitter and that LTP occurs at all of the synaptic connections depicted. However, the majority of LTP studies have focused on the pyramidal neurons of the CA1 region of the hippocampus, which receive excitatory glutamatergic inputs from CA3 neurons via the Schaffer collaterals.

FIGURE 8.11 depicts what happens to a typical synapse on a CA1 pyramidal neuron before, during, and after the tetanic stimulus. A test pulse (single electrical stimulus) of the presynaptic cell is used to assess the strength of the synaptic connection. The test pulse elicits the release of a small amount of glutamate from the presynaptic nerve endings. As shown in the left panel, this glutamate binds to both AMPA and NMDA receptors in the postsynaptic membrane. A small EPSP is produced mainly by activation of the AMPA receptors. However, the NMDA receptor channels fail to open because the membrane is not depolarized sufficiently to release the Mg^{2+} block of those channels. As long as test pulses are separated in time, you can give many of these pulses and not see any enhancement of the EPSP. But look at what happens in response to a tetanic stimulus (middle panel). Much more glutamate is released, and this causes prolonged activation of the AMPA receptors and greater postsynaptic depolarization. This permits Mg^{2+} ions to dissociate from the NMDA receptor channels and Ca^{2+} ions to enter the cell through these channels. Acting as a second messenger, these Ca^{2+} ions alter the functioning of the postsynaptic cell so that the same test pulse given before now produces an enhanced EPSP (right panel).

E-LTP can be divided into two phases: an **induction phase**, which takes place during and immediately after the tetanic stimulation, and an **expression phase**, which represents the enhanced synaptic strength measured at a later time. NMDA receptors play a critical role in the induction phase but not in the expression phase. We know this because application of an NMDA receptor antagonist to the hippocampal slice during the tetanus blocks induction, but the same drug applied during the test pulse does not prevent the enhanced

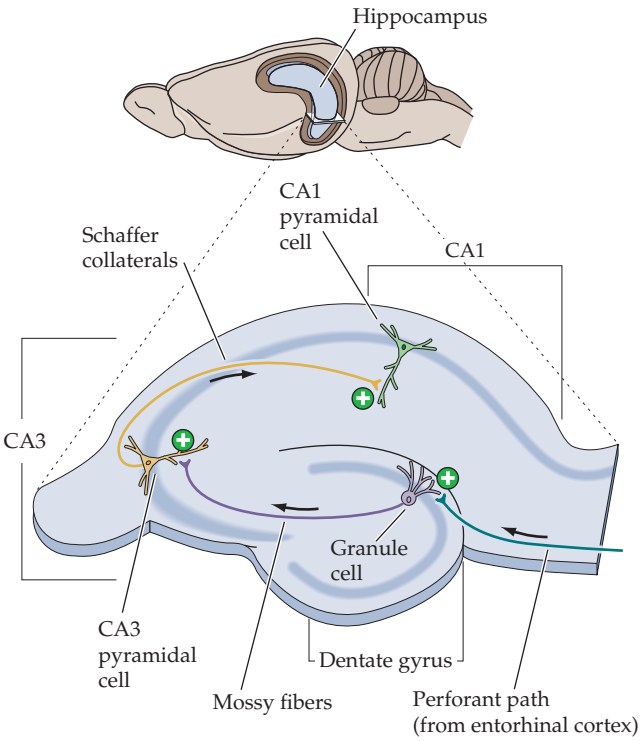


FIGURE 8.10 Long-term potentiation of synaptic transmission LTP can be studied in vitro using the hippocampal slice preparation.

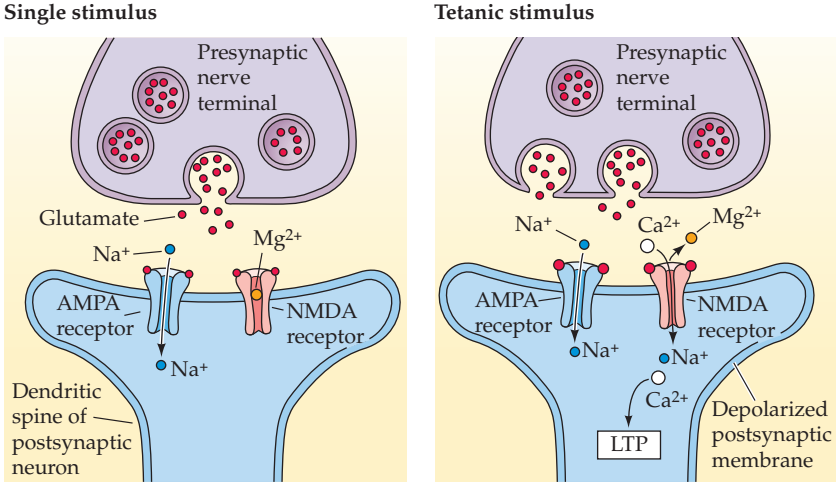


FIGURE 8.11 Induction of long-term potentiation LTP is induced by a tetanic stimulation of the presynaptic input.

EPSP. In contrast, AMPA receptors are necessary for LTP expression, since it is an AMPA receptor–mediated EPSP that is facilitated in LTP.

The biochemical mechanisms thought to underlie E-LTP are illustrated in **FIGURE 8.12**. The influx of Ca^{2+} ions through the NMDA receptor channels activates several protein kinases, including one type of calcium/calmodulin protein kinase called CaMKII (see Chapter 3). CaMKII is an unusual enzyme in that it can remain activated well after the level of intracellular Ca^{2+} has returned to baseline (Lisman et al., 2002). There are several consequences of postsynaptic Ca^{2+} influx and CaMKII activation, the most important of which is insertion of additional AMPA receptors into the membrane of the dendritic spine (Herring and Nicoll, 2016). This insertion is actually a modulation of the normal process that we call **receptor trafficking**, in which neurotransmitter receptors (in this case, AMPA receptors) are continuously moved into and out of the cell membrane (**FIGURE 8.13**, middle). In LTP, the rate of receptor insertion is increased (Figure 8.13, right), thereby enhancing sensitivity of the cell to glutamate. In contrast, some forms of long-term depression (LTD) involve withdrawal of AMPA receptors from the membrane (Figure 8.13, left), thereby reducing the cell's sensitivity (Volk et al., 2015). As the figure illustrates, the AMPA receptors are transported by vesicle-like structures, and indeed, receptor insertion into the membrane uses a process of exocytosis similar to the exocytosis of synaptic vesicles at nerve terminals (Jurado, 2014).

Thus far, our discussion has covered the mechanisms underlying E-LTP, which is a key form of synaptic strengthening in the nervous system. However, we have not yet explained L-LTP, nor have we touched on how LTP may be related to learning and memory. These topics are taken up in Web Box 8.1.

High levels of glutamate can be toxic to nerve cells

Despite the vital role of glutamate in normal neural and behavioral functioning, this neurotransmitter system also has a dark side. More than 50 years ago, two researchers published a report showing retinal damage in mice following subcutaneous injection of the sodium salt of glutamic acid—monosodium glutamate (MSG) (Lucas

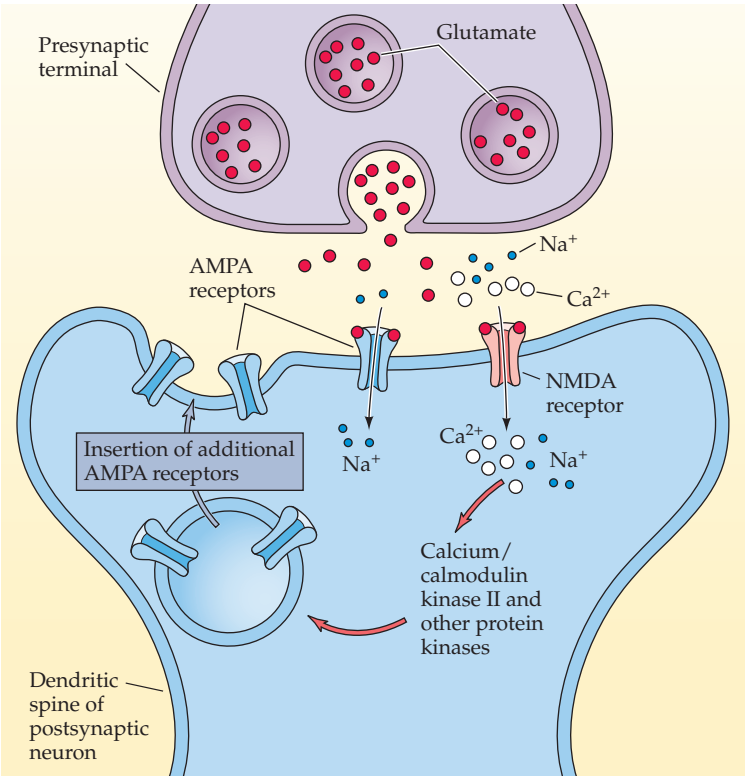


FIGURE 8.12 The underlying mechanism LTP involves modification of AMPA receptors in the postsynaptic cell.

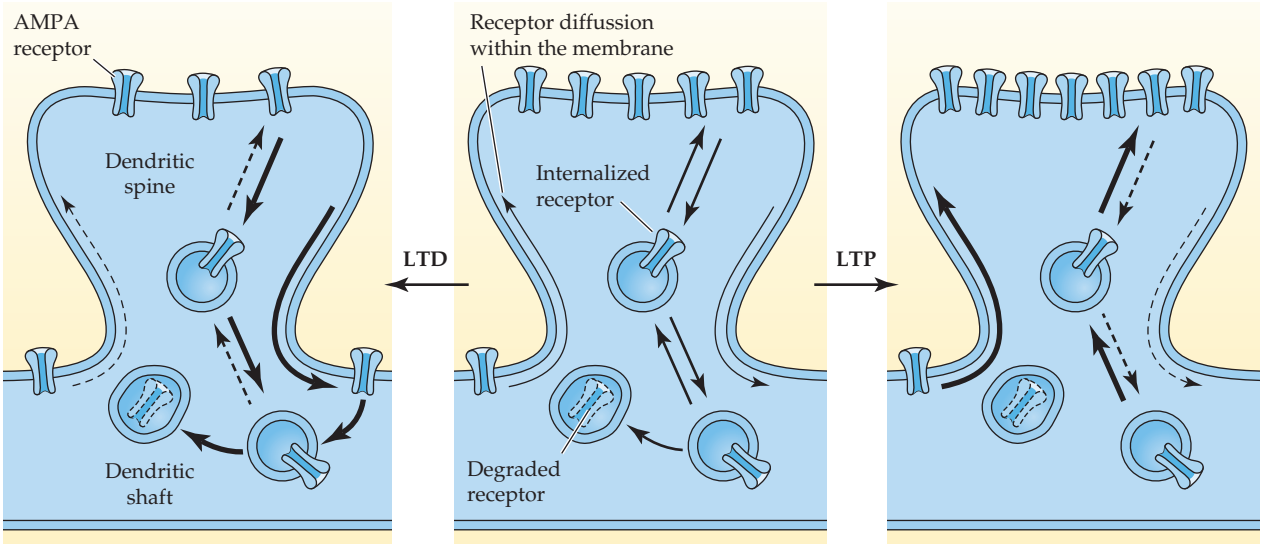


FIGURE 8.13 AMPA receptor trafficking Movement of AMPA receptors in dendritic spines can be altered under conditions of synaptic plasticity. The middle spine depicts normal receptor trafficking, which is maintained by an equilibrium of receptor movement into and out of the membrane and a low rate of receptor degradation. LTP favors increased receptor insertion into the spine membrane and reduced degradation, whereas LTD favors increased receptor removal from the membrane and degradation. Arrows additionally depict movement of receptors into and out of the spine from the membrane of the dendritic shaft, with thicker arrows reflecting greater rates of movement. (After Volk et al., 2015.)

and Newhouse, 1957). Furthermore, this toxic effect of glutamate was more severe in infant than in adult mice. Twelve years later, Olney (1969) presented the first evidence that MSG also produces brain damage in young mice. Subsequent research showed that glutamate could lesion any brain area of adult animals when injected directly into that structure. This effect was shared by other excitatory amino acids, including kainate and NMDA, and the damage was shown to occur at postsynaptic sites but not at nerve terminals. These and other findings led to the **excitotoxicity hypothesis**, which proposed that the effects produced by excessive exposure to glutamate and related excitatory amino acids are caused by a prolonged depolarization of receptive neurons that in some way leads to their eventual damage or death. Administration of an excitatory amino acid kills nerve cells but spares fibers of passage (i.e., axons from distant cells that are merely passing through the lesioned area). Thus excitotoxic lesions are more selective than lesions produced by passing electrical current through the targeted area (called electrolytic lesions), since the latter method damages both cells and fibers of passage. For this reason, excitotoxic lesions have replaced electrolytic lesions in many research applications.

MECHANISMS OF EXCITOTOXICITY The mechanisms underlying amino acid excitotoxicity have been studied primarily using cultured nerve cells. In such tissue culture models, neuronal cell death is most readily triggered by strong activation of NMDA receptors. Nevertheless, non-NMDA receptors (AMPA and/or kainate receptors) may contribute to the excitotoxic effects of glutamate, and under certain conditions, these receptors can even mediate cell death themselves without NMDA receptor involvement. When both NMDA and non-NMDA receptors are subjected to prolonged stimulation by a high concentration of glutamate, a large percentage of the cells die within a few hours. The mode of cell death in this case is called **necrosis**, which is characterized by **lysis** (bursting) of the cell due to osmotic swelling and other injurious consequences of prolonged glutamate receptor activation. But a different pattern occurs if either the neurotransmitter concentration or the time of exposure is significantly reduced. In this case, the osmotic swelling is temporary, and the cells appear to return to a normal state. However, there may be a delayed response that emerges over succeeding hours and that is characterized by a gradual disintegration of the cells and their eventual death. This delayed excitotoxic reaction is highly dependent on NMDA receptor activation, since it can be elicited by the selective application of NMDA or blocked by the presence of an NMDA receptor antagonist such as MK-801.

In contrast to the necrotic reaction, which occurs relatively quickly, the later-appearing type of cell death is known as **programmed cell death**. To add to the complexity, there are two types of programmed cell death. The first type, which is seen in vitro using cultured cells and in vivo when the subjects are young animals, is called **apoptosis**. Apoptosis involves a

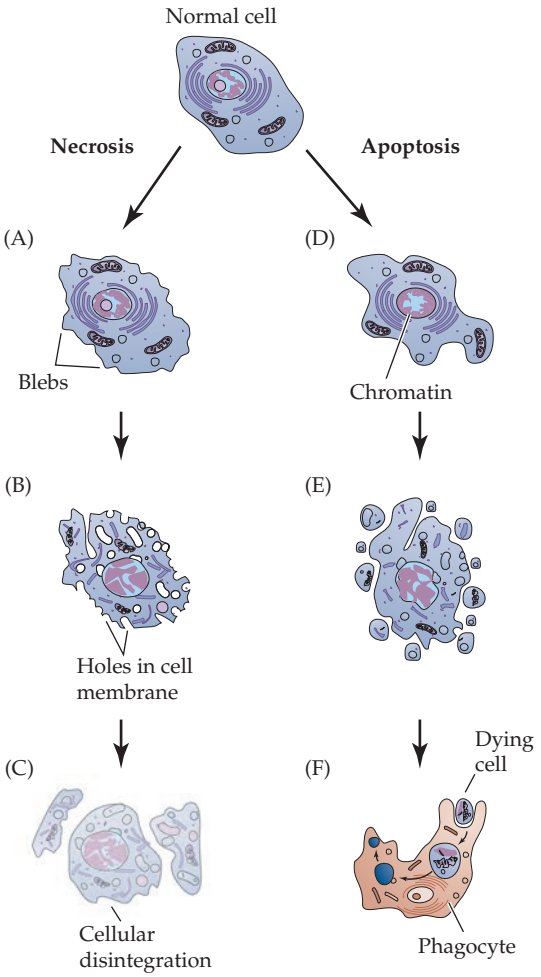


FIGURE 8.14 Cell death by necrosis versus apoptosis
During the initial stages of necrosis (A), the cell swells and the membrane forms protrusions called blebs. In the next stage (B), the membrane begins to break up and release the contents of the cell cytoplasm. Finally, the cell disintegrates completely (C). In apoptotic cell death, the cell also blebs (D), but instead of swelling, it shrinks. At the same time, the chromatin (genetic material) condenses within the cell nucleus. The cell then breaks up into smaller pieces (E) that are subsequently engulfed and digested by phagocytes (F).

complex cascade of biochemical events that lead to disruption of the cell's nucleus, DNA breakup, and ultimately cell death. One of the differences between necrosis and apoptosis is that apoptotic cells do not lyse and spill their contents into the extracellular space. Instead, they are cleared away by other cells through a process called phagocytosis (**FIGURE 8.14**). A significant amount of apoptosis occurs normally during fetal brain development because the brain generates more cells than will be needed later on. However, excitotoxic treatment of young animals can also activate the apoptotic cell death program, thereby leading to inappropriate and excessive loss of nerve cells. The second type of programmed cell death, which is provoked by excitotoxic treatment of adult animals, has been termed either **programmed necrosis** or **necroptosis** (Fayaz et al., 2014) because the appearance of the dying neurons is different from the appearance of neurons undergoing apoptosis. Apoptosis and programmed necrosis also differ significantly in the biochemical mechanisms by which the cell is killed.

Fujikawa (2015) has summarized evidence that excitotoxicity in the adult brain is typically mediated by programmed necrosis rather than apoptosis. Furthermore, glutamate triggering of this cell death program depends on NMDA receptors with a specific subunit composition and cellular localization. We mentioned earlier that NMDA receptors contain two GluN1 and, usually, two GluN2 subunits. However, the situation is more complicated in that four different kinds of GluN2 subunits exist, designated A to D. NMDA receptors localized to the area of the synapse primarily contain GluN2A subunits. These receptors mediate NMDA-dependent LTP, and they also participate in a signaling pathway that promotes neuronal survival. In contrast, there exist "extrasynaptic" NMDA receptors that primarily contain GluN2B subunits. These receptors can be activated during times of abnormally high glutamate levels in the extracellular space, due to excessive glutamate release and/or inadequate uptake by EAATs. Although both synaptic and extrasynaptic NMDA receptors permit Ca^{2+} influx when activated, the Ca^{2+}-dependent signaling pathway linked to extrasynaptic NMDA receptors is the one responsible for triggering excitotoxic cell death (Lai et al., 2014).

EXCITOTOXIC CELL DEATH IN CLINICAL MEDICINE

Mechanisms of excitotoxicity have been studied most extensively in cell culture and in experimental animals. But this phenomenon also occurs in humans, with significant clinical implications. One of the clearest examples of excitotoxic brain damage in humans is the damage produced by ingesting large amounts of a toxic excitatory amino acid called **domoic acid**. This toxin is made by several species of marine algae; is taken up and concentrated by certain shellfish, crabs, and fish; and is passed on to humans who eat the tainted food. Domoic acid poisoning first came to the attention of health officials in 1987, when more than 100 people in Prince Edward Island, Canada, were afflicted after consuming blue mussels contaminated with domoic acid. The victims developed various neurological symptoms such as headache, dizziness, muscle weakness, mental confusion, and, in some cases, permanent loss of short-term memory. Three people died. Since that time, unsafe levels of domoic acid in seafood have periodically been found off the west coast of the United States and Canada. Local and national governments regulate seafood consumption when domoic acid levels are high. However, groups that routinely eat large amounts of

shellfish (e.g., many Native Americans living in the Pacific Northwest) experience long-term exposure to lower amounts of this toxin, and the consequences of such exposure for neurobehavioral functioning in adults or for brain development in children are not yet known (Lefebvre and Robertson, 2010). Furthermore, because it is impossible to prevent all wildlife from being exposed to domoic acid, many dolphins, sea lions, and seabirds have become ill and died from ingesting this substance. In fact, Alfred Hitchcock's film *The Birds* is believed to have been based on a 1961 incident in Santa Cruz County, California, in which domoic acid–poisoned seabirds began to crash into pedestrians, automobiles, and buildings (Bargu et al., 2012). In reality, the birds were not attacking the town, but rather had become weak and disoriented because of the effects of the toxin.

Excitotoxic brain damage also occurs in people who experience brain **ischemia**, which is an interruption of blood flow to the brain, and in some cases of traumatic brain injury (Lau and Tymianski, 2010). Ischemia can result from either a stroke (focal ischemia, usually caused by an embolism that impedes blood flow to a specific region of the brain) or, less commonly, a heart attack (global ischemia, where blood flow to the entire brain is interrupted). Depending on the affected area, stroke may cause severe impairments in motor function, sensory mechanisms, language, or memory. This neurological disorder is also the second most frequent cause of mortality beyond the age of 60 years (Chamorro et al., 2016). When a stroke occurs, brain tissue that is completely or nearly completely deprived of oxygen rapidly dies. This is the **core** area of the stroke, and no treatment can prevent this brain damage from occurring. In contrast, there is a larger volume of tissue surrounding the ischemic core, called the **penumbra**, that experiences only partial oxygen deprivation (**FIGURE 8.15**). Since cells within the penumbra do not die right away, there is hope that this brain tissue can be salvaged, thereby limiting the consequences of the stroke to the patient. The problem with attempting to rescue the cells within the penumbra is that the partial oxygen deprivation leads to massive neuronal depolarization because the ion pumps that maintain the resting potential require a constant generation of ATP, which, in turn, requires oxygen. This membrane depolarization causes a massive release of glutamate in the affected area, thereby leading to prolonged activation of NMDA receptors, including those that trigger the excitotoxic cell death pathway. Using animal models of focal ischemia, researchers have tested the effects of blocking NMDA receptor activation with drugs that target glutamate binding, binding at the co-agonist (glycine/D-serine) site, or the PCP binding site within the receptor channel. These approaches have all proved successful in reducing the amount of ischemic cell death. Unfortunately, a large number of human clinical trials with the same drugs have thus far been largely disappointing (Chamorro et al., 2016). Compounds that appeared promising in preclinical studies failed to show therapeutic benefit in patients and sometimes led to severe side effects. Indeed, noncompetitive NMDA receptor antagonists that bind to the PCP site within the receptor channel can produce psychotic-like symptoms in people (see Chapter 15). Some researchers have theorized that even when a drug is well tolerated by patients, the treatment may have failed because of the time lag between the stroke and beginning the treatment (i.e., it may be too late to prevent excitotoxicity by blocking the NMDA receptors). This idea has led to new treatment approaches, still under development, that are aimed at inhibiting the cell death pathways *triggered* by NMDA receptor activation (Lai et al., 2014; Hoque et al., 2016). Another interesting idea is to enhance clearance of the excessive glutamate by increasing expression or activity of glutamate transporters, especially the key astrocyte transporter EAAT2

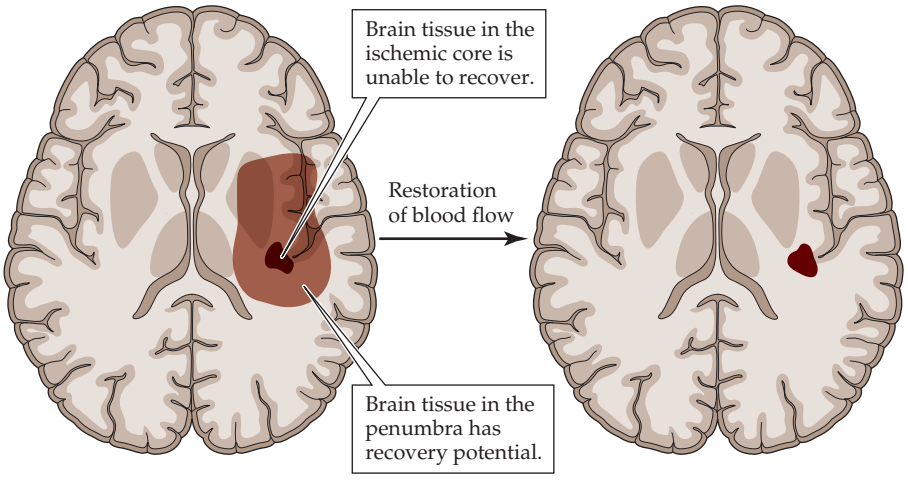


FIGURE 8.15 The brain tissue affected by an ischemic stroke can be separated into the ischemic core and the penumbra The ischemic core consists of the tissue that has suffered the greatest interruption of blood flow and is destined to die regardless of treatment. The penumbra consists of a larger area of tissue surrounding the core that has suffered less severe interruption of blood flow and is believed to have the potential to recover if appropriate medications can be developed. (After Hooper, 2015.)

(Krzyżanowska et al., 2014; Fontana, 2015). Given the aging population in the United States and other developed countries, finding more effective therapies for stroke patients is of prime importance in the area of drug development.

Excitotoxic cell death in ischemic stroke is an acute process provoked by rapid increases in extracellular glutamate levels. Domoic acid poisoning similarly occurs over a relatively short time period. On the other hand, there is also evidence for a chronic, slower-acting excitotoxic process that may be present in other CNS disorders (Lewerenz and Maher, 2015). For many years, excitotoxicity has been hypothesized to occur in **amyotrophic lateral sclerosis** (**ALS**), also known as motor neuron disease or, colloquially, Lou Gehrig's disease. ALS is a fatal neurological disorder involving a slow but progressive degeneration of motor neurons in the spinal cord and cortex (Zarei et al., 2015; Balendra and Patani, 2016; see Chapter 20 for a more detailed description of the disorder). Some cases of ALS can be traced to genetic mutations; however, the majority have no known cause. Evidence for glutamate-mediated excitotoxicity in ALS is based largely on abnormalities of the glutamate system in postmortem brain tissue from patients with ALS (Blasco et al., 2014; King et al., 2016) and findings of cortical hyperexcitability in patients tested using transcranial magnetic stimulation (TMS; Vucic et al., 2014). Moreover, the only treatment available at this time is **riluzole**, which is believed to exert its therapeutic action by reducing glutamate release (Blasco et al., 2014). Importantly, none of this evidence is conclusive, which leaves unproven the glutamate excitotoxicity hypothesis of ALS. Nevertheless, researchers are continuing to explore novel therapeutic approaches for this disorder that target the glutamate system. One such target is EAAT2, which was mentioned above in our discussion of new directions in treating ischemic stroke. For example, a recent study demonstrated that administration of a drug that enhances EAAT2 expression caused a significantly slower loss of motor function and a prolonged life span in a mouse model of ALS (**FIGURE 8.16**; Kong et al., 2014). Even though the results were obtained in an animal model, they provide new support for the notion that excitotoxicity may play an important role in the loss of motor neurons in ALS.

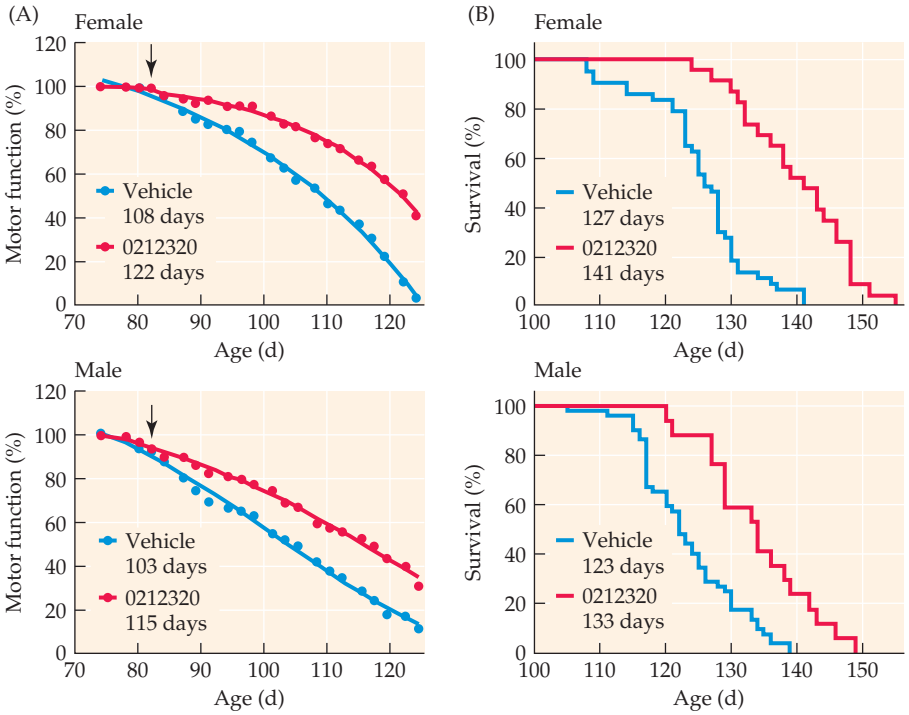


FIGURE 8.16 Pharmacological enhancement of EAAT2 expression improves motor function and prolongs the life span in a mouse model of ALS
Female and male transgenic mice that reproduce the symptoms of ALS were given daily IP injections of LDN/OSU-0212320 (40 mg/kg) or vehicle beginning at 84 days of age (shown by the arrow) and continuing until the animals were dead. Motor function was determined using a test of grip strength. (A) Drug treatment (red circles) significantly slowed the loss of motor function in both female and male mice compared with vehicle (blue circles). The values above the *x*-axes of the graphs indicate the average number of days until a 50% decline in grip strength occurred in each group. (B) Survival curves show that life span was also increased in the treated mice (red line). The values above the *x*-axes of the graphs indicate the average number of days of survival in each group. (After Kong et al., 2014.)

Finally, although space limitations do not permit further discussion of slow excitotoxic cell death, it should be noted that this process may also occur in Alzheimer's disease (Ong et al., 2013; Rudy et al., 2015; see Chapter 20) and in refractory temporal lobe epilepsy with hippocampal sclerosis (i.e., loss of neurons in the hippocampus) (Cendes et al., 2014; Walker, 2015; see Box 8.2 later in this chapter).

Section Summary

- Glutamate is the workhorse for fast excitatory signaling in the nervous system. There are numerous glutamatergic pathways in the brain, including the projections of the pyramidal neurons of the cerebral cortex, the parallel fibers of the cerebellar cortex, and several excitatory pathways within the hippocampus.
- AMPA, kainate, and NMDA receptors constitute the three subtypes of ionotropic glutamate receptors. Each is named for an agonist that is relatively selective for that subtype. All of these receptors permit Na^+ ions to cross the cell membrane, thereby producing membrane depolarization and an excitatory postsynaptic response. NMDA receptors also conduct Ca^{2+} ions and can trigger Ca^{2+}-dependent second-messenger actions within the postsynaptic cell.
- Most NMDA receptors are composed of two GluN1 subunits and two GluN2 subunits.
- AMPA and kainate receptors possess different protein subunits that give them somewhat different electrophysiological and pharmacological properties. Behavioral functions of AMPA receptors have been revealed through the use of the antagonist NBQX. Administration of high doses of this compound to rodents leads to sedation, ataxia, deficient rotarod performance, and protection against seizures, indicating involvement of this receptor subtype in locomotor activity, coordination, and brain excitability.
- NMDA receptors are distinct from the AMPA and kainate receptor subtypes in several ways, in addition to the difference in ionic conductances. First, the opening of NMDA receptor channels requires a co-agonist in addition to glutamate. This co-agonist may be either glycine or D-serine. Second, NMDA receptors possess a binding site for Mg^{2+} ions within the receptor channel. When the cell membrane is at the resting potential, this site is occupied and the channel is blocked even if the receptor has been activated by agonists. However, depolarization of the membrane reduces Mg^{2+} binding, thus allowing the channel to open. Consequently, for the NMDA receptor to function, some other synaptic input must excite the cell at the same time that glutamate and either glycine or D-serine bind to the receptor. Third, NMDA receptors also possess a channel binding site that recognizes PCP, ketamine, memantine, and MK-801. These compounds act as noncompetitive antagonists of the NMDA receptor.
- There are also eight different metabotropic receptors for glutamate, designated mGluR1 to mGluR8. Group I metabotropic glutamate receptors, consisting of mGluR1 and mGluR5, are located postsynaptically and mediate excitatory responses by stimulating the phosphoinositide second-messenger system. Group II receptors, consisting of mGluR2 and mGluR3, and group III receptors, consisting of mGluR4 and mGluR6–8, are typically located presynaptically where they reduce transmitter release by inhibiting cAMP formation.
- Compounds acting at various mGluRs are being tested for their possible usefulness in treating a variety of neuropsychiatric disorders, including schizophrenia. One potential application is in fragile X syndrome, a leading genetic cause of intellectual disability and autistic symptoms. This syndrome is caused by mutations in the *fragile X mental retardation 1* gene, which codes for the fragile X mental retardation protein (FMRP). Researchers proposed a metabotropic glutamate receptor theory of fragile X syndrome, in which loss of FMRP causes exaggerated group I mGluR–related functions, leading to dendritic spine abnormalities and elevated rates of LTD. However, clinical trials with mavoglurant, an mGluR5 antagonist, failed to produce significant symptom improvement in patients with fragile X syndrome.
- NMDA and AMPA receptors are believed to play an important role in learning and memory. NMDA receptor antagonists impair the acquisition of various learning tasks. Activation of this receptor is necessary for the *induction* of hippocampal LTP, a mechanism of synaptic strengthening. The Ca^{2+}-activated enzyme CaMKII has an obligatory role in LTP induction. LTP *expression*, which is independent of NMDA receptors, involves increased trafficking of AMPA receptors into the postsynaptic membrane. LTP can be divided into two phases: E-LTP, which has just been described and only persists for a few hours, and the longer-lasting L-LTP, which involves protein synthesis, release of the neuropeptide BDNF, activation of atypical PKCs, and dendritic spine growth. LTP may occur in the human brain, particularly a form of LTP induced by theta burst stimulation.

- Excessive exposure to glutamate and other excitatory amino acids can damage or even kill nerve cells through a process of depolarization-induced excitotoxicity. This process is usually mediated primarily by NMDA receptors, with some contribution from AMPA and/or kainate receptors. One type of excitotoxic cell death occurs via rapid necrosis, which involves cellular swelling and eventual lysis. Additionally, there are slower programmed forms of cell death that either have some of the features of necrosis or are mediated by apoptosis, which involves disruption of the cell nucleus and breakdown of DNA.
- In humans, excitotoxic cell death can be caused by ingestion of food contaminated with the algal toxin domoic acid. Excitotoxicity is also thought to be a major contributory factor to the brain damage that occurs in the penumbra of a focal ischemic event (e.g., stroke) and in some kinds of traumatic brain injury. This type of excitotoxic cell death is mediated by Ca^{2+} entry through extrasynaptic NMDA receptors containing GluN2B subunits. A slower form of excitotoxicity may be a contributing factor in the loss of motor neurons in ALS and the loss of hippocampal neurons in refractory temporal lobe epilepsy. Therapeutic approaches to reduce excitotoxic cell death include blockade of glutamate receptors, inhibition of glutamate release (e.g., by riluzole), and enhancement of glutamate uptake (e.g., by EAAT2 up-regulation).

GABA

Earlier in this chapter we saw that glutamate plays a dominant role in fast excitatory transmission in the central nervous system (CNS). Inhibitory transmission is equally important in behavioral control mechanisms. The significance of neural inhibition is evident from the fact that blocking the action of either of the two major inhibitory amino acid transmitters leads to convulsions and even death. These two transmitters are **GABA** (**γ-aminobutyric acid**) and glycine. The remainder of this chapter focuses primarily on GABA, which is the more important of the two transmitters in the brain.

GABA Synthesis, Release, and Inactivation

Whereas the amino acid glutamate participates widely in cellular metabolism, including protein synthesis, the only function of GABA is to serve as a neurotransmitter. Hence, it is manufactured only by GABAergic neurons. This section of the chapter will discuss the synthesis, release, and inactivation of GABA.

GABA is synthesized by the enzyme glutamic acid decarboxylase

GABA is synthesized from glutamate in a single biochemical step, which is catalyzed by the enzyme **glutamic acid decarboxylase** (**GAD**) (**FIGURE 8.17**). It is interesting to note that the principal inhibitory neurotransmitter in the brain, namely GABA, is made from the principal excitatory transmitter, glutamate. GAD is localized specifically to GABAergic neurons; therefore, researchers can identify such neurons by staining for GAD.

Several drugs, including **allylglycine**, **thiosemicarbazide**, and **3-mercaptopropionic acid**, are known to block GABA synthesis. As noted earlier, a significant reduction in GABA synthesis leads to convulsions; this indicates the importance of this transmitter in regulating brain excitability. On the other hand, it also shows that GAD inhibitors are normally used to study GABAergic transmission only in vitro, not in vivo.

GABA packaging into vesicles and uptake after release are mediated by specific transporter proteins

Like the vesicular transporters that take up glutamate into synaptic vesicles, the **vesicular GABA transporter** (**VGAT**) was discovered later than some of the other vesicular transporters. Subsequent studies revealed an interesting and unexpected feature of this protein, namely, that it is also found in neurons that use glycine as a transmitter. Thus the same transporter is used to load GABA or glycine into synaptic vesicles. For this reason, this transporter is sometimes referred to as **VIAAT** (**vesicular inhibitory amino acid transporter**) instead of VGAT. This situation of transport of multiple neurotransmitters by the same protein is similar to the previously discussed example of VMAT, the vesicular monoamine transporter, which is responsible for vesicle filling of three different neurotransmitters: dopamine (DA), norepinephrine (NE), and serotonin (5-HT).

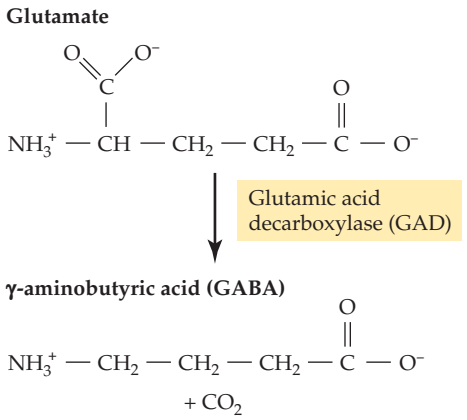


FIGURE 8.17 GABA is synthesized from glutamate
The synthesis is catalyzed by the enzyme glutamic acid decarboxylase (GAD).

Following the synaptic release of GABA, it is removed from the extracellular space by three different transporters on the membranes of nerve cells and glia, designated **GAT-1**, **GAT-2**, and **GAT-3**. GAT-1 and GAT-2 appear to be expressed in both neurons and astrocytes, whereas GAT-3 is found in astrocytes only. GAT-1 has received particular attention for two reasons. First, this transporter has been found at the nerve terminals of GABAergic neurons; therefore, it is likely to be important for GABA reuptake by these cells. Second, in contrast to GAT-2 and GAT-3, a selective inhibitor of GAT-1 is available for pharmacological study. Administration of this compound, **tiagabine**, elevates extracellular GABA levels and enhances GABAergic transmission in several brain areas, including the cortex and the hippocampus. Given the fact that depleting GABA (e.g., by blocking GAD activity) causes seizures, we might predict that tiagabine protects against seizure onset. Indeed, tiagabine was licensed in 1997 under the trade name **Gabitril** for use as an adjunctive therapy (an additional treatment given along with more-standard antiepileptic drugs) in treatment-resistant patients with partial seizures (seizures involving only part of the brain). Tiagabine appears to be clinically beneficial in this role, and it is being tested as a monotherapy (single treatment) for certain kinds of epilepsy.

Whereas the immediate inactivation of GABA in the synapse occurs through a combination of neuronal and astroglial uptake, there is also a cellular mechanism for metabolizing and recycling this neurotransmitter. GABA breakdown occurs through several steps, beginning with the enzyme **GABA aminotransferase** (**GABA-T**) and leading eventually to the final product, succinate. It is worth noting that a by-product of this metabolic pathway is the formation of one molecule of glutamate for every molecule of GABA that is broken down. GABA-T is found in both GABAergic neurons and astrocytes. Hence, within GABAergic neurons, some of the glutamate regenerated by the action of GABA-T could be used to synthesize more GABA. Moreover, some of the glutamate produced by GABA-T in astrocytes could be converted to glutamine by astrocytic glutamine synthetase, and the glutamine could subsequently be transported to the GABAergic neurons to be converted back to glutamate by the enzyme glutaminase (**FIGURE 8.18**). This shows that the metabolic interplay between neurons and glial cells discussed earlier for glutamate is equally important for GABA.

Vigabatrin is an irreversible inhibitor of GABA-T. By preventing GABA metabolism, administration of this drug leads to a buildup of GABA levels within the brain. By now, you should be able to predict correctly that vigabatrin has anticonvulsant effects in animals and humans. Like tiagabine, vigabatrin (trade name, **Sabril**) is being used clinically as either an adjunctive treatment or the primary therapeutic agent for certain types of epilepsy, particularly infantile spasms (repeated generalized seizures in young infants). However,

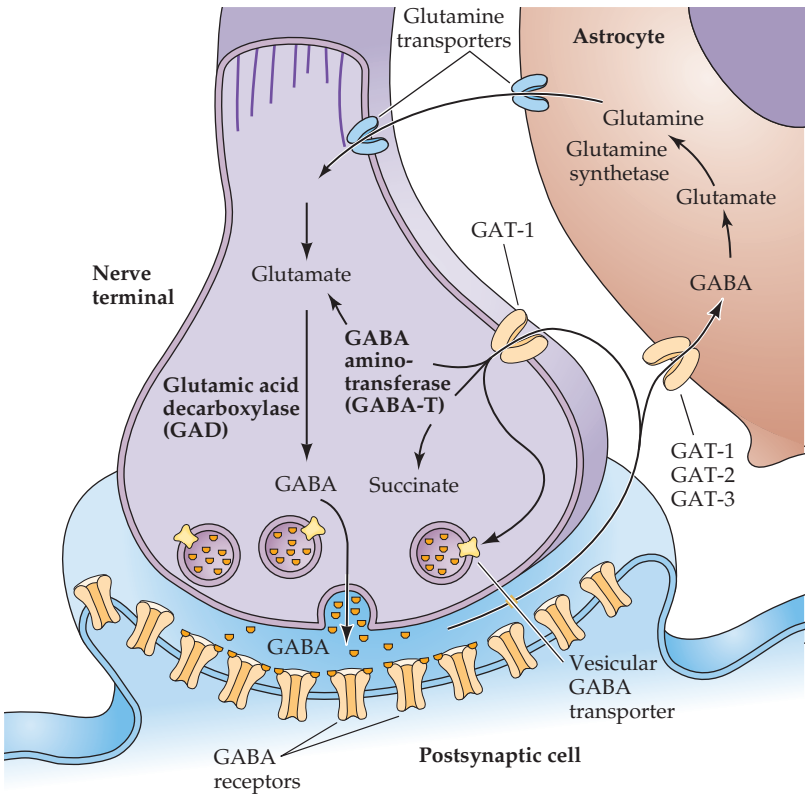


FIGURE 8.18 Cycling of GABA between glutamatergic neurons and astrocytes After neurons release GABA, it can be transported back into the nerve terminal by GAT-1 or transported into nearby astrocytes by GAT-2 or GAT-3. Inside the cell, GABA is metabolized to glutamate and succinate by GABA aminotransferase (GABA-T). In the case of astrocytes, the glutamate is converted into glutamine by the enzyme glutamine synthetase. The glutamine can later be released by the astrocytes, taken up by neurons, converted back into glutamate by the enzyme glutaminase, and finally used to resynthesize GABA.

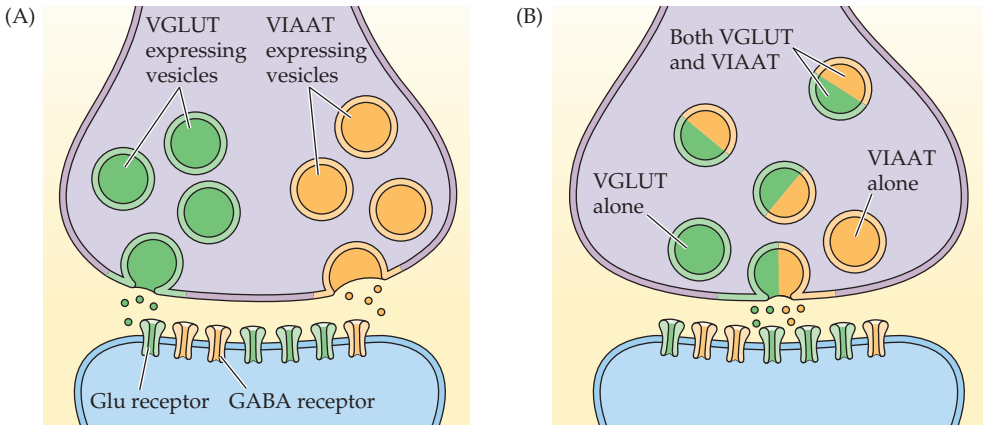


FIGURE 8.19 Possible segregated versus combined synaptic vesicle loading and transmitter release sites in axon terminals that corelease GABA and glutamate (A) This figure depicts a situation in which separate synaptic vesicles express VIAAT or a VGLUT, and these different vesicle populations and their respective release sites are segregated spatially in the terminal. (B) This figure depicts an alternative situation in which vesicles are intermingled that express VIAAT alone, VGLUT alone, or both transporters. Transmitter release sites are also intermingled in this case. (After Münster-Wandowski et al., 2016, CC-BY 4.0.)

there are a number of recent reports that use of vigabatrin can lead to constriction of the visual field in both adults and children. Since there are GABAergic interneurons in the retina, visual abnormalities could be related to drug effects on these cells. In light of these findings, physicians must consider the risk-to-benefit ratio when considering vigabatrin treatment of patients with epilepsy.

GABA is coreleased with several other classical neurotransmitters

Researchers have increasingly become aware that GABA is synthesized and coreleased by many neurons that were previously characterized as using a different classical transmitter. Such co-expression and corelease of GABA has been demonstrated for certain neurons that also use glycine, acetylcholine, and dopamine as neurotransmitters (Tritsch et al., 2016). Corelease is accomplished, in part, by co-expression of multiple vesicular transporters by the same neuron (e.g., VIAAT together with the vesicular acetylcholine transporter, VAChT). A neurotransmitter role for glycine mainly occurs in the brainstem and spinal cord, and current evidence suggests that inhibitory neurons in these areas of the CNS may release GABA only, glycine only, or a combination of both (Aubrey, 2016). Because VIAAT can fill vesicles with either transmitter, it is easy to imagine how a given neuron could regulate the relative availability of one substance or another for the purpose of vesicle filling and release.

More complicated are the situations in which GABA is coreleased with a neurotransmitter other than glycine. In fact, researchers have surprisingly found instances in which the same neuron expressed both VIAAT for GABA and VGLUT1 or VGLUT2 for filling vesicles with glutamate (Münster-Wandowski et al., 2016; Tritsch et al., 2016). **FIGURE 8.19** illustrates two distinct possibilities for GABA–glutamate corelease, namely separate vesicle populations with distinct release sites on the nerve terminal, or a mixture of vesicles that, due to the transporter(s) on their membrane, contain GABA alone, glutamate alone, or a mixture of both. It may seem paradoxical for the same cell to release an excitatory and an inhibitory transmitter at the same time, but such a situation allows for a fine-tuning of the postsynaptic response that would be difficult to achieve using a single neurotransmitter (for more information, see Tritsch et al., 2016).

Section Summary

- GABA is the major inhibitory amino acid neurotransmitter in the brain. This transmitter is synthesized from glutamate in a single biochemical reaction catalyzed by GAD, an enzyme found only in GABAergic neurons.
- Because of the widespread inhibitory effects of GABA on neuronal excitability, treatment with drugs that inhibit GABA synthesis by blocking GAD leads to seizures.
- GABA is taken up into synaptic vesicles by the vesicular transporter VGAT (also known as VIAAT because the same vesicular transporter is used by glycine).
- After release into the synaptic cleft, GABA is removed from the cleft by three different transporters designated GAT-1, GAT-2, and GAT-3. Astrocytes express all three of these transporters and therefore must play a significant role in GABA

uptake. GAT-1 is also found in GABAergic neurons, and the GAT-1 inhibitor tiagabine (Gabitril) is used clinically in the treatment of some patients with epilepsy.

- GABA is co-expressed and coreleased with several other classical neurotransmitters, including glycine, acetylcholine, dopamine, and glutamate. This is accomplished, in part, by co-expression of the vesicular transporters for multiple transmitters in the same neuron. Corelease of glutamate is of particular interest, as it involves a combination of inhibitory and excitatory neurotransmitter signaling.
- In addition to uptake, the other process that regulates GABAergic transmission is GABA metabolism. The key enzyme in GABA breakdown is GABA-T, which is present in both GABAergic neurons and astrocytes. A by-product of the reaction catalyzed by GABA-T is glutamate, which is the precursor of GABA. Hence, GABA breakdown in neurons or in glial cells may involve a recycling process that assists in the formation of new GABA molecules.
- Vigabatrin (Sabril) is an irreversible inhibitor of GABA-T and thereby elevates GABA levels in the brain. Like tiagabine, vigabatrin has been licensed for the treatment of certain types of epilepsy. However, there are reports that repeated vigabatrin use can lead to visual system abnormalities in adults and children; therefore, caution should be exercised when this compound is administered to patients.

Organization and Function of the GABAergic System

Like glutamate, GABA is used by many populations of neurons in the brain. Here we discuss some features of the anatomy of the GABAergic system, subtypes of GABA receptors, and a few basic functions of GABAergic transmission.

Some GABAergic neurons are interneurons, while others are projection neurons

Fonnum (1987) has estimated that as many as 10% to 40% of the nerve terminals in the cerebral cortex, hippocampus, and substantia nigra use GABA as their neurotransmitter. Even the lower range of this estimate indicates that a lot of GABAergic transmission takes place, when you consider the dozens of different neurotransmitters that may be present within a specific brain region. In addition to the three structures just mentioned, other brain areas rich in GABA are the cerebellum, striatum, globus pallidus, and olfactory bulbs.

In some structures, such as the cortex and the hippocampus, GABA is found in large numbers of local interneurons. However, there are also GABAergic projection neurons that carry inhibitory information longer distances within the brain. For example, GABAergic neurons of the striatum project to the globus pallidus and the substantia nigra. When DA input to the striatum is damaged in Parkinson's disease, the result is abnormal firing of the striatal GABAergic neurons, which causes the motor abnormalities seen in this neurological disorder (see Chapter 20). GABA is also the transmitter used by Purkinje cells of the cerebellar cortex. These neurons, which project to the deep cerebellar nuclei and to the brainstem, have an important function in fine muscle control and coordination. This is illustrated in a rare disorder involving degeneration of cerebellar Purkinje cells. Patients with this disorder, which is known as Holmes cerebellar degeneration, show ataxia when walking, impaired fine hand movements, defective speech, and tremors.

The actions of GABA are primarily mediated by ionotropic $GABA_A$ receptors

Like glutamate, GABA makes use of both ionotropic and metabotropic receptors. However, only one type of each is used: the **$GABA_A$ receptor**, which is ionotropic, and the **$GABA_B$ receptor**, which is metabotropic. Our discussion will concentrate on the $GABA_A$ receptor because of its prominent role in GABAergic transmission and because it is a crucial target of many important psychoactive drugs.

STRUCTURE AND FUNCTION OF THE $GABA_A$ RECEPTOR

$GABA_A$ receptors are ion channels that permit Cl^- ions to move across the cell membrane from outside to inside. This causes inhibition of the postsynaptic cell as the result of membrane hyperpolarization. More Cl^- ions flow through open $GABA_A$ receptor channels when the membrane has previously been depolarized by excitatory synaptic inputs. In such cases, these receptors function to blunt depolarization and prevent the cell from firing an action potential. Although we normally think about the $GABA_A$ receptor mediating Cl^- influx and, consequently, membrane hyperpolarization, there are circumstances under which the opposite occurs, namely Cl^- outflow that leads to membrane depolarization and cellular excitation instead of inhibition. One such circumstance may be seizure-prone areas of the brain in patients with epilepsy. **BOX 8.2** discusses the potential role of GABA, $GABA_A$ receptors, and drugs acting on these receptors in the genesis of seizure activity and in pharmacotherapy for epilepsy.

Structurally, each $GABA_A$ receptor contains five subunits. Three or four different *kinds* of subunits may

BOX 8.2 Clinical Applications

GABA and Epilepsy

The term *epilepsy* refers to a class of neurological disorders characterized by recurrent convulsive and nonconvulsive seizures. It is estimated that seizure disorders affect roughly 3 million Americans and 65 million people worldwide (Epilepsy Foundation, n.d.). The seriousness of this disorder can be seen in the fact that postmortem studies of patients with epilepsy, especially temporal lobe epilepsy, have shown brain cell loss in the hippocampus (termed **hippocampal sclerosis**) and that an excitotoxic, NMDA receptor–mediated process may be involved in such loss (Thom, 2014). In the present discussion, we will focus on the possible role of GABA in the etiology and maintenance of seizure disorders.

One of the characteristic features of epilepsy is that during the **interictal period** (the period between seizures), principal neurons (i.e., pyramidal cells) in the cortex and certain subcortical structures exhibit periodic episodes of prolonged depolarization called a **paroxysmal depolarization shift** (**PDS**). During a PDS, the cell membrane is depolarized much longer than for a typical EPSP, and as a result the cell fires a burst of action potentials (Browne and Holme, 2008). This massive depolarization is followed by the second phase, which is a period of hyperpolarization that reflects the activation of inhibitory mechanisms impinging on the cells. One of the processes thought to underlie the transition from interictal discharges to the generation of a full-blown seizure is a decrease in the hyperpolarizing phase, and thus a failure of inhibition. Given that GABA is one of the main transmitters involved in neuronal inhibition, it seems possible that GABAergic dysfunction might play a role in the epileptogenic process (Sperk et al., 2004; Blauwblomme et al., 2014).

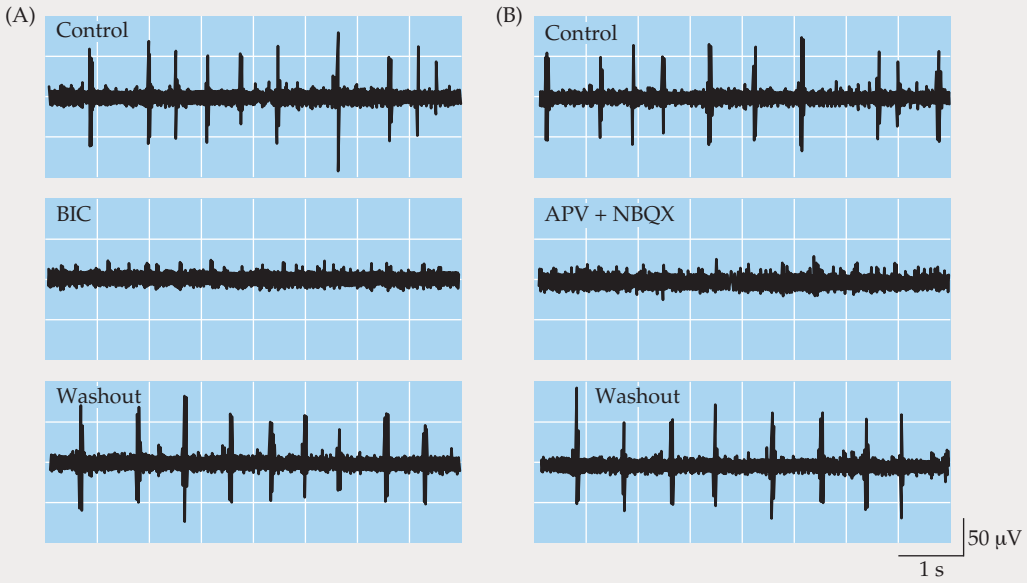
Some of the evidence for GABA involvement in epilepsy comes from studies of epileptic tissue obtained surgically from patients who failed to respond to standard drug treatments. Surgical intervention occurs most commonly in cases of treatment-resistant temporal lobe epilepsy. Although surgical removal of brain tissue is obviously of serious concern to the patient, researchers have utilized this opportunity to study living human epileptic brain tissue to help determine how it may be malfunctioning. One approach used in this research was to perform electrical recordings of hippocampal slices obtained from patients. These recordings showed that pyramidal cells and interneurons from the subiculum (an area responsible for most of the output of the hippocampal formation) exhibited spontaneous activity similar to interictal discharges seen in EEGs from patients with epilepsy, and, most importantly, this activity was mediated not only by ionotropic glutamate receptors (as might be expected) but also by $GABA_A$ receptors (Cohen et al., 2002; Huberfeld et al., 2007) (see **Figure**). Additional experiments demonstrated that the shift in $GABA_A$-mediated responses from inhibitory to excitatory was due to the fact that opening of the receptor channel resulted in Cl^- *efflux* from the cell instead of the normal Cl^- *influx*, thus causing membrane depolarization instead of hyperpolarization. Interestingly, it turns out that $GABA_A$ receptors behave this way during early brain development because the ratio of Cl^- ions inside versus outside the cells differs from that ratio in the mature brain (i.e., during development, the Cl^- ion concentration inside these neurons is much greater than it is later on; Ben-Ari et al., 2012; Kaila et al., 2014). These findings suggest that one factor in this form of severe temporal lobe epilepsy may be the return of at least some hippocampal $GABA_A$ receptor–expressing neurons to an earlier developmental state, at least with respect to their regulation of Cl^- ion distribution.

A loss of GABA-mediated inhibition is particularly significant in the development of **status epilepticus**, a highly dangerous condition characterized either by a continuous seizure lasting longer than 30 minutes or by episodes of repeated seizures that recur so quickly that the patient has insufficient time to recover from one seizure before another one begins. Diazepam and other benzodiazepines (BDZs), compounds that potentiate the action of GABA on the $GABA_A$ receptor (see the section entitled Allosteric Modulators of the $GABA_A$ Receptor), are usually effective in suppressing seizures in patients with epilepsy. However, these compounds are much less effective in patients with a history of status epilepticus. Such resistance to BDZs has been linked to seizure-induced internalization (i.e., endocytosis) of BDZ-sensitive $GABA_A$ receptors from the cell surface (Goodkin and Kapur, 2009; Deeb et al., 2012). Fortunately, $GABA_A$ receptors that are responsive to other anticonvulsant drugs (e.g., barbiturates, anesthetics, and neurosteroids) are still available, providing the opportunity for seizure suppression by these other drug classes. Indeed, Schipper and colleagues (2015) recently proposed targeting the BDZ-insensitive extrasynaptic $GABA_A$ receptors that mediate tonic neuronal inhibition for the treatment of temporal lobe epilepsy.

Most cases of epilepsy have no known genetic cause; however, in some instances mutations have been discovered either in the GABAergic system or in other systems (e.g., voltage-gated ion channels)

(*Continued*)

BOX 8.2 Clinical Applications (*continued*)



Neurotransmitter modulation of spontaneous neuronal activity in brain slices from patients with epilepsy Patients with temporal lobe epilepsy who responded poorly to antiepileptic drug treatment underwent surgery for unilateral removal of the tissue responsible for seizure initiation. After this tissue had been sliced and maintained temporarily in vitro, electrical activity was recorded from the subiculum under baseline (control) conditions and in response to (A) the $GABA_A$ receptor antagonist bicuculline (BIC) or (B) a combination of the glutamate NMDA receptor antagonist 2-amino-5-phosphonovalerate (APV) plus the non-NMDA antagonist NBQX. Both pharmacological treatments completely blocked spontaneous electrical discharges—an effect that was reversed when the drugs were washed out of the slice. (From Cohen et al., 2002.)

that can be causally linked to the seizure disorder. Epilepsy-related mutations in $GABA_A$ receptor subunits include those in γ2 (childhood absence epilepsy [CAE], generalized epilepsy with febrile seizures [GEFS], Dravet syndrome, infantile spasms [IS], and genetic generalized epilepsy [GGE]), α1 (CAE, GGE, IS, and juvenile myoclonic epilepsy [JME]), α6 (CAE), β2 (IS), β3 (CAE, IS, and Lennox-Gastaut syndrome), and δ (GEFS and JME) (Hirose, 2014). Although space considerations do not permit us to describe the features of each of these types of epilepsy, we note that all of these disorders begin in infancy or childhood, they exhibit a range of different physical manifestations, and many of them are characterized by additional adverse health outcomes such as developmental delay or disability, and shortened life span.

To summarize, evidence for GABA involvement in epilepsy comes from many sources. First, a failure of GABA-mediated inhibition is thought to be one of the mechanisms underlying the transition from interictal discharges to the generation of a full-blown seizure. Second, biopsies from patients with treatment-resistant temporal lobe epilepsy have shown excitatory, instead of inhibitory, responses to $GABA_A$ receptor activation. This change is due to Cl^- ion efflux instead of influx through the open receptor channel. Third, $GABA_A$ receptor internalization off the cell membrane occurs in status epilepticus, thereby reducing the efficacy of BDZs to suppress seizure activity. Finally, mutations in the genes coding for several $GABA_A$ receptor subunits can lead to early-onset epileptic disorders. Taken together, this evidence clearly confirms that ongoing GABAergic activity and normal $GABA_A$ receptor functioning are necessary to prevent abnormal, uncontrolled increases in brain excitability.

be found within a particular $GABA_A$ receptor complex. These different kinds of subunits are designated by the Greek letters α, β, γ, and δ.[2] Most $GABA_A$ receptors are thought to contain two α subunits, two β subunits, and one γ subunit (**FIGURE 8.20**). There are multiple isoforms of all of these subunits, consisting of six different αs (designated α1–α6), three different βs (β1–β3), and three different γs (γ1–γ3). According to Jembrek and Vlainić (2015), approximately 60% of $GABA_A$ receptors in the brain have a subunit composition of $(\alpha1)_2(\beta2)_2(\gamma2)$ (this means two α1 subunits,

[2]There are additional kinds of subunits designated with other Greek letters, but these subunits are found in relatively few $GABA_A$ receptors in the brain and, therefore, are not discussed here.

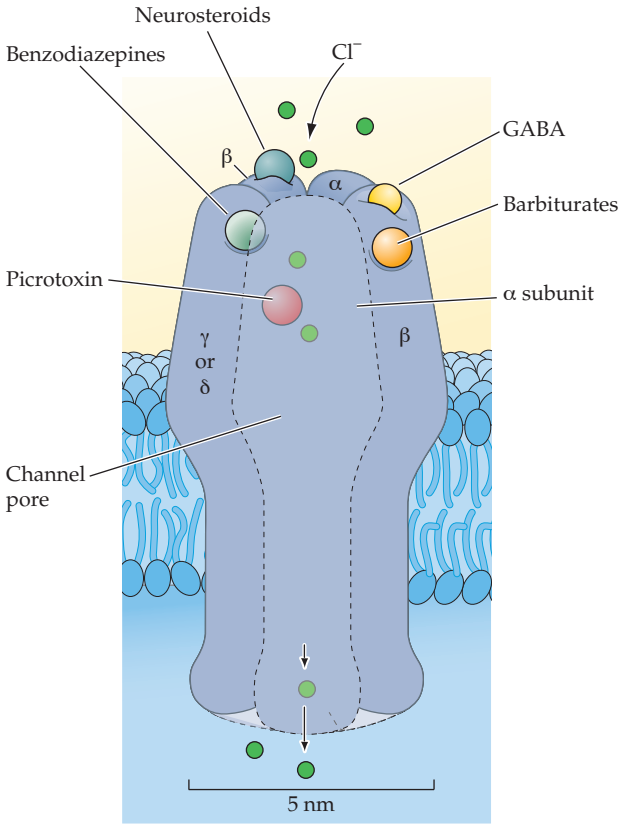


FIGURE 8.20 The $GABA_A$ receptor The receptor consists of five subunits that form a Cl^--conducting channel. In addition to the GABA binding site on the receptor complex, there are additional modulatory sites for benzodiazepines, barbiturates, neurosteroids, and picrotoxin. Note that the locations of the various binding sites are depicted arbitrarily and are not meant to imply the actual locations of these sites on the receptor.

two β2 subunits, and one γ2 subunit). At 15% to 20%, the next most common receptor composition is $(\alpha2)_2(\beta3)_2(\gamma2)$, followed by receptors that contain two α3 subunits, two β subunits of some kind, and one γ2 subunit (10%–15%). A small number of receptors contain a δ subunit instead of a γ subunit. Such receptors typically are found in combination with two α4 or α6 subunits (along with the normal two β subunits) and are localized outside the normal synaptic area (Belelli et al., 2009). These extrasynaptic receptors respond to low levels of GABA that have avoided immediate uptake following release and have escaped from the synaptic cleft. Their role is to exert a mild tonic (continuous) hyperpolarizing and thus inhibitory effect on the cells that express those receptors.

The classic agonist for the $GABA_A$ receptor is a drug called **muscimol**. This compound is found in the mushroom *Amanita muscaria* (**FIGURE 8.21**), which was mentioned in Chapter 7 as the original source of the cholinergic agonist muscarine. In earlier times, this mushroom apparently would be chopped up and placed in a dish with milk to attract flies. Ingestion of muscimol and the related compound ibotenic acid would make the flies stuporous and easy to catch. The common name for *A. muscaria* is therefore "fly agaric" (*agaric* is an old term meaning "mushroom"). Rudgley (1999) discusses the custom of eating fly agaric, as practiced by various Siberian peoples for hundreds of years. The mushroom was prized for its stimulatory and hallucinogenic qualities—effects that can also be obtained by drinking urine from an intoxicated individual (not a very appealing idea to most Westerners!).[3] One of the interesting hallucinatory effects produced by the fly agaric is macroscopia, which refers to the perception of objects as being larger than they really are. When administered to humans at relatively high doses, pure muscimol causes an intoxication characterized by hyperthermia (elevated body temperature), pupil dilation, elevation of mood, difficulties in concentration, anorexia (loss of appetite), ataxia, catalepsy, and hallucinations. Many of these effects are similar to those associated with more traditional hallucinogenic drugs such as lysergic acid diethylamide (LSD; see Chapter 15).

Bicuculline is the best-known competitive antagonist for the $GABA_A$ receptor. It blocks the binding of GABA to the $GABA_A$ receptor, and when taken systemically, it has a potent convulsant effect. **Pentylenetetrazol** (**Metrazol**) and **picrotoxin** are two other convulsant drugs that inhibit $GABA_A$ receptor function by acting at sites distinct from the binding site of GABA itself. Pentylenetetrazol is a synthetic compound that once was used as convulsant therapy for major depression. Although it is no longer used for that purpose, it still has value for the induction of experimental seizures in laboratory animals. Picrotoxin is obtained from the

[3]Fly agaric intoxication was apparently enjoyed not only by Siberians but by their reindeer as well. Animals were observed to eat the mushrooms of their own accord and were also sometimes given urine to drink from a person who had previously partaken.

FIGURE 8.21 The fly agaric mushroom, *Amanita muscaria* (© Arie v.d. Wolde/Shutterstock.)

seeds of the East Indian shrub *Anamirta cocculus*. Because neither pentylenetetrazol nor picrotoxin prevents GABA from interacting with the $GABA_A$ receptor, these agents are noncompetitive rather than competitive receptor antagonists.

ALLOSTERIC MODULATORS OF THE $GABA_A$ RECEPTOR For psychopharmacologists, the most remarkable property of the $GABA_A$ receptor is its sensitivity to certain CNS-depressant drugs that display an anxiolytic (antianxiety), sedative–hypnotic (sedating and sleep-inducing), and anticonvulsant profile. Among such drugs are the **benzodiazepines** (**BDZs**) and the **barbiturates**. There is overwhelming evidence that the principal mechanism of action of BDZs and barbiturates is positive allosteric modulation of the $GABA_A$ receptor, thereby potentiating GABA-mediated synaptic inhibition. **Ethanol** is another CNS-depressant drug that has many of the same properties as BDZs and barbiturates. Although the actions of ethanol are complex, one of its major effects is likewise to enhance $GABA_A$ receptor activity (Förstera et al., 2016). Chronic administration of any of these $GABA_A$ receptor positive modulators can lead to the development of tolerance, dependence, and withdrawal symptoms if drug treatment is suddenly terminated (Calixto, 2016; Gravielle, 2016). Various studies have shown that these phenomena are mediated, at least in part, by changes in $GABA_A$ receptor expression and function. Interactions of $GABA_A$ receptors with sedative–hypnotic and anxiolytic drugs and with ethanol are discussed in later chapters that cover these compounds. Below we discuss two other groups of positive modulators: anesthetics such as **propofol** (**Diprivan**), an intravenously administered drug frequently used for surgical anesthesia and also the drug responsible for the overdose death of Michael Jackson, and **neurosteroids**, which consist of steroid hormones synthesized in the brain that act locally on the $GABA_A$ receptor.

How do BDZs, barbiturates, ethanol, anesthetics, and neurosteroids exert their influence on the $GABA_A$ receptor? All of these substances interact with sites on the receptor complex that are distinct from the GABA binding site. This concept is illustrated in Figure 8.20, which depicts binding sites for BDZs and barbiturates, as well as a negative modulatory site for picrotoxin and related convulsant drugs. Ethanol, anesthetics, and neurosteroids are not shown in the figure, as these compounds are thought to interact with multiple binding sites on the receptor (Sieghart, 2015). There are also important differences among these compounds in their ability to affect $GABA_A$ receptor activity. BDZs and ethanol can only modulate receptor activity, regardless of the administered dose. In contrast, sufficiently high doses of barbiturates, anesthetics, and neurosteroids can open the receptor channel even in the absence of GABA (Sieghart, 2015).

BENZODIAZEPINE INTERACTIONS WITH THE $GABA_A$ RECEPTOR Although the pharmacology of BDZs is covered extensively in Chapter 17, it is worth noting here some of the key features of the interactions of BDZs with the $GABA_A$ receptor. The recognition site for BDZs on the $GABA_A$ receptor complex is considered a bona fide BDZ receptor because these drugs bind directly to the site, and such binding is necessary for BDZs to exert their behavioral and physiological effects. When a BDZ such as **diazepam** (**Valium**) binds to the BDZ receptor, the potency of GABA to activate the receptor is increased. If we examine this interaction by measuring the amount of inward current flow carried by Cl^- ions across the cell membrane, we can see that the current flow produced by a given concentration of GABA is greatly enhanced by the simultaneous application of diazepam (**FIGURE 8.22A**; Sigel and Steinmann, 2012). Note that application of diazepam alone has no effect, showing that BDZs can only modulate $GABA_A$ receptors and have no effect in the absence of GABA. Another way of visualizing the interaction of GABA with BDZs is to hold the concentration of diazepam constant while varying the GABA concentration. In this case, we see that the BDZ shifts the concentration–response curve to the left (**FIGURE 8.22B**).

It is important to recognize that not all $GABA_A$ receptors are sensitive to BDZs. Genetic engineering studies in mice have shown that BDZ binding and functional sensitivity to BDZs require the presence of a γ subunit (usually $\gamma 2$), along with $\alpha 1$, $\alpha 2$, $\alpha 3$, or $\alpha 5$ subunits and any β subunit (Sieghart and Sperk, 2002). Studies with genetically engineered strains of mice demonstrated that the sedating actions of BDZs strongly depend on receptors containing $\alpha 1$ subunits, whereas the anxiolytic actions depend on receptors containing $\alpha 2$ subunits (Rudolph and Knoflach, 2011). These findings further illustrate the functional importance of the subunit composition of $GABA_A$ receptors. Most $GABA_A$ receptors in the brain have the requisite composition for BDZ sensitivity, but there are exceptions. For example, the extrasynaptic receptors that contain $\alpha 4$ or $\alpha 6$ subunits are not affected by administration of a BDZ. On the other hand, these extrasynaptic receptors are an important target for ethanol, anesthetics, and neurosteroids (Brickley and Mody, 2012). Consequently, enhancement of tonic cellular inhibition, mediated by extrasynaptic $GABA_A$ receptors, is a significant contributor to the sedating, sleep-promoting, and other behavioral effects of many non-BDZ positive allosteric modulators.

Diazepam and related BDZs are agonists at the BDZ receptor on the $GABA_A$ receptor complex. Researchers have discovered certain compounds that

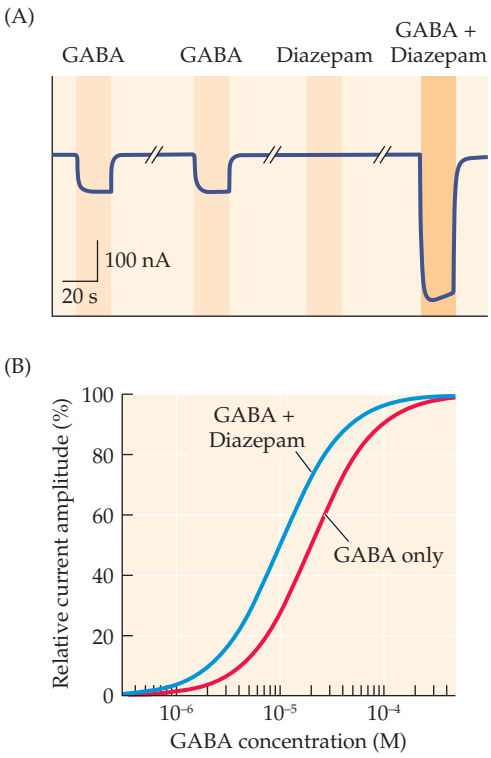


FIGURE 8.22 Positive allosteric modulation of the $GABA_A$ receptor by diazepam (A) Downward traces depict inward current flow across the membrane of a $GABA_A$ receptor–expressing cell. Currents are elicited by application of a low concentration of GABA (left two traces). Application of diazepam (1 μM) by itself has no effect (third trace), but diazepam applied together with GABA elicits a large increase in current amplitude (rightmost trace). (B) The graph illustrates a concentration–response curve in the absence (red line) and presence (blue line) of a fixed concentration of diazepam. Note that the curve is shifted to the left in the presence of diazepam, indicating an enhancement of current amplitude in response to low to moderate concentrations of GABA. However, the maximum current amplitude elicited by a high concentration of GABA is unaffected by diazepam. (After Sigel and Steinman, 2012.)

modulate the $GABA_A$ receptor in a direction opposite that of a BDZ agonist. Such compounds have been termed **inverse agonists** at the BDZ receptor. If a BDZ agonist enhances the effectiveness of GABA on the receptor, then a BDZ inverse agonist reduces the effectiveness of GABA, although it doesn't actually block GABA in the way that a GABA receptor antagonist does. Like a BDZ agonist, an inverse agonist has no effect in the absence of GABA. The behavioral profile of a BDZ inverse agonist is the opposite of that of a BDZ agonist. Consequently, BDZ inverse agonists are anxiogenic (anxiety producing), arousing, and proconvulsant (seizure promoting) instead of anxiolytic, sedating, and anticonvulsant (see Chapter 17 for further discussion). Finally, let's think about what it means for the brain to have a receptor that responds to a class of synthetic drugs.[4] This raises the important question of whether there is an endogenous ligand for the BDZ receptor (i.e., a neurotransmitter made by the brain that signals via this receptor), which is the subject of **Web Box 8.2**.

ANESTHETIC AND NEUROSTEROID INTERACTIONS WITH THE $GABA_A$ RECEPTOR Anesthetics are drugs that not only induce a state of unconsciousness, but also block sensory awareness. Lack of pain sensitivity is what enables patients to undergo surgery without discomfort and without waking up. Anesthetics act on a complex array of molecular targets, including both voltage-gated ion channels (Covarrubias et al., 2015) and ligand-gated channels like the $GABA_A$ receptor (Antkowiak and Rudolph, 2016; Forman and Miller, 2011). As mentioned above, anesthetic agents allosterically modulate $GABA_A$ receptor channel opening and, at high concentrations, can open the channel even in the absence of GABA. As with BDZs and other modulatory compounds, anesthetics differentially affect receptors with a particular subunit composition. An interesting example of this concerns a sparsely expressed family of $GABA_A$ receptors that contain an α5 subunit. This subunit is most heavily expressed in the hippocampus and the olfactory bulb (Rudolph and Möhler, 2014). Hippocampal expression is noteworthy because of this brain area being a key site for LTP and memory encoding. If you have ever undergone a medical procedure (e.g., major surgery) that required general anesthesia, you may have experienced a loss of memory of events that occurred just *before* administration of the anesthetic agent. A combination of human and animal research strongly suggests that anesthetic potentiation of extrasynaptic hippocampal α5 receptor activity plays an important role in this kind of amnesia (Perouansky and Pearce, 2011; Rudolph and Möhler, 2014). This is yet another example of the functional significance of $GABA_A$ receptor subunit composition.

$GABA_A$ receptors are additionally modulated by neurosteroids. These substances are made from cholesterol and possess a steroid structure similar to that of the glucocorticoids and gonadal steroids (see Chapter 3). However, they are synthesized in the brain (hence the term *neuro*steroid) by neurons and glial cells, and they act as local signaling molecules rather than as hormones that circulate throughout the body by means of the bloodstream. Allopregnanolone, allotetrahydrodeoxycorticosterone, and androstanediol are among the most

[4]BDZs were developed by pharmaceutical companies and do not occur naturally in the brain, except perhaps in very small quantities (and evidence for that is inconclusive).

extensively studied neurosteroids. These compounds enhance the functioning of both synaptic and extrasynaptic $GABA_A$ receptors, acting as positive allosteric modulators at low doses but having the capability to directly open the receptor channel at high doses (Reddy and Estes, 2016). As depicted in Figure 8.20, neurosteroids interact with the receptor at a site other than the BDZ binding site, and their ability to increase GABAergic activity does not require the specific $GABA_A$ receptor subunits that are needed for BDZ sensitivity. Similar to the drugs discussed above, neurosteroids reduce brain excitability and produce sedative–hypnotic and anxiolytic effects behaviorally. These properties have led to the development of synthetic neurosteroid-like compounds such as alfaxolone (Alfaxan), which is an intravenous anesthetic used in cats and dogs. Other synthetic neurosteroids are being tested for the possible treatment of epilepsy and a variety of other neuropsychiatric disorders (Reddy and Estes, 2016).

GABA also signals using metabotropic $GABA_B$ receptors

STRUCTURE AND FUNCTION OF THE $GABA_B$ RECEPTOR

As mentioned earlier, the other GABA receptor subtype is a metabotropic receptor termed $GABA_B$. Interestingly, unlike virtually all other known metabotropic receptors, the $GABA_B$ receptor requires two different subunits in order to assemble in the membrane and work properly (Bettler and Tiao, 2006). $GABA_B$ receptors are located both postsynaptically and presynaptically. The postsynaptic receptors inhibit neuronal firing through stimulation of K^+ channel opening (Heaney and Kinney, 2016). Presynaptic $GABA_B$ receptors are found on axon terminals of GABAergic neurons (autoreceptors) and on some terminals of cells using a different neurotransmitter such as glutamate (heteroreceptors). These presynaptic receptors reduce neurotransmitter release from the nerve terminal by inhibiting Ca^{2+} channel opening. Both post- and presynaptic $GABA_B$ receptors also inhibit adenylyl cyclase, thereby reducing the rate of cAMP formation.

Behavioral functions of $GABA_B$ receptors have been studied using pharmacological and genetic engineering approaches. The classical agonist at the $GABA_B$ receptor is **baclofen** (**Lioresal**), which has been used for many years as a muscle relaxant and an antispastic agent. **Saclofen** and **2-hydroxysaclofen**, which are chemical analogs of baclofen, are competitive antagonists at the $GABA_B$ receptor. Studies of knockout mice lacking one of the $GABA_B$ receptor subunits or animals given a $GABA_B$ agonist or antagonist have demonstrated a role for this receptor in a wide variety of behavioral functions, including learning and memory, anxiety- and depression-like behaviors, and responses to drugs of abuse. Readers interested in learning more about these topics are referred to recent reviews by Agabio and Colombo (2014), Kasten and Boehm II (2015), and Heaney and Kinney (2016).

Section Summary

- Many brain areas, including the cerebral cortex, hippocampus, substantia nigra, cerebellum, striatum, globus pallidus, and olfactory bulbs, are rich in GABA. GABAergic neurons may function as interneurons, as in the cortex and hippocampus, or they may function as projection neurons, as in pathways originating in the striatum and in the cerebellar Purkinje cells.
- There are two general GABA receptor subtypes: ionotropic $GABA_A$ receptors and metabotropic $GABA_B$ receptors.
- $GABA_A$ receptors conduct Cl^- ions into the postsynaptic cell, causing membrane hyperpolarization and an inhibitory effect on cell excitability. Each receptor is composed of five subunits, usually including two α subunits, two βs, and one γ. A small number of $GABA_A$ receptors contain a δ subunit instead of γ.
- The most common synaptic $GABA_A$ receptor composition is $(\alpha1)_2(\beta2)_2(\gamma2)$. There are also extrasynaptic receptors containing two $\alpha4$ or two $\alpha6$ subunits, along with two β subunits and a δ subunit. Such receptors are sensitive to low GABA concentrations and mediate a mild tonic inhibitory effect on cell firing.
- Muscimol is a $GABA_A$ receptor agonist derived from the mushroom *Amanita muscaria* (fly agaric). Ingestion of this mushroom or of pure muscimol causes hallucinations (including macroscopia) and other behavioral and physiological effects similar to those associated with LSD.
- $GABA_A$ receptor antagonists include the competitive antagonist bicuculline and the noncompetitive inhibitors pentylenetetrazol (Metrazol) and picrotoxin, all of which are seizure inducing.
- BDZs, barbiturates, ethanol, anesthetics, and neurosteroids all act as positive allosteric modulators of the $GABA_A$ receptor, which means that they enhance the action of GABA on the receptor. At high doses, barbiturates, anesthetics, and neurosteroids can open the receptor channel in the absence of GABA. Functionally, all of these compounds exert a CNS depressant effect that is manifested behaviorally as anxiolytic, sedative–hypnotic, and anticonvulsant properties.
- BDZs bind to a specific site on the $GABA_A$ receptor complex that is considered to be a BDZ receptor. BDZ sensitivity requires the presence of a γ subunit (usually $\gamma2$), any β subunits, and an $\alpha1$,

α2, α3, or α5 subunit. Specific behavioral effects of BDZs and other allosteric modulators can be attributed to receptors with a particular α subunit composition: α1 for BDZ-mediated sedation, α2 for BDZ-mediated anxiety reduction, and α5 for anesthetic-mediated amnesia.

- Inverse agonists at the BDZ receptor also require the presence of GABA, but such compounds reduce instead of enhance the effectiveness of GABA in activating the $GABA_A$ receptor. BDZ inverse agonists produce behavioral effects opposite to those produced by BDZ agonists, namely, anxiety, arousal, and increased susceptibility to seizures.
- The metabotropic $GABA_B$ receptor is composed of two different subunits and is located both post- and presynaptically. Postsynaptic $GABA_B$ receptors inhibit neuronal firing by stimulating K^+ channel opening, whereas presynaptic $GABA_B$ receptors (acting as either autoreceptors or heteroreceptors) inhibit neurotransmitter release by inhibiting Ca^{2+} channel opening. The receptors additionally inhibit cAMP formation.
- $GABA_B$ receptors contribute to many behavioral functions, including learning and memory, anxiety- and depression-like behaviors, and responses to drugs of abuse. This information has been obtained from studies of knockout mice lacking one of the receptor subunits and from pharmacological studies involving the selective agonist baclofen (Lioresal), which is used clinically as a muscle relaxant and an antispastic agent, or the competitive antagonists saclofen or 2-hydroxysaclofen.

STUDY QUESTIONS

1. Show the reactions for glutamate interconversion with glutamine, including the name of the enzyme catalyzing each reaction.
2. Describe the function of vesicular glutamate transporters, including which ones are most widely expressed in the brain. Provide an example of co-expression of a VGLUT with a vesicular transporter for a different transmitter or group of transmitters. What is the functional significance of such co-expression?
3. What is meant by the term *gliotransmission*? Which kind of glial cell has been implicated in this process?
4. Discuss (a) the function and cellular expression of excitatory amino acid transporters and (b) the interplay between neurons and glial cells in the regulation of glutamate metabolism and signaling.
5. The text states, "Glutamate is the workhorse transmitter for fast excitatory signaling in the nervous system." What is the evidence for this statement?
6. Describe the three subtypes of ionotropic glutamate receptors, including how they were named, their subunit structure, and their ion conductances.
7. Describe the properties of the NMDA receptor, including its binding sites both outside of and within the ion channel. What conditions are necessary for the receptor channel to open, and how are these conditions related to the idea that the NMDA receptor is a "coincidence detector"?
8. List the ionotropic glutamate receptor antagonists mentioned in the chapter, including their channel selectivity and use, either as an abused drug or as a therapeutic agent.
9. Describe the family of metabotropic glutamate receptors, including their signaling mechanisms.
10. Discuss the use of positive allosteric AMPA receptor modulators as cognitive enhancers.
11. Discuss the phenomenon of hippocampal long-term potentiation. Include in your answer the specific roles of NMDA and AMPA receptors in LTP, changes in AMPA receptor trafficking, differences between early and late LTP in their characteristics and underlying mechanisms, and evidence for an involvement of LTP in encoding of episodic memories.
12. What is excitotoxicity? Include in your answer a discussion of the types of excitotoxic neuron cell death, underlying mechanisms (including the role of ionotropic glutamate receptors), and evidence for excitotoxic cell death in clinical medicine.

(*Continued*)

STUDY QUESTIONS (*continued*)

13. What is fragile X syndrome? What is the genetic basis of this disorder? Discuss the metabotropic glutamate receptor theory of fragile X syndrome, and the current status of medications that have been developed on the basis of this theory.
14. Describe the processes of GABA synthesis, packaging into synaptic vesicles, and uptake from the extracellular space. For GABA uptake, include the cell types that express each GABA transporter.
15. List the drugs mentioned in the text that affect GABA synthesis, metabolism, or uptake. Include in your answer any therapeutic applications of the listed drugs.
16. Discuss the phenomenon of GABA corelease with other classical transmitters. Provide examples of such corelease.
17. Where in the brain are GABA projection neurons and/or GABA interneurons found?
18. Describe the structure and function of the $GABA_A$ receptor, including subunits and binding sites for allosteric modulators.
19. How do benzodiazepines enhance $GABA_A$ receptor function? Are all $GABA_A$ receptors sensitive to benzodiazepines? If not, what aspect of the receptor determines its sensitivity to this class of drugs?
20. What is meant by an "inverse agonist" at the benzodiazepine site on the $GABA_A$ receptor? What is the behavioral profile of such compounds?
21. Discuss the involvement of $\alpha 5$ subunit–containing $GABA_A$ receptors in anesthetic-mediated amnesia.
22. Describe the properties of the $GABA_B$ receptor, including its signaling mechanisms and agonist and antagonist drugs mentioned in the text.
23. Discuss the evidence for an involvement of GABA in certain types of epileptic disorders.

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CHAPTER 9

One of the most addicting routes of drug administration is intravenous injection. (Paula Bronstein/Getty Images.)

Drug Abuse and Addiction

"CHRIS THRALL LEFT THE ROYAL MARINES [a unit of the Royal Navy of the United Kingdom] to find fortune in Hong Kong but a year later was suffering from drug induced psychosis resulting from his crystal meth addiction. He became homeless, lost his sanity and almost his life after becoming addicted to the deadly drug, also known as 'ice.' Chris, from Plymouth, joined the Marines at 18 and served 7 years. He got an opportunity to get involved in a new business venture, left the Forces and moved to Hong Kong. Unfortunately, the venture failed, he was in debt and jobless. He found a job in marketing for a Hong Kong firm, a few months after starting he walked in on a colleague in the toilets smoking meth. Chris accepted the offer to try some, the next day he wanted more...His life went out of control and in an attempt to get straight; he went from a day job to working as nightclub doorman with the intention that if he worked at night he wouldn't be able to take drugs. But his addiction was life consuming; he was on it constantly, hallucinating and became psychotic. He got sacked and several other jobs followed, he was living on the streets and believed he was part of a global underground conspiracy. He put his life at risk deciding to climb a huge construction crane 'for a laugh.' 13 months after arriving in Hong Kong Chris booked a return flight to the UK paid for by his worried family. He was ravaged, 4 stone [56 pounds] lighter, wild-eyed and gaunt. He returned to Plymouth and even though he was ashamed of the addiction he continued to score the drug and carried on using for another 18 months. Crystal meth wasn't hard to find. One day [he] looked in the mirror and didn't know himself anymore; after a long battle and several attempts, he slowly started his recovery. He has now been clean for over 10 years." ■

— Love PR & Communications, London, 2012

The above is the true story of a young Englishman whose life unraveled after he became addicted to methamphetamine. In 2011, Thrall published an account of his ordeal entitled *Eating Smoke: One Man's Descent into Drug Psychosis in Hong Kong's Triad Heartland*. His story dramatically illustrates what we might call the "paradox" of addiction. That is, how can a person develop and maintain a pattern of behavior (in this case, repeated methamphetamine use) that is so obviously destructive to the individual's life? No one has a complete explanation for this paradox, but a variety of theories have been proposed. The aim of this chapter is to introduce you to several facets of this intriguing problem, including various psychosocial, genetic, and neurobiological factors thought to underlie the development and maintenance of an addicted state. We will describe the history and current prevalence of drug use in the United States, as well as the laws that determine which substances are legal and which are not. Finally, we will consider the important issue of whether drug addiction should be considered a disease, and how this issue bears on the kinds of treatments used to combat this disorder.

Introduction to Drug Abuse and Addiction

The first part of this chapter considers the prevalence of psychoactive drug use in our society, the discovery and use of such drugs in earlier times, the history of drug laws in the United States, and the definition and characteristics of drug addiction.

Drugs of abuse are widely consumed in our society

Drugs of abuse may be defined as psychoactive substances that have the potential to be used in a problematic way, usually because the substance affects mood or behavior in a manner that is desirable to the user. Each year, the Substance Abuse and Mental Health Services Administration conducts a National Survey on Drug Use and Health, which estimates the prevalence and incidence of drug use among civilians 12 years of age and older. According to the 2016 survey, approximately 28.6 million Americans (10.6% of the population) were current users[1] of at least one illicit drug[2] at that time (Substance Abuse and Mental Health Services Administration, 2017; **FIGURE 9.1A**). Of those 28.6 million people, 24 million (83.9%) were users of marijuana either alone or with one or more other illicit substances. The second highest category after marijuana was misuse of prescription pain relievers, such as oxycodone, codeine, and fentanyl. The large number of people in this category reflects the recent epidemic of recreational opioid use that is plaguing the country. Moreover, misuse of other prescription medications classified as tranquilizers or stimulants is another important aspect of illicit drug use. We have long known that illicit drug use occurs most prominently in young adults. This fact is illustrated is **FIGURE 9.1B**, which shows that the

[1]In this survey, "current use" is defined as use of a drug during the past month.

[2]For purposes of the survey, illicit drugs include marijuana/hashish, cocaine (including crack), ecstasy (not shown on the graph), heroin, hallucinogens, and inhalants. Also shown are prescription psychotherapeutic agents (e.g., sedatives or stimulants) used recreationally instead of for medicinal purposes.

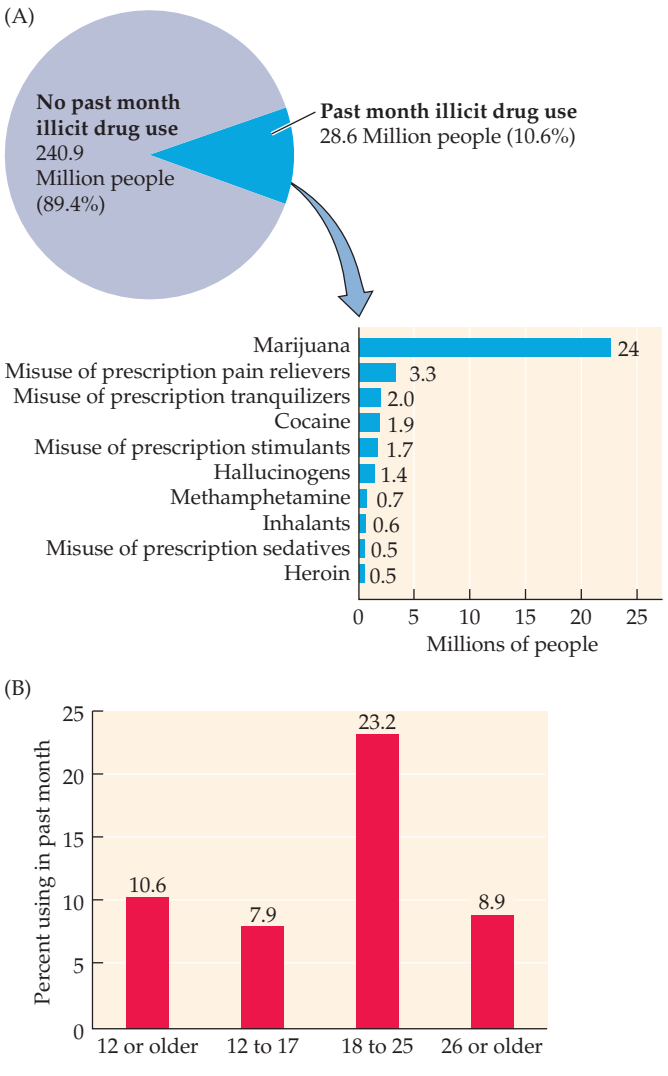

FIGURE 9.1 Data on illicit drug use in the United States (A) Results from the 2016 National Survey on Drug Use and Health include the overall numbers and percentages of surveyed people age 12 or older who were current (defined as past month) illicit drug users at the time of the survey (top) and the numbers of users of specific substances (bottom). (B) Depicted is the age distribution of illicit drug users expressed as the percentage of surveyed people. (From the Substance Abuse and Mental Health Services Administration, 2017.)

prevalence of illicit drug use in 2016 was greatest in the age range of 18 to 25 years, with a significantly lower prevalence both at younger and older ages.

It is no surprise that legal drugs such as tobacco and alcohol are consumed even more widely than illicit substances. The 2016 survey estimated that more than 63 million Americans were current tobacco users (mostly cigarette smokers), 137 million drank alcohol, and, of the latter, over 16 million were heavy drinkers (defined as consuming 5 or more drinks at one time on 5 or more days within a single month). How did we reach a state where psychoactive drug use and abuse are so prevalent?

Drug use in our society has increased and has become more heavily regulated over time

Psychoactive drugs have been a part of human culture since antiquity. However, the substances available to drug users and the prevalence of use have varied in different periods.

HISTORICAL TRENDS Many psychoactive substances such as nicotine, caffeine, morphine, cocaine, and tetrahydrocannabinol are made by plants and were available to ancient peoples in their native forms. Likewise, alcohol is a naturally occurring product of sugar fermentation by yeast. In his 1989 book *Intoxication: Life in Pursuit of Artificial Paradise*, Ronald Siegel presents many anecdotal accounts of wild animals becoming intoxicated after eating drug-containing plants. He goes on to suggest that early societies may have come to identify the pharmacological properties of such plants after first observing the behaviors of these intoxicated animals. More historical information on individual abused drugs is provided in later chapters. Here, we will focus on the history of drug use and cultural attitudes in the United States over the past 200 years.

Compared with the wide variety of psychoactive drugs available (legally or illegally) to twenty-first-century Americans, the situation was quite different 200 years ago. Alcohol and caffeine were widely used, and although the modern cigarette had not yet been invented, tobacco chewing was becoming increasingly popular within a large segment of the male population. Opium, either alone or in the form of laudanum (opium extract in alcohol), was available for the purpose of pain relief. On the other hand, there was no cocaine, heroin, marijuana, 3,4-methylenedioxymethamphetamine (MDMA, or "ecstasy"), methamphetamine, barbiturates, lysergic acid diethylamide (LSD), or phencyclidine (PCP). There were also few drug control laws, especially none at the federal level.

As time went on, however, a number of events and cultural trends set us on the path to where we are today. First, the alcohol temperance movement started to gain strength. The founding of this movement, which promoted abstention from hard liquor in favor of moderate beer or wine consumption, is generally credited to a highly regarded Philadelphia physician named Benjamin Rush. Rush not only identified a number of adverse physiological consequences of excessive drinking, he also argued that such consumption impaired the drinker's moral faculty, leading to irresponsible and even criminal acts. Although the temperance movement reached its height in the failed twentieth-century attempt at complete alcohol prohibition (except for "medicinal" use), its aftermath still colors the attitudes of many people toward the problems of alcohol and drug abuse. In particular, the equating of drug use with criminal behavior can be seen in the "War on Drugs" that has pervaded our society for so many years.

Second, advances in chemistry during the nineteenth century made it possible to purify the primary active ingredient of opium, namely, morphine, and then later the active ingredient of coca, which of course is cocaine. This allowed the drugs to be taken in much more concentrated form, increasing their addictive potential. But since the route of administration is also important, an equally significant event was the development of the hypodermic syringe in 1858 (**FIGURE 9.2**), which permitted the purified substances to be injected directly into the bloodstream. One consequence of this marriage of purified drug and improved delivery vehicle was the widespread use of morphine to treat wounded and ill soldiers during the Civil War. Many developed an opiate addiction, which was called "soldier's disease" in the common parlance.

Third, the increasing availability of purified drugs combined with the lack of drug control laws led to growing use of these substances in many different forms. Cocaine was the major ingredient in a variety of tonics and patent medicines sold over the counter. The most notorious of these was *Vin Mariani*, which was made by the French chemist Angelo Mariani by soaking coca leaves in wine along with spices and other flavorings. Heroin was synthesized by Bayer Laboratories

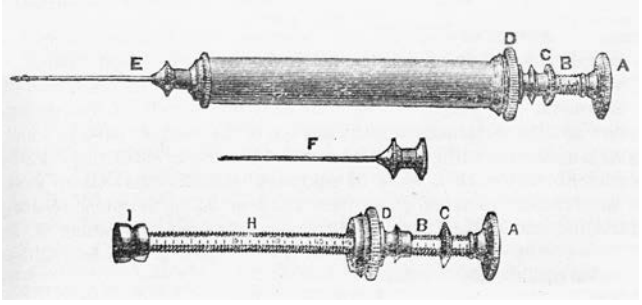

FIGURE 9.2 An early hypodermic syringe and needle used in the 1870s (From Morgan, 1981; original photograph from H. H. Kane, *The Hypodermic Injection of Morphia*, 1880, p. 21.)

in 1874 and was first marketed 14 years later as a nonaddicting (!) substitute for codeine. If you've ever had a severe cough, the doctor probably prescribed a codeine-containing cough syrup. At the turn of the twentieth century, you could purchase heroin-containing cough syrup at any neighborhood pharmacy without a prescription (**FIGURE 9.3**). It does not surprise us now that the ready availability of these substances led many people to become dependent on them.

The last factor that we wish to mention here is the medicalization of drug addiction that occurred primarily in the second half of the twentieth century. The medicalization of addiction had two components: that addiction was now thought of as a disease, and that drug addicts should be treated by the medical establishment. We can trace the origin of these views to the American Association for the Cure of Inebriates, an organization founded in 1870, which stated that inebriety (excessive drinking) was a disease and proposed that inebriates (alcoholics) be admitted to hospitals and sanitaria for help. However, this medical approach to alcoholism and drug abuse later faded and was not seriously revived until the early 1950s, when alcoholism was declared a disease first by the World Health Organization and subsequently by the American Medical Association. Alcoholics Anonymous (AA), which embraces many aspects of the disease model of alcoholism, was gaining prominence during this period. The disease model of drug addiction continues to be strongly promoted by the treatment community, by self-help groups such as AA and Narcotics Anonymous, and by much of the research establishment, including the National Institute on Drug Abuse, an agency of the National Institutes of Health. It is also the view most widely accepted by the lay public. On the other hand, this model has also been the subject of some criticism, as we shall see later in the chapter.

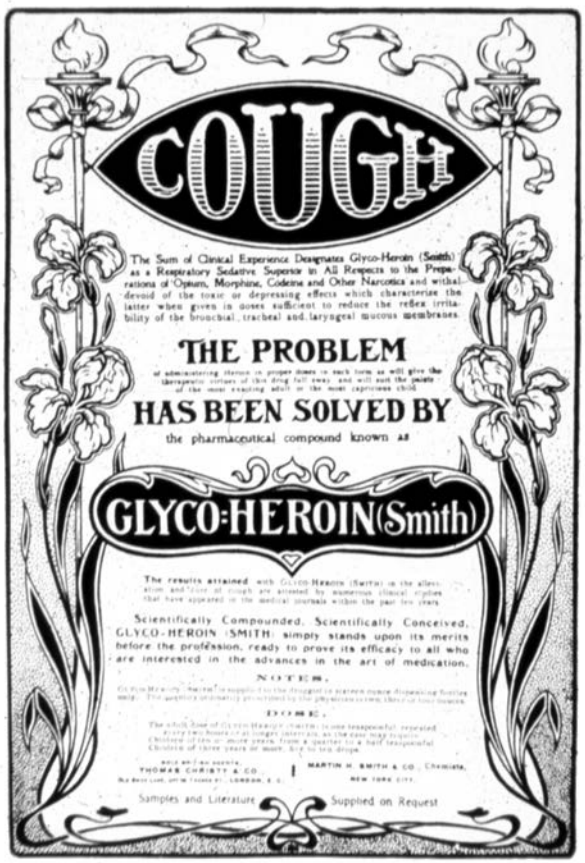

FIGURE 9.3 An early ad for heroin-containing cough syrup

DRUGS AND THE LAW Since 1980, the United States has witnessed the introduction of "crack" cocaine, the increased potency of heroin and marijuana sold on the street, an upsurge in methamphetamine use, and the rise of so-called club drugs such as MDMA and γ-hydroxybutyrate (GHB). In just the past few years, we have seen a veritable epidemic of opiate use (both heroin and prescription opiates) and the appearance of novel compounds like synthetic marijuana ("spice") and cathinone-based stimulants ("bath salts," "flakka," and others). Although these developments led to the establishment and continuation of our government's "War on Drugs," illicit drug use continues on a massive scale despite the best efforts of the drug warriors. At the federal level, the political climate remains strongly against any consideration of legalization or even decriminalization of any currently illegal drugs, including marijuana. In contrast, many states have moved toward decriminalization or even legalization of marijuana, sometimes restricted to medical use but in other cases extending all the way to recreational use of the drug.

Given the recent history of drug politics in the United States, it is easy to forget that things were not always this way. As discussed earlier, by the beginning of the twentieth century, there was widespread use of cocaine, opium, and heroin in over-the-counter or patent medicines. In the absence of any federal regulations governing the marketing, distribution, or purchase of these preparations, sales increased from $3.5 million in 1859 to $74 million in 1904 (Hollinger, 1995). Over time, however, the federal government became increasingly involved in controlling the commercialization of drugs (**TABLE 9.1**). This involvement began with an initial concern about the quality and purity of both medications and food, which led to the passage in 1906 of the Pure Food and Drug Act. The new law mandated the accurate labeling of patent medicines so that the consumer would be aware of the presence of alcohol, cocaine, opiates, or marijuana in such products. It also created the Food and Drug Administration (FDA), which is charged with assessing the potential hazards and benefits of new medications and with licensing their use. The Pure Food and Drug Act was clearly an

TABLE 9.1 History of Federal Drug Legislation in the United States

Name of law	Year enacted	Purpose
Pure Food and Drug Act	1906	Regulated labeling of patent medicines and created the FDA
Harrison Act	1914	Regulated dispensing and use of opioid drugs and cocaine
Eighteenth Constitutional Amendment (Prohibition)	1920	Banned alcohol sales except for medicinal use (repealed in 1933)
Marijuana Tax Act	1937	Banned nonmedical use of cannabis (overturned by U.S. Supreme Court in 1969)
Controlled Substances Act	1970	Established the schedule of controlled substances and created the DEA

educational approach to the drug problem, since the law did not make any of the abovementioned substances illegal to sell or use.

Around the same time that drug labeling and purity were under discussion, concern over the burgeoning problem of drug abuse and dependency was growing. For example, importation of opium grew rapidly between 1870 and 1900. Rightly or wrongly, opium smoking had become associated with Chinese laborers who had immigrated to the United States following the Civil War to work on the railroads. Meanwhile, China itself began a vigorous campaign against opium, viewing it as a symbol of Western imperialism.[3] These events culminated in the convening of the first International Opium Conference in 1911, where each participating country agreed to enact its own legislation restricting narcotic drug use except for legitimate medical purposes.

It took the United States several more years to pass its antinarcotic law, the Harrison Act of 1914. A major reason for the delay was lobbying by drug and patent medicine manufacturers who objected to the initial wording of the bill. The Harrison Act was designed to regulate the dispensing and use of opiates (opium and its derivatives such as morphine) and cocaine by means of the following provisions:

1. Use of these substances for nonmedical purposes was prohibited.
2. Pharmacists and physicians were required to register with the Treasury Department and to keep records of their inventory of narcotics.
3. Retail sellers of narcotics and practicing physicians had to pay a yearly $1 tax to the federal government.
4. Patent medicines containing small amounts of opium, morphine, heroin, or cocaine remained legal and could continue to be sold by mail order or in retail establishments.

Because of the third and fourth provisions, we can see that the principal aims of the Harrison Act were to control rather than abolish the use of opiates and cocaine and to link narcotic use with government revenue through taxation (it's worth noting that during the Prohibition era, the federal government finally managed to convict the notorious gangster Al Capone not on alcohol trafficking or murder, but rather on tax evasion!).

Many physicians had previously been providing maintenance doses of opiates or cocaine to patients who were addicted to these substances. However, because addiction was not considered a disease by government authorities at that time, one immediate effect of the Harrison Act was to cut addicts off from this source of drugs. The consequences are easily predicted. Addicts were forced to turn to street dealers and prices skyrocketed. According to Hollinger (1995), the cost of heroin increased from $6.50 per ounce to roughly $100 (a huge sum in the early 1900s). People who could not afford to pay these prices were forced into abstinence and withdrawal. One result of these events was the establishment of many municipal clinics to treat drug addicts. The clinic in New York City registered approximately 7700 patients between April 1919 and March 1920 (Morgan, 1981). Unfortunately, these clinics failed to solve the problem; most addicts went back to using street drugs soon after their treatment, and illegal drug sales continued to flourish.

The Harrison Act did not regulate the use of alcohol, which had nevertheless been a major social problem in the United States for many years. As a consequence of increasing support for the alcohol temperance movement mentioned previously, the Eighteenth Amendment to the Constitution went into effect in 1920. The new Prohibition law banned the dispensing of any beverage with alcohol content greater than 0.5% (1/10 the typical alcohol content of regular beer) except

[3]In the eighteenth and nineteenth centuries, England profited enormously from Chinese purchase of opium obtained through the British colony in India. The Opium Wars of the mid-1800s at least partially revolved around the desire of Western powers to maintain this lucrative drug trade against China's wishes. Although the United States was not a participant in the wars, it benefited from favorable trade agreements negotiated after the conclusion of hostilities.

by physicians for medicinal purposes. Once again, the consequences were disastrous. Speakeasies (establishments where alcohol was sold illegally) sprang up everywhere, and the organized crime movement really took off during this period. The experiment in Prohibition finally ended in 1933.

Cannabis was another substance disregarded by the Harrison Act. The practice of marijuana smoking was initially associated with Mexican immigrants who entered the United States in the early 1900s. After World War I, public opposition to marijuana grew, and eventually numerous state laws were passed against its possession or sale. The federal government subsequently entered the picture with passage of the Marijuana Tax Act of 1937. This law was similar to the Harrison Act in banning nonmedical use of cannabis and levying a tax on importers, sellers, and dispensers of marijuana.

The Marijuana Tax Act was declared unconstitutional and was overturned by the U.S. Supreme Court in 1969. But in the very next year, the federal government passed a much broader law meant to apply to all potentially addictive substances. This was the Comprehensive Drug Abuse Prevention and Control Act (also called the Controlled Substances Act [CSA]) of 1970. The CSA replaced or updated virtually all previous federal legislation concerning narcotic drugs and other substances thought to have abuse or addiction potential. Among other provisions, the CSA established five schedules of controlled substances, which are discussed later in this chapter. It also created the Drug Enforcement Administration (DEA), which was charged with enforcement of the CSA. Although this law has been revised several times since its inception, it remains the cornerstone of federal drug control legislation. Moreover, many states have adopted the Uniform Controlled Substances Act, a model drug control law patterned after the CSA.

Several conclusions can be drawn from this brief survey of the history of federal drug laws. The first is that each time the federal government became more involved in drug regulation, the action resulted from increases in drug use and/or perceived societal dangers posed by such use. Those who wish to make drug laws more lenient must start by changing such antidrug perceptions. The second conclusion is that existing laws are not entirely consistent with available medical and scientific evidence. For example, we will see later that nicotine (obtained via tobacco) is more addictive than marijuana by all established criteria, yet tobacco smoking is legal, whereas marijuana smoking is still not legal according to federal law. A final conclusion is that legal mechanisms have only limited effectiveness in preventing drug use. This is most obvious in the events that occurred during Prohibition, as well as in the current widespread use of marijuana. We acknowledge that cocaine and heroin use would almost certainly be greater than it is now if those substances were legal. But millions of people have little trouble obtaining cocaine, heroin, or any other illicit drug they desire. Many would argue that a more important restraint on drug use is the individual's own concerns that a particular substance might harm her health, jeopardize other important goals or values, or put her at risk for becoming addicted to that substance.

Features of Drug Abuse and Addiction

Before proceeding further, try writing down your definition of drug addiction in one or two sentences. Were you easily able to come up with a satisfactory definition? If not, don't be concerned, because addiction is not a simple concept. This problem was highlighted by Burglass and Shaffer (1984) in the following (not entirely frivolous) description of addiction: "Certain individuals use certain substances in certain ways thought at certain times to be unacceptable by certain other individuals for reasons both certain and uncertain." The medical establishment has attempted to develop a broadly acceptable definition, yet many experts continue to disagree about exactly what it means to be addicted to a drug (Walters and Gilbert, 2000).

Drug addiction is considered to be a chronic, relapsing behavioral disorder

Early views of drug addiction emphasized the importance of physical dependence. As you learned in Chapter 1, this means that abstinence from the drug leads to highly unpleasant withdrawal symptoms that motivate the individual to reinstate his drug use. It is true that some drugs of abuse, such as alcohol and opiates, can create strong physical dependence and severe withdrawal symptoms in dependent individuals. Certain other substances, however, produce relatively minor physical dependence. It may surprise you to learn that cocaine is one such substance, and that there was a time when cocaine was not considered to be addictive because of this lack of an opiate-like withdrawal syndrome.

Modern conceptions of addiction have focused more on other features of this phenomenon. First, there is an emphasis on behavior, specifically, the compulsive nature of drug seeking and drug use in the addict. The addict is often driven by a strong urge to take the drug, which is called drug **craving**. Second, addiction is thought of as a chronic, relapsing disorder. This means that individuals remain addicted for long periods of time and that drug-free periods (**remissions**) are often followed by **relapses** in which drug use recurs despite negative consequences. This is the paradox of addiction mentioned earlier in

the chapter. The current scientific view of addiction is encompassed by the following definition: "Drug addiction can be defined as a chronically relapsing disorder, characterised by compulsion to seek and take the drug, loss of control in limiting intake, and emergence of a negative emotional state (e.g., dysphoria, anxiety, irritability) when access to the drug is prevented" (Koob and Volkow, 2016, p. 760). This definition will serve as a framework for most of the discussion that follows in later sections of the chapter.

The term *addiction* has strong negative emotional associations for most of us. Despite the fact that drug addicts live in all parts of the country and come from all walks of life, we usually think of them as urban and poor. Although some drug addicts fit this description, many others do not. For this reason, and because of conflicting definitions of addiction, the American Psychiatric Association (APA) stopped using the terms *addiction* and *addict* in its professional writings. This can be seen in the fifth edition of the APA's *Diagnostic and Statistical Manual of Mental Disorders*, known as *DSM-5*® (American Psychiatric Association, 2013). The *DSM* represents an attempt to classify the entire range of psychiatric disorders, with objective criteria provided for the diagnosis of each disorder. Instead of using the term *drug addiction*, the *DSM-5* specifies a group of **substance-related disorders** that cover 10 designated classes of drugs: (1) alcohol, (2) caffeine, (3) cannabis, (4) hallucinogens, (5) inhalants, (6) opioids, (7) sedative–hypnotic and anxiolytic drugs, (8) stimulants, (9) tobacco, and (10) other substances (note that all nine named drug classes are discussed in depth in this textbook). The *DSM-5* posits that all of these substances share the ability to activate the neural circuitry that mediates "reward," an issue that we will elaborate on in a subsequent section of the chapter. It is this ability, which is commonly experienced as a drug-induced "high," that confers upon these substances a risk for misuse and, in some cases, the development of an addictive pattern of behavior. Substance-related disorders are further broken down in the *DSM-5* into **substance use disorders** and **substance-induced disorders**. A substance use disorder is characterized by "a cluster of cognitive, behavioral, and physiological symptoms indicating that the individual continues using the substance despite significant substance-related problems" (American Psychiatric Association, 2013, p. 483). A substance-induced disorder is characterized by "the development of a reversible substance-specific syndrome due to recent ingestion of a substance" (American Psychiatric Association, 2013, p. 485). The substance-induced syndromes mentioned in the *DSM-5* consist of "intoxication, withdrawal, and other substance/medication-induced mental disorders (e.g., substance-induced psychotic disorder, substance-induced depressive disorder)" (American Psychiatric Association, 2013, p. 485).

The *DSM-5* provides a specific set of criteria to diagnose a substance use disorder for eight of the nine drug classes listed above. Caffeine is excluded in this case because it is considered to produce only substance-induced disorders, namely caffeine intoxication (i.e., symptoms produced by excessive caffeine consumption) and caffeine withdrawal (i.e., symptoms produced by acute abstinence in a regular caffeine user). As the criteria for the remaining eight are very similar, we have chosen to illustrate the basic diagnostic approach by listing the specific criteria for alcohol use disorder in **TABLE 9.2**. A few key points need to be made about these criteria. First, the *DSM-5* states that for an alcohol use disorder to be diagnosed, the pattern of use must be "problematic" and it must lead to "clinically significant impairment or distress." This may seem fairly obvious when considering a legal drug like alcohol that is used regularly by millions of people without adverse consequences. But the same ideas pertain to dangerous illicit drugs like cocaine or heroin. Thus, mere use of either cocaine or heroin does not constitute a psychiatric disorder as long as the use does not lead to significant impairment or distress (although it is always possible that the current use pattern could evolve into a more problematic pattern that *does* meet *DSM-5* criteria for a substance use disorder). Second, you can see from the table that there is no single criterion for determining the presence of an alcohol use disorder; this is true for any substance use disorder. This reflects the fact that problematic drug use may result in many different adverse consequences, depending on which drug is being taken and on the amount and pattern of drug taking. Of course, someone with a long-standing, severe case of substance use disorder may meet virtually all of the listed criteria, not just a few. To reflect this fact, the *DSM-5* adds an important severity component to the diagnosis. Specifically, individuals who meet only two or three of the specified criteria are considered to have a "mild" substance use disorder, individuals who meet four or five criteria are considered to have a "moderate" disorder, and individuals who meet six or more criteria are considered to have a "severe" disorder.

The substance-related disorders in *DSM-5* fall within a larger classification called substance-related and addictive disorders. The reason for this is the continually growing discussion over whether the concept of addiction should be applied to other uncontrolled or compulsive behaviors such as binge eating, sexual preoccupation, compulsive gambling, excessive internet use, and so forth. These uncontrolled behaviors are sometimes called **behavioral addictions**. After considerable deliberation, the authors of the *DSM-5* decided to add a category called non-substance-related

TABLE 9.2 *DSM-5* Diagnostic Criteria for Alcohol Use Disorder

A problematic pattern of alcohol use leading to clinically significant impairment or distress, as manifested by at least two of the following, occurring within a 12-month period:

1. Alcohol is often taken in larger amounts or over a longer period than was intended.
2. There is a persistent desire or unsuccessful efforts to cut down or control alcohol use.
3. A great deal of time is spent in activities necessary to obtain alcohol, use alcohol, or recover from its effects.
4. Craving, or a strong desire or urge to use alcohol.
5. Recurrent alcohol use resulting in a failure to fulfill major role obligations at work, school, or home.
6. Continued alcohol use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of alcohol.
7. Important social, occupational, or recreational activities are given up or reduced because of alcohol use.
8. Recurrent alcohol use in situations in which it is physically hazardous.
9. Alcohol use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by alcohol.
10. Tolerance, as defined by either of the following:
    a. A need for markedly increased amounts of alcohol to achieve intoxication or desired effect.
    b. A markedly diminished effect with continued use of the same amount of alcohol.
11. Withdrawal, as manifested by either of the following:
    a. The characteristic withdrawal syndrome for alcohol [listed elsewhere in *DSM-5* as an alcohol-induced disorder].
    b. Alcohol (or a closely related substance, such as a benzodiazepine) is taken to relieve or avoid withdrawal symptoms.

Source: Reprinted with permission from the *Diagnostic and Statistical Manual of Mental Disorders*, 5th ed., © 2013. American Psychiatric Association. All rights reserved.

disorders, which includes gambling disorder as its sole current entry. In **BOX 9.1**, we discuss the rationale for specifying gambling disorder as a non-substance-related disorder in *DSM-5*, the current status of behavioral addictions more generally, and the extent to which these behavior patterns show similarities to classical substance-based addictions.

Before we conclude this section, a brief consideration of terminology is in order. The *DSM-5* not only substituted the term *substance use disorder* for *addiction*, it also eliminated the previously used term *substance abuse* from its lexicon of psychiatric disorders. Nevertheless, that terminology can be seen in the older published literature, and the notions of drug abuse and addiction continue to be present in popular parlance and in the lay press. For these reasons, and also because using the formal *DSM-5* diagnosis of "substance use disorder" can be clumsy at times, we will continue to use the terms *drug abuse* (or *substance abuse*) and *drug addiction* in the remainder of this chapter except when we are referring to a study or review that specifically uses the *DSM-5* terminology. It should be understood by the reader that when the terms *drug abuse* or *substance abuse* are used, we are generally referring to a kind of problematic drug use that might, for example, meet the *DSM-5* criteria for a mild or (at most) moderate substance use disorder. The term *addiction* will generally refer to instances of problematic drug use that likely meet the *DSM-5* criteria for a severe substance use disorder.

There are two types of progression in drug use

Drug use can involve two different kinds of progression. In one type of progression characteristic of many young people, the individual starts out taking a legal substance such as alcohol or tobacco, later progresses to marijuana, and in a small percentage of cases moves on to cocaine, heroin, other illicit substances, or illegally obtained prescription drugs. One of the theories that attempts to account for this type of progression, namely, the **gateway theory**, is discussed in **Web Box 9.1**.

The second kind of progression pertains to changes in the amount, pattern, and consequences of drug use as they affect the user's health and functioning.

When an individual first experiments with an abused drug, he may or may not progress to regular, nonproblematic use or beyond. Despite the popular view that drugs like cocaine and heroin are instantly and automatically addictive, this is not the case. Indeed, one of the central unresolved questions in the addiction field is why some individuals eventually develop a pattern of compulsive drug use, whereas many others do not (Swendsen and Le Moal, 2011). **FIGURE 9.4** depicts a contemporary view of pathological drug use as a cyclical pattern that comprises three components: (1) periods of preoccupation with drugs and anticipation of upcoming use; (2) periods of drug intoxication that, in some cases, are associated with "bingeing" on the drug; and (3) periods following drug use that are characterized by withdrawal symptoms and negative affect (e.g., depressed mood or irritability). The figure includes some of the features of the cycle (e.g., taking drugs in larger amounts than intended and development of drug tolerance) that represent criteria for developing a substance use disorder in *DSM-5*. Over time, repeated cycles constitute a downward spiral that can ultimately result in addiction.

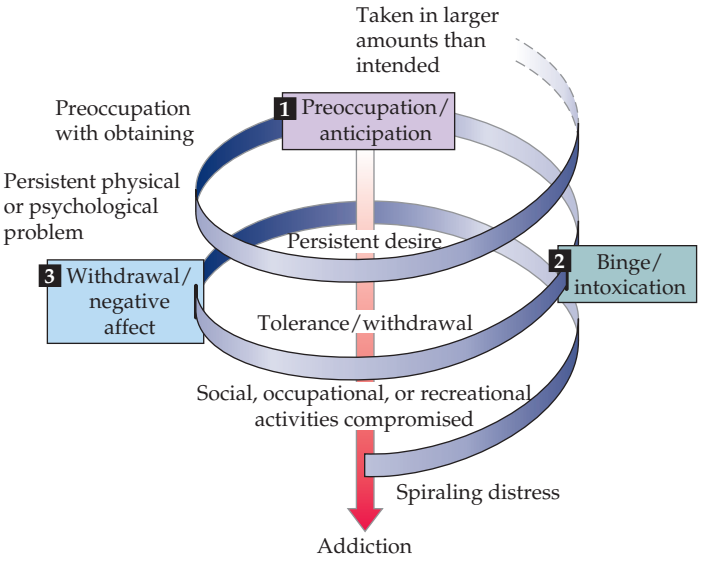

FIGURE 9.4 Cycles of pathological drug use that can lead to the development of addiction (After Koob and LeMoal, 1997.)

BOX 9.1 Of Special Interest

Should the Term *Addiction* Be Applied to Compulsive Behavioral Disorders That Don't Involve Substance Use?

The term *addiction* has traditionally been associated with harmful use of substances such as alcohol, cocaine, and heroin. But articles in the mainstream media now commonly use the same term when referring to behavioral problems that don't involve chemical substances. Examples include *gambling addiction*, *sex addiction*, *food addiction*, *internet addiction*, and so forth. On what basis can we determine whether this is a legitimate use of *addiction* or whether we should restrict the term to its original usage?

In a search of the addiction literature, the first use of the term *behavioral addictions* we could find was in the title of a 1990 editorial in the *British Journal of Addiction* by Isaac Marks (Marks, 1990). Marks argued that behavioral addictions shared important similarities with drug addictions, which is generally consistent with current theories; however, his classification of behavioral addictions included obsessive-compulsive disorder, Tourette syndrome, and trichotillomania (compulsive hair pulling), none of which are considered to be addiction-type disorders in the *DSM-5*. Instead, the *DSM-5* places only gambling disorder within the broad category of substance-related and addictive disorders. The major criteria for a diagnosis of gambling disorder are presented in the **Table**. These criteria have several similarities to the diagnostic criteria for alcohol use disorder (see Table 9.2), including the presence of tolerance (item 1), withdrawal (items 2 and 3), and craving (items 4 and 5). Understandably, there are additional criteria that are specific to gambling. The decision to include gambling disorder within the same category as substance-related disorders stemmed not only from similarities in phenomenology but also from research demonstrating neurobiological and cognitive dysfunction in compulsive gamblers that overlaps with the neurobiological and cognitive features of drug addiction (el-Guebaly et al., 2012; Clark, 2014).

A number of other behavioral disorders are likely candidates for future classification as behavioral addictions. Nevertheless, the relevant working group tasked with writing this part of the *DSM-5* felt that other disorders required more research, particularly in the areas of neurobiology and genetics, before inclusion with substance-related disorders was

(*Continued*)

BOX 9.1 Of Special Interest (*continued*)

warranted. Examples of other addiction-like behavioral patterns are "internet addiction" (sometimes characterized more specifically as addiction to massively multiplayer online role-playing games such as World of Warcraft) (Ko, 2014; Cerniglia et al., 2017), "food addiction" (sometimes characterized more specifically as "sugar addiction") (Moore et al., 2017; Rogers, 2017; but see Westwater et al., 2016, for arguments against the notion of sugar addiction), "exercise addiction" (Berczik et al., 2012; Weinstein and Weinstein, 2014), "shopping addiction" (also called "compulsive buying") (Rose and Dhandayudham, 2014; Maraz et al., 2016), "cell phone addiction" (De-Sola Gutiérrez et al., 2016; but see Billieux et al. 2015a, for arguments against considering excessive cell phone use to be a behavioral addiction), "sex addiction" (Garcia and Thibaut, 2010; Karila et al., 2014), and "pornography addiction," which is usually internet related and thus considered separately from sex addiction (Love et al., 2015; Duffy et al., 2016). A few other putative disorders such as "work addiction" (i.e., "workaholism") (Quinones and Griffiths, 2015) and "study addiction" (Atroszko et al., 2015) have been discussed in the literature but are far away from official acceptance as behavioral addictions.

The above list of proposed behavioral addictions is already long, but papers have appeared in the recent literature that would add even more candidates to the list, such as "dance addiction" (Maraz et al., 2015), "tango addiction" (presumably a variety of dance addiction) (Targhetta et al., 2013), "tanning addiction" (Kourosh et al., 2010), and "fortune-telling addiction" (Grall-Bronnec et al., 2015). The proliferation of proposed behavioral addictions raises the obvious question of whether, as suggested by Billieux and colleagues (2015b), we are "overpathologizing everyday life." Mihordin (2012) raised similar concerns that strong enthusiasts of pastimes such as model railroading exhibit some of the features characteristic of a behavioral addiction. Does that mean that we need to consider a category called "model railroading addiction"? A young person who has a passion to become a world-class gymnast, dancer, or solo violinist may similarly feel compelled to practice her skill to the exclusion of almost any other activity. Such individuals may additionally feel irritable or depressed if prevented from practicing, say due to sickness or injury. Are these feelings pathological?

To summarize, researchers continue to debate whether excessively performed behaviors should be labeled as addictions (Potenza, 2015; Grant and Chamberlain, 2016). For any given proposed disorder and for people deemed to be suffering from that disorder, at least two questions must be resolved. First and most important, how problematic is the behavior, that is, does it reach the threshold for consideration as a psychopathology? Second, even if the answer to the first question is affirmative, do the behavioral and neurobiological features of the proposed disorder align more closely with the *DSM-5* substance-related and addictive disorders category or with the obsessive-compulsive and related disorders category? This distinction may not mean a lot to the patient, but it may help guide the practitioner in determining what kind of therapeutic intervention is most likely to help that patient.

DSM-5 Diagnostic Criteria for Gambling Disorder

Persistent and recurrent problematic gambling behavior leading to clinically significant impairment or distress, as indicated by the individual exhibiting four (or more) of the following in a 12-month period:

1. Needs to gamble with increasing amounts of money in order to achieve the desired excitement.
2. Is restless or irritable when attempting to cut down or stop gambling.
3. Has made repeated unsuccessful efforts to control, cut back, or stop gambling.
4. Is often preoccupied with gambling (e.g., having persistent thoughts of reliving past gambling experiences, handicapping or planning the next venture, thinking of ways to get money with which to gamble).
5. Often gambles when feeling distressed (e.g., helpless, guilty, anxious, depressed).
6. After losing money gambling, often returns another day to get even ("chasing" one's losses).
7. Lies to conceal the extent of involvement with gambling.
8. Has jeopardized or lost a significant relationship, job, or educational or career opportunity because of gambling.
9. Relies on others to provide money to relieve desperate financial situations caused by gambling.

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Interestingly, even after an individual becomes addicted to a drug, he may show intermittent periods of reduced use or even abstinence. Such periods may be related to repeated attempts to stop drug use without help, or they may be associated with participation in a treatment program or incarceration in prison. **FIGURE 9.5** illustrates an example of this phenomenon taken from a longitudinal study of male opioid (heroin) addicts in San Antonio, Texas (Maddux and Desmond, 1981). The figure presents drug status data over periods ranging from 7 to 20 years for 10 representative individuals out of a total of nearly 250. Particularly striking is the diversity of patterns among these heavy drug users. For example, person number 111 exhibited periods of either occasional or daily use interspersed with long intervals of abstinence. In contrast, number 162 used daily for most of the 20-year period except when he was institutionalized in a hospital or prison. When considered across all 10 individuals, the numerous instances of abstinence followed by renewed drug use support the view mentioned earlier that addiction is a chronic, relapsing disorder.

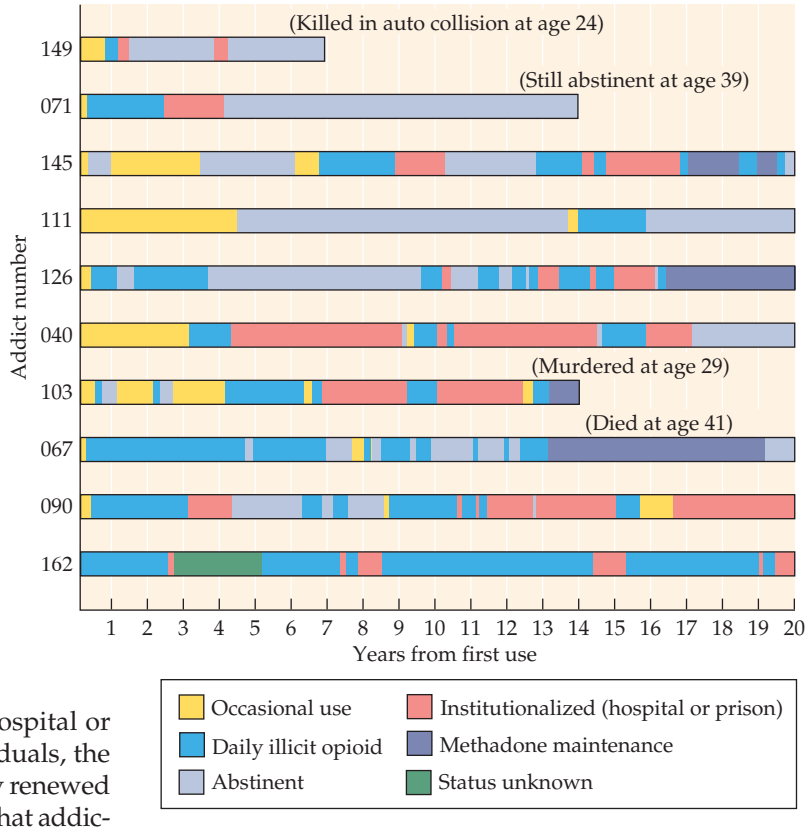


FIGURE 9.5 Patterns of opioid drug use over a 20-year period in ten heroin addicts (After Maddux and Desmond, 1981.)

For many people, the use of both alcohol and illicit drugs such as marijuana naturally declines once they reach adulthood and begin to take on the responsibilities associated with earning a living and having a family. This pattern is consistent with data from longitudinal studies documenting a reduction in drug use beyond the period of adolescence (Chen and Kandel, 1995). Some writers have called this process "maturing out" of a drug-using lifestyle, and studies have associated recovery from alcohol dependence with a variety of transitional life events (Dawson et al., 2006). Of course, not all individuals stop or reduce their substance use in this way; recovery from addiction is a complex process that cannot be accounted for by any single factor (Waldorf, 1983).

Which drugs are the most addictive?

Just as we all have mental images of drug addicts, we also have ideas about which drugs are the most addictive. Drugs thought to have high addictive potential are sometimes called "hard drugs." Aside from popular opinion, however, there are legal standards meant to classify drugs according to their addictive potential. The Controlled Substances Act of 1970 established a system by which most substances with abuse potential are classified into one of five different schedules. These schedules, along with representative drugs, are shown in **TABLE 9.3**. Schedule I substances are considered to have no medicinal value and thus can be obtained only for research use by registered investigators.[4] Items listed under Schedules II to V are available for medicinal purposes with a prescription from a medical professional such as a physician, dentist, or veterinarian. They can also be obtained for research use. Note that the **Schedule of Controlled Substances** specifically excludes alcohol and tobacco, thus permitting these substances to be purchased and used legally without registration or prescription. The Schedule of Controlled Substances was formulated almost 50 years ago and was based not only on the scientific knowledge of that time but also partly on political considerations. Although it has been updated periodically since its inception, we may still ask whether this classification system accurately reflects our current understanding of various abused substances, or whether it continues to be too politicized. This issue is discussed in **Web Box 9.2**.

[4]For all controlled substances, but particularly for Schedule I and II items, there are strict federal requirements for investigator registration, ordering, and record keeping. The substances must be maintained securely, as in a locked safe, with careful control over who has access to the drug supply.

TABLE 9.3 Schedule of Controlled Substances

Schedule	Description	Representative substances
I	Substances that have no accepted medical use in the United States and have a high abuse potential	Heroin, LSD, mescaline, marijuana, THC, MDMA
II	Substances that have a high abuse potential with severe psychic or physical dependence liability	Opium, morphine, codeine, meperidine (Demerol), cocaine, amphetamine, methylphenidate (Ritalin), pentobarbital, phencyclidine (PCP)
III	Substances that have an abuse potential less than those in Schedules I and II, including compounds containing limited quantities of certain narcotics and nonnarcotic drugs	Paregoric, barbiturates other than those listed in another schedule
IV	Substances that have an abuse potential less than those in Schedule III	Phenobarbital, chloral hydrate, diazepam (Valium), alprazolam (Xanax)
V	Substances that have an abuse potential less than those in Schedule IV, consisting of preparations containing limited amounts of certain narcotic drugs generally for antitussive (cough suppressant) and antidiarrheal purposes	

Section Summary

- High levels of drug use continue in our society despite significant governmental attempts to control such use.
- Although early ideas about addiction emphasized the role of physical dependence, more recent conceptions have focused on the compulsive features of drug seeking and use (despite the potentially harmful consequences) and on the concept of drug addiction as a chronic, relapsing disorder characterized by repeated periods of remission followed by relapses.
- In the *DSM-5*, problematic patterns of drug use are categorized by a disorder called substance use disorder. In its severe form, substance use disorder has characteristics that correspond closely to those usually associated with addiction.
- The *DSM-5* also contains a diagnostic category called gambling disorder. This disorder falls within a group of compulsive non-substance-related behaviors sometimes called behavioral addictions.
- Young people often progress from legal substances like alcohol or tobacco to marijuana, and some even go on to try cocaine, heroin, or illegally obtained prescription drugs. The gateway theory attempts to account for this progression, although other explanations have been offered to explain the same findings.
- A second kind of progression consists of cycles of pathological drug use consisting of three components: preoccupation–anticipation, binge–intoxication, and withdrawal–negative affect. Repeated cycles can cause spiraling distress in the user that eventually leads to addiction. Nevertheless, even addicts may show periods of abstinence interspersed among the intervals of regular drug consumption.
- The Schedule of Controlled Substances classifies potentially abused drugs into five categories, or schedules, on the basis of their degree of abuse potential and medicinal value. Alcohol and tobacco are not listed on the schedule, so they can be purchased for recreational use and without a prescription.
- Debate has arisen about whether the Schedule of Controlled Substances is a reasonable classification system based on current scientific knowledge, or whether it has been driven too much by sociopolitical considerations.

Factors That Influence the Development and Maintenance of Drug Abuse and Addiction

Over the years, researchers have discovered a variety of different factors that contribute to the development and maintenance of drug abuse and addiction. In this section, we will discuss the most important of these factors, including how they are studied in animal models of addiction. Such models are critical for our ability to investigate the neurobiological underpinnings of addiction and to screen new pharmacological agents that might have therapeutic value in treating drug addicts. Later in the chapter, we will consider how different

contributory factors are brought together in current theories of addiction.

The addiction potential of a substance is influenced by its route of administration

In Chapter 1, you learned about the various routes by which drugs can enter a person's body. Routes of administration such as oral or transdermal result in relatively slow absorption of the drug and, therefore, equally slow drug availability to the brain. In contrast, intravenous (IV) injection or inhalation/smoking yields rapid drug entry into the brain and a fast onset of drug action. Conversely, a fast onset is associated with a shorter duration of action. Researchers and addiction treatment providers have known for many years that the addiction potential of a substance is related to the route of administration, with routes that cause a fast onset of drug action having the greatest addiction potential (Allain et al., 2015). **FIGURE 9.6** illustrates this principle for opiate drugs, cocaine, and nicotine. Although we don't fully understand why IV injection and inhalation are associated with the greatest vulnerability to addiction, we do know that these routes produce the strongest euphoric effects (for a given dose) as a result of rapid drug delivery to the brain. It is also likely that repeated exposure of the brain to rapid, as opposed to gradual, drug delivery produces long-term neurobiological changes that are necessary for addiction to develop. A later section of this chapter discusses our current understanding of addiction neurobiology.

Most abused drugs exert rewarding and reinforcing effects

Most abused drugs act as **positive reinforcers**. This means that consuming the drug strengthens whatever preceding behavior was performed by the organism. **Drug reward** is a different but related concept that refers to the positive subjective experience (i.e., pleasure) associated with the drug. Examples include the euphoric feeling or "high" induced by a variety of drugs, and the feeling of relaxation induced by alcohol, marijuana, and sedative drugs such as barbiturates. Obviously, the rewarding effects of a substance are likely to play an important role in its reinforcing properties, but other factors (e.g., increased alertness produced by stimulant drugs) may also play a role.

DRUG SELF-ADMINISTRATION Drug reinforcement is most often studied operationally by investigating an organism's propensity to self-administer the substance using procedures introduced in Chapter 4. As you will recall, an experimental animal such as a rat, mouse, or monkey is typically fitted with an IV catheter attached to a drug-filled syringe. When the animal performs an operant response such as pressing a lever or poking its nose into a hole in the wall of the apparatus, a pump is briefly activated that slightly depresses the plunger of the syringe and infuses a small dose of the drug directly into the animal's bloodstream. This is directly analogous to a drug addict giving himself an IV drug injection. The ability of animals to learn and maintain the lever-pressing/nose-poking response or of addicts to learn and maintain drug-seeking and drug-using behaviors means that the drug is acting as a reinforcer, just as food is a reinforcer for those who are hungry. Substances that are strong reinforcers in the IV self-administration paradigm virtually always have great abuse liability in humans, particularly when taken intravenously or by smoking (the two routes of administration that produce the most rapid uptake of the drug by the brain). Classic examples of this kind are cocaine, heroin, and amphetamine or methamphetamine. In contrast, drugs that are not readily self-administered by animals, such as antidepressant or antipsychotic medications, are generally not addictive in humans.

One of the many factors that can be manipulated in the IV self-administration paradigm is the amount of drug (dose) given in each infusion. Studies on a

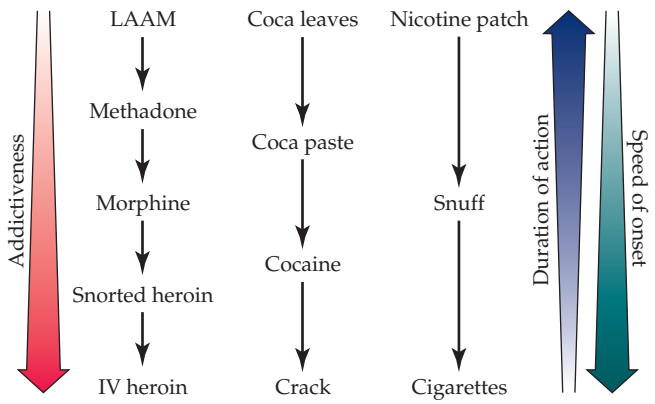


FIGURE 9.6 Relationship between route of administration and addiction potential of opiates, cocaine, and nicotine Opiates: LAAM (levo-alpha-acetyl-methadol) and methadone are orally administered treatments for opiate addiction; morphine taken orally has a more rapid onset and a shorter duration than methadone; and heroin may be taken by snorting, IV injection, or smoking (not shown) (see Chapter 11). Cocaine: coca leaves are chewed, coca paste is a crude extract of the leaves, powdered cocaine is often snorted, and crack cocaine is smoked (see Chapter 12). Nicotine: the nicotine patch is a transdermal delivery system used for smoking cessation, snuff is powdered tobacco leaves that are snorted, and cigarettes are smoked (see Chapter 13). (After Melichar et al., 2001.)

variety of drugs have shown that when the drug is delivered using a simple schedule of reinforcement such as a fixed-ratio (FR) schedule (see Chapter 4), the typical dose–response function is an inverted U-shaped curve (**FIGURE 9.7A**). The ascending part of the curve is thought to reflect increasing reinforcing effectiveness of the drug over that range of doses. On the other hand, the descending limb is potentially attributable to multiple factors, including satiation to the drug, aversive reactions, or behaviorally disruptive side effects (e.g., extreme hyperactivity in the case of stimulant drugs, or sedation in the case of sedative–hypnotic agents). Because of the complications introduced by these factors, simple dose–response functions are not very useful for determining the reinforcing strength of one substance versus another. A better measure of the relative strength of drug reinforcement makes use of a **progressive-ratio procedure**. In this procedure, animals are initially trained to lever press on a continuous-reinforcement (CR) schedule, which means that each press is followed by drug delivery. In the second phase, the animals are switched to a low FR schedule, such as an FR-5 (one drug delivery for every five responses). Finally, the FR schedule is progressively increased until the animals stop responding, presumably because the dose being delivered is not sufficiently reinforcing to support the amount of effort required. The response ratio at which responding ceases is called the **breaking point** (sometimes termed **breakpoint** instead). Breaking points vary across drugs and also across doses. What do you think is the relationship between dose and breaking point? If you guessed that breaking point generally increases with higher doses, you were correct. This is illustrated in a study conducted by Weeks and Collins (1987) in which they compared the approximate breaking points for a range of morphine doses in rats. As the dose of morphine was increased, the breaking point also rose by a large amount (**FIGURE 9.7B**). Keep in mind, however, that if the dose of the drug being tested becomes too high, determination of the breaking point may be compromised by some of the same factors that can interfere with dose–response analyses.

Just as mere drug use by humans does not constitute a psychiatric disorder, self-administration of a drug by a rat, mouse, or monkey is not a model of addiction per se even though it demonstrates that the substance has positive reinforcing properties. To produce a more convincing model of addiction, researchers must implement procedures that result in additional features such as escalating self-administration, relapse to renewed drug-seeking behavior after a period of abstinence, and preference of drug taking over alternative reinforcers available to the animal (Ahmed, 2012; Ahmed et al., 2013; Vanderschuren and Ahmed, 2013). Chapter 12 provides examples of procedures that lead to escalating cocaine self-administration by rats. Relapse is typically modeled in self-administration studies by stopping drug delivery (thus producing a forced abstinence that results in extinction of the operant response) and then exposing the animals to stimuli that are known to provoke renewed responding in an attempt to obtain the drug again. Such renewed responding is termed **reinstatement of drug-seeking behavior**. Three main types of stimuli are effective in reinstating drug-related

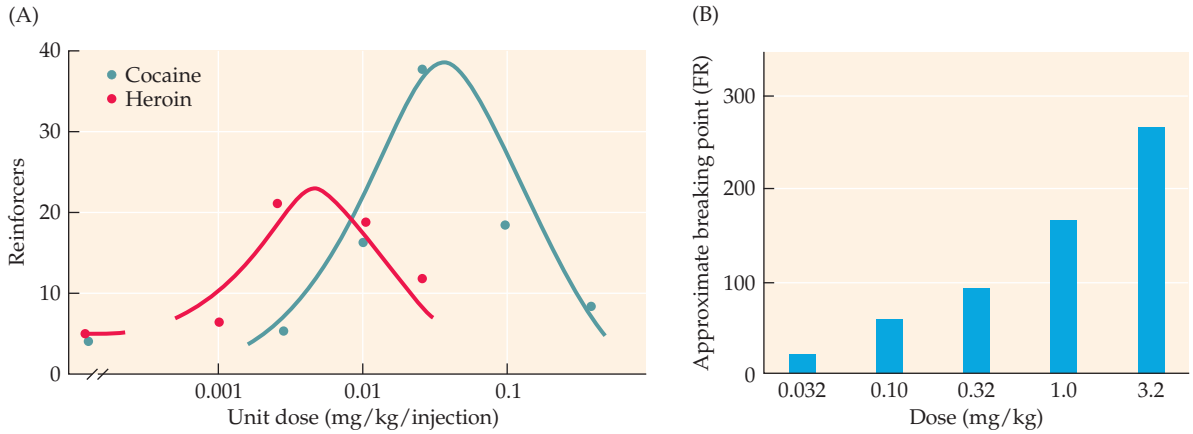


FIGURE 9.7 Features of intravenous drug self-administration by laboratory animals (A) Representative data for cocaine and heroin illustrate the typical inverted U-shaped curve relating dose per infusion to number of reinforcers (drug deliveries) obtained during a self-administration session. (B) Breaking point on a progressive-ratio schedule is an index of the reinforcing effectiveness of different doses of a drug. In this experiment, rats were trained to self-administer morphine on a continuous-reinforcement schedule, then were switched to a fixed-ratio (FR) schedule. The FR was increased daily (progressive-ratio schedule) until the breaking point was reached, as defined in this study by fewer than four drug injections in a day. The graph illustrates the estimated breaking points according to morphine dose per infusion. Within the dose range tested, it is clear that the drug's reinforcing properties rise dramatically as the dose is increased. (A after Bergman and Paronis, 2006; B after Weeks and Collins, 1987.)

responding: (1) the experimenter delivering a small dose of the drug to the animal, known as **drug priming**; (2) subjecting the animal to stress; and (3) exposing the animal to environmental cues that were previously paired with drug delivery (prior to extinction). The general paradigm used in this type of research is illustrated in **FIGURE 9.8**.

The scientific literature on drug self-administration consists almost entirely of studies on experimental animals, yet this procedure is also applicable to humans. It is important to note, however, that study participants are usually individuals who are already experienced users of the substance being investigated (Haney, 2009). In some cases, self-administration may be used to test the effectiveness of current or prospective medications for individuals addicted to a particular drug. For example, **FIGURE 9.9** shows the results of a study in which heroin addicts were given the choice of an IV injection of heroin or money while receiving one of three doses of methadone maintenance treatment. It can be seen that participants given a sufficiently large amount of methadone almost never chose the heroin because that dose of the methadone was effective in blocking the heroin-induced "high" (Comer et al., 2008).

The positive reinforcing effects of drugs undoubtedly play a significant role in addiction. Nevertheless, we may ask why the negative consequences of drug addiction (which can include such extreme effects as family breakup, loss of one's job and financial destitution, engaging in criminal activity to support drug purchases, damage to one's health, contracting needle-borne diseases such as AIDS or hepatitis, and even fatal overdose for some drugs) don't effectively counteract the positive reinforcement so that the individual stops using and remains abstinent. One possible answer concerns the temporal relationship between drug consumption and the positive or negative effects. Drug-induced euphoria occurs very quickly after consumption, particularly in the case of IV injection or inhalation (including smoking). In contrast, the negative consequences occur later in time and, in most cases, are linked to a long pattern of use rather than to a specific occasion of drug consumption. According to well-established principles of reinforcement, an event (euphoria) that occurs very soon after a response (drug consumption) exerts much greater control over that response than events (negative consequences) that occur later in time. Even for humans, who have the ability to plan and to foresee the consequences

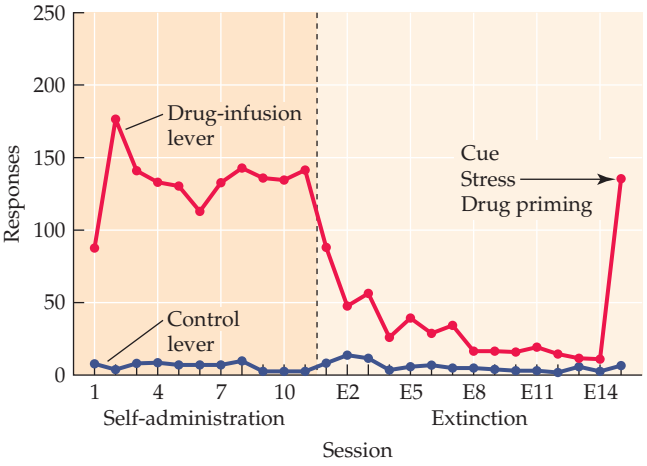


FIGURE 9.8 Representative data showing extinction and reinstatement of drug self-administration by drug-related cues, stress, or drug priming The graph depicts typical data from rats that were first stabilized on IV drug (e.g., cocaine) self-administration using an FR schedule of reinforcement. The red data points show the number of responses per session on a lever that is linked to drug delivery. Each drug infusion is accompanied by presentation of a cue, which is typically a light or a tone. The purple data points show the number of responses per session on a control lever that has no consequences when pressed. After 12 days of self-administration in this particular paradigm, the animals are switched to a 14-day extinction (E) phase in which pressing the drug lever no longer elicits either drug delivery or cue presentation. Note the rapid falloff of responding during this phase. However, if the drug-paired cue is presented, if the animals are subjected to stress (e.g., foot shock), or if a priming dose of the drug is administered by the experimenter, the animals resume a high rate of responding on the drug lever despite the fact that the drug is still withheld (E14). This phenomenon, which is termed reinstatement, is considered to be an animal model of relapse to drug use after abstinence in drug addicts. (After Kalivas et al., 2006.)

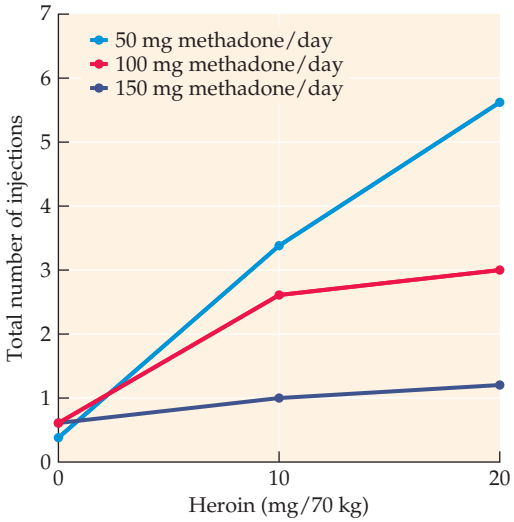


FIGURE 9.9 Choice of heroin infusions as a function of methadone maintenance dose Heroin addicts were stabilized on 50, 100, or 150 mg of methadone daily, after which they were tested for their choice of either an IV dose of heroin (0, 10, or 20 mg/70 kg) or a cash payment ranging from $2 to $38. The graph shows the number of heroin injections chosen as a function of heroin and methadone dose. (After Donny et al., 2005.)

of their actions in ways that animals cannot, this property of reinforcers has considerable influence over many behaviors, not just drug use. On the other hand, many individuals who take drugs once or even a few times stop their drug use before they develop a compulsive pattern and become addicted. This is true even for highly reinforcing drugs like cocaine and heroin. Thus, additional factors must contribute to the development of addiction in some drug users, but not in others.

PROCEDURES USED TO STUDY DRUG REWARD Two different procedures are commonly used to study the rewarding properties of drugs (see Chapter 4). The first is a classical conditioning procedure called **place conditioning**. Animals (typically rats and mice) are trained in a two- or three-compartment apparatus in which one of the compartments is paired with the presence of a drug over several conditioning sessions. A test session is then conducted under drug-free conditions. If the drug produced a rewarding effect during training, the animal will spend more time in the drug-paired compartment than in a compartment that had been paired with a placebo. The second procedure makes use of **electrical self-stimulation** of the brain—a method in which the performance of an operant response causes the delivery of an electrical stimulus to a part of the brain's reward circuit (see later section for additional details on this circuit). Numerous studies have shown that the threshold for rewarding brain stimulation is reduced when animals have been treated acutely with various drugs of abuse (**TABLE 9.4**). Because a lower threshold indicates a more sensitive system, such results indicate that the underlying neural circuitry for drug reward overlaps with the circuitry for brain stimulation reward. On the other hand, withdrawal of animals from chronic treatment with these same substances causes an increased threshold for electrical self-stimulation. A similar phenomenon occurring in a drug-dependent addict might contribute to the negative mood state and difficulty in experiencing pleasure (**anhedonia**) that are often reported during drug withdrawal.

TABLE **9.4** Drug Effects on Thresholds for Rewarding Brain Stimulation

Drug class	Acute administration	Withdrawal from chronic treatment
Psychostimulants (cocaine, amphetamine)	↓	↑
Opiates (morphine, heroin)	↓	↑
Nicotine	↓	↑
Sedative–hypnotic drugs (ethanol)	↓	↑
THC	↓	↑

DRUGS OF ABUSE CAN BE AVERSIVE RATHER THAN REWARDING Interestingly, drugs of abuse sometimes can be shown to exert aversive effects on animals or humans. For example, even though rats will self-administer nicotine under some conditions, they will also learn to press a lever to prevent experimenter-controlled infusions of the same drug. Considerable evidence suggests that a number of substances are reinforcing when under the animal's control but either are not as reinforcing or are even aversive when administered by the experimenter. For humans as well, drugs may produce aversive psychological or behavioral effects in addition to their rewarding effects. One example is the ability of cocaine to bring about feelings of anxiety that follow soon after the initial period of drug-induced euphoria. However, even though such aversive effects presumably inhibit the tendency toward future drug seeking and drug use, they may not be sufficient to outweigh the many factors promoting these behaviors.

Drug dependence leads to withdrawal symptoms when abstinence is attempted

Certain drugs of abuse such as alcohol and opiates (e.g., heroin or morphine) can lead to physical dependence when taken repeatedly. Some researchers have proposed that this process plays a key role in the establishment and maintenance of drug addiction. According to this idea, once an individual has become physically dependent as the result of repeated drug use, attempts at abstinence lead to highly unpleasant withdrawal symptoms (also called an **abstinence syndrome**). This motivates the user to take the drug again (relapse) to alleviate the symptoms. In the language of learning theory, relief of withdrawal symptoms promotes drug-taking behavior through a process of negative reinforcement, thus leading ultimately to a continuous behavioral loop consisting of repeated abstinence attempts followed by relapses.[5]

Koob and Le Moal (2005) have proposed that in the development of addiction, drug-taking behavior progresses from an "impulsive" stage, in which the primary motivation for drug use is the substance's positive reinforcing effects, to a "compulsive" stage, in which the primary motivation is the negative reinforcement obtained by relief from drug withdrawal (**FIGURE 9.10**). The instigation of an aversive state (negative affect) of withdrawal that underlies such negative reinforcement is termed the "dark side" of addiction by Koob and Le Moal, and they further propose that this process

[5]Recall that negative reinforcement refers to the concept of reinforcement by removal of an undesirable stimulus (in this case, painful or distressing withdrawal symptoms).

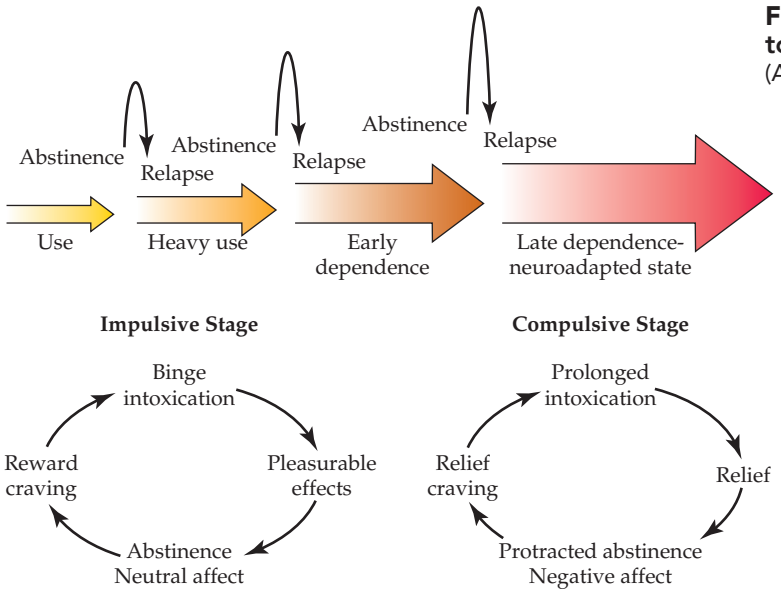


FIGURE 9.10 Transition from the impulsive to the compulsive phase of drug addiction
(After Heilig and Koob, 2007.)

is due to the gradual recruitment of an "antireward" system in the brain (depicted as a "neuroadapted state" in the figure). We will discuss this theory later in the section on addiction neurobiology.

One of the early proponents of an important role for physical dependence was Abraham Wikler, who studied and treated heroin addicts over a period of several decades. Because of lack of money or other factors, addicts do not have constant access to heroin. Over time, therefore, they are likely to undergo many episodes of withdrawal. Wikler argued that if these withdrawal reactions repeatedly occur in specific environments, such as the places where the addict either "hustles" for or takes the drug, the responses will become classically conditioned to the stimuli associated with those environments (Wikler, 1980). Consequently, even if an addict has been drug-free for some length of time and is therefore no longer experiencing an acute abstinence syndrome, withdrawal symptoms can be triggered by exposure to the conditioned stimuli (**FIGURE 9.11**).

As shown in the figure, drug craving is considered to be one of the crucial symptoms associated with conditioned withdrawal. Numerous studies have used neuroimaging methods to investigate the activation of specific brain areas during craving induced by presentation of drug-associated cues to dependent users of that drug. **FIGURE 9.12** presents the results from a recent example of this experimental approach (Malcolm et al., 2016). Methamphetamine-dependent men along with healthy non-methamphetamine-using controls were presented with two different sets of visual stimuli: one set consisted of pictures of methamphetamine use by IV injection, intranasal application (i.e., "snorting"), or smoking, whereas the other set consisted of pictures of neutral stimuli. While the pictures were being viewed, the participants reported their feelings of drug craving while simultaneously being scanned for regional brain activation by means of functional magnetic resonance imaging (fMRI). As expected, the methamphetamine-dependent men, but not the controls, experienced significant drug craving while viewing the methamphetamine-related stimuli. The fMRI data further revealed concurrent activation of the ventral striatum (a region that includes the nucleus accumbens) and the medial frontal cortex in the methamphetamine-dependent participants. Later in the chapter, we shall see that both of these brain areas have been implicated in various aspects of the addictive process.

For drugs that produce significant physical and/or psychological dependence, such dependence undoubtedly is an important contributor to the maintenance of addiction because unpleasant withdrawal symptoms provide motivation to obtain and take the drug again. Nevertheless, the relative importance of this factor varies among different drugs. As mentioned earlier, some major drugs of abuse, including cocaine, do not produce strong physical dependence, although psychological dependence does occur in some users. In addition,

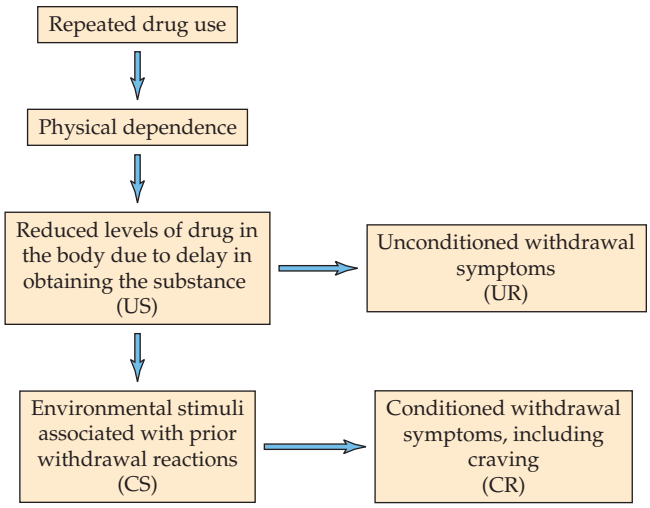


FIGURE 9.11 Development of conditioned drug withdrawal responses in dependent drug users
CR, conditioned response; CS, conditioned stimulus; UR, unconditioned response; US, unconditioned stimulus.

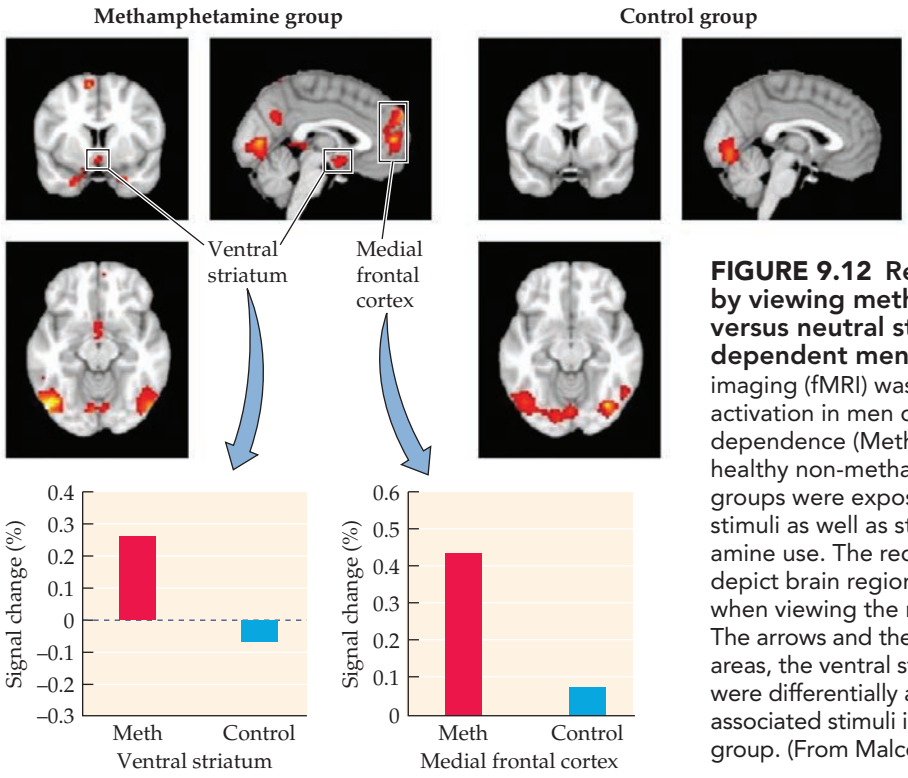


FIGURE 9.12 Regional brain activation produced by viewing methamphetamine-related stimuli versus neutral stimuli in methamphetamine-dependent men Functional magnetic resonance imaging (fMRI) was used to measure regional brain activation in men diagnosed with methamphetamine dependence (Meth) compared with brain activation in healthy non-methamphetamine-using controls. Both groups were exposed to an assortment of neutral visual stimuli as well as stimuli associated with methamphetamine use. The red and yellow areas in the fMRI scans depict brain regions in which activation was greater when viewing the methamphetamine-associated stimuli. The arrows and the graphs below illustrate that two brain areas, the ventral striatum and the medial frontal cortex, were differentially activated by the methamphetamine-associated stimuli in the meth group but not in the control group. (From Malcolm et al., 2016, CC-BY 4.0.)

drug-seeking behavior to obtain relief from withdrawal symptoms fails to explain relapse of addicts after they have gone through **drug detoxification** (elimination of the drug from one's system and passage through the abstinence syndrome). After the withdrawal symptoms have gone away, what still motivates an addict to take heroin again, for example? Research on addicts' self-reported reasons for returning to drug use as well as animal model studies such as those depicted in Figure 9.8 suggest that many factors can contribute to relapse, including exposure to drug-related stimuli, stress, boredom, lack of other reinforcers, or simply a desire to "get high" again.

Discriminative stimulus effects contribute to drug-seeking behavior

Psychoactive drugs, including drugs of abuse, often produce powerful discriminative stimulus effects in animal studies (Solinas et al., 2006). As summarized in Chapter 4, this means that drugs can produce internal states that can serve as cues controlling the animal's behavior in a learning task. The drug discrimination paradigm can be used in a few different ways. For example, not only can researchers determine whether an animal can reliably discriminate a drug from a drug vehicle such as saline, they can also test for stimulus generalization. In a stimulus generalization task, animals are first trained to discriminate a drug from the vehicle. Following the training, the subjects are administered varying doses of a test compound. If any doses of the test compound produce an internal state (i.e., stimulus cue) similar to that produced by the training drug, then the subjects will make the drug-correct response. The results of one such stimulus generalization experiment are shown in **FIGURE 9.13** (Smith et al., 2017). In this experiment, rhesus monkeys were trained to press one key when injected with cocaine and a different key when given saline vehicle. Once the cocaine discrimination had been well established, the monkeys were tested for their responses to three different doses of intramuscularly injected amphetamine. The figure illustrates the time course of responding on the cocaine-correct key versus the saline-correct key at each amphetamine dose. It can be seen that the monkeys given the highest dose of amphetamine responded almost entirely on the cocaine-correct key for more than 180 minutes after injection, after which the effect of the drug began to wear off. An intermediate effect of about 70% responding on the cocaine-correct key was initially observed at the middle dose of amphetamine, whereas the lowest dose produced 100% responding on the saline-correct key. These results show that at an appropriate dose, amphetamine produces a stimulus cue that is similar to the stimulus cue produced by cocaine. Similar results were obtained for methamphetamine. In contrast, when the monkeys were tested on different test doses of 3,4-methylenedioxymethamphetamine

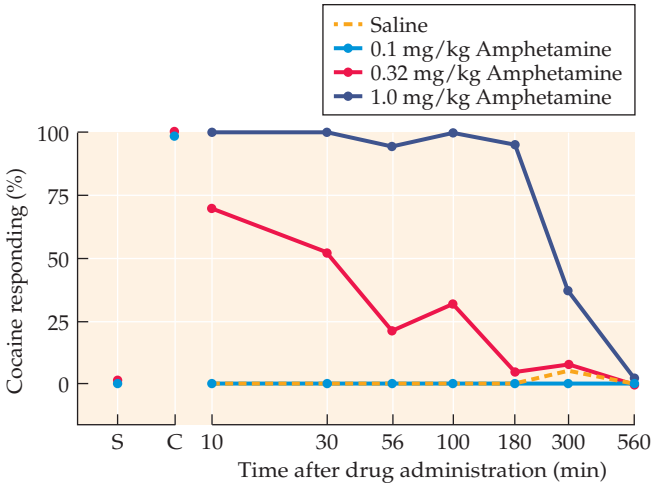


FIGURE 9.13 Dose-dependent stimulus generalization to amphetamine in monkeys trained to discriminate cocaine from saline Male rhesus monkeys were trained on a two-choice operant task to discriminate cocaine (0.32 mg/kg IM) from saline vehicle. The task required the animals to press the correct key to deliver food reinforcement depending on whether or not they perceived the interoceptive stimulus produced by the cocaine training drug. The symbols depicted over "S" and "C" show the mean percent baseline responding on the cocaine-correct key when the monkeys were given saline (S) or cocaine (C) prior to the drug discrimination test. The remaining data points show the mean percent responding on the cocaine key at various time points after administration of amphetamine (0.1 mg/kg, 0.32 mg/kg, or 1.0 mg/kg IM; solid lines) or saline (dashed line). At all time points, the lowest amphetamine dose produced virtually no responding on the cocaine key, similar to the effect of administering saline. An intermediate level of cocaine responding was produced by the middle amphetamine dose, whereas the highest dose initially produced 100 percent responding on the cocaine key. Note that the cocaine-like stimulus effect gradually dissipated over time, leading to eventual responding on the saline-correct key at the latest time points in both the middle and high amphetamine dose conditions. (After Smith et al., 2017.)

(MDMA) or 3,4-methylenedioxyamphetamine (MDA), even the highest doses produced only partial generalization to cocaine (data not shown). These findings are in accord with the biochemical mechanisms of action of these drugs, with cocaine, amphetamine, and methamphetamine primarily activating the catecholamine systems (Chapter 12), but MDMA and MDA primarily acting on the serotonergic system (Chapter 6). It is important to note that the discriminative stimulus effects of drugs in animals are considered to be analogous to the subjective effects that people experience when they take the same substances. Experienced users come to expect these subjective effects, and such expectations are thought to contribute to the persistence of drug-seeking and drug-using behaviors.

Genetic factors contribute to the risk for addiction

Genetic variation is one of the likely sources of individual differences in vulnerability to addiction. Adoption and twin studies have yielded estimates of 0.3 to 0.8 for the **heritability** of substance use disorders (Urbanoski and Kelly, 2012). In the field of genetics, *heritability* is a mathematical term representing the estimated contribution of genetic variation within a population to the total population variation for a particular trait (in this case, the trait is the presence or absence of a substance use disorder). Because the maximum heritability value is 1.0, it is clear that the remaining variability is due to environmental influences and gene–environment interactions.[6]

Genetic research into neuropsychiatric disorders like addiction has been based on two different hypotheses. The first is the **common disease–common variant hypothesis** (Yu and McClellan, 2016). According to this hypothesis, the genetically based susceptibility to a particular neuropsychiatric disorder stems from a pool of risk-conferring gene alleles that are possessed in common throughout the population. Each of these "risk alleles" that you may carry confers a small increase in susceptibility to developing the disorder. Your overall genetically based susceptibility, therefore, is determined by the *sum* of all the risk alleles you carry. A more recently proposed alternative, called the **common disease–rare variant hypothesis**, arose as researchers determined that neuropsychiatric disorders tend to be extremely heterogeneous in their genetic risk alleles. That is, different individuals diagnosed with the same disorder may carry vastly different genetic profiles. According to this hypothesis, therefore, a significant portion of the genetic risk for a neuropsychiatric disorder stems from rare mutations or other genetic anomalies such as copy number variations (variable numbers of repeated stretches of DNA). Note that findings supporting the rare variant hypothesis have been obtained largely from studies of nonaddictive disorders such as autism rather than studies of addiction itself. Nevertheless, researchers believe that those findings are likely to be applicable to addiction as well. Moreover, the two hypotheses just outlined are not mutually exclusive; indeed, the risk of developing an addictive disorder may well depend on a combination of both common and rare risk alleles.

[6] A gene–environment interaction occurs when a genetic contribution to a trait is only manifested in individuals who also experience a specific type of life event. For example, there might be evidence that a particular gene contributes to vulnerability for developing addiction in people who experience major stressful events in their life but not in people who don't have such experiences.

Three main approaches are used to study the genetics of neuropsychiatric disorders, such as addiction (Yu and McClellan, 2016). These are **candidate gene analysis**, **linkage analysis**, and **genome-wide association studies** (**GWAS**). Although these terms may sound daunting at first, they are not so difficult to understand. When researchers use the candidate gene approach, they are focusing on the potential involvement of genes that have been preselected based on the biochemical mechanisms thought to underlie the disorder. In the case of addiction, for example, we shall see later in the chapter that the neurotransmitter dopamine (DA) plays a key role in the rewarding and reinforcing effects of many abused drugs. Based on this information, scientists interested in the genetics of addiction might start by determining whether drug addicts, compared with healthy controls, tend to carry certain alleles for genes that code for proteins that participate in dopaminergic transmission. The relevant proteins in this case would include enzymes involved in DA synthesis and/or metabolism, the membrane DA transporter protein, and the family of DA receptors. The second approach, linkage analysis, seeks to find chromosomal regions that tend to associate with the disorder being studied. Once such a region has been identified, the next step is to identify a particular mutation or group of mutations present within that region of DNA that enhances susceptibility to the disorder. The third approach, GWAS, is the most favored of the three in current research efforts. As the name implies, this approach investigates the entire genome in search of alleles that associate with a disorder. Multiple alleles of a gene often differ by single nucleotides (the AGCTs of the genetic code) at various positions along the DNA sequence that comprises the gene. These variations of individual nucleotides are called **single-nucleotide polymorphisms** (abbreviated **SNPs** and pronounced "snips").[7] To be considered as having a SNP, a gene must not only possess alternate forms, but every form must be present in at least 1% of the population. For this to be the case, the allele must have persisted for a significant period of evolutionary time and have been inherited across many generations. When a very rare (much less than 1%) nucleotide change is observed, such a change is interpreted as a recent mutation, in either the carrier of that gene or a relatively recent ancestor like a parent or grandparent. **FIGURE 9.14** shows the distinction between SNPs and mutations. Despite this difference, both SNPs and mutations are important because changing just a single nucleotide usually changes the identity of one of the amino acids in the protein coded for by that gene, and this lone amino acid alteration may significantly modify the functioning of the protein. To illustrate this last point using our example of the dopaminergic system, researchers have found SNPs in the human tyrosine hydroxylase gene that result in lower enzymatic activity and, therefore, a slower rate of DA synthesis (Haavik et al., 2008). Moreover, a SNP in the gene for the human D_2 DA receptor causes a reduction in DA binding. Carriers of this form of the receptor appear to exhibit poorer cognitive function and may be at increased risk for ADHD (Gluskin and Mickey, 2016).

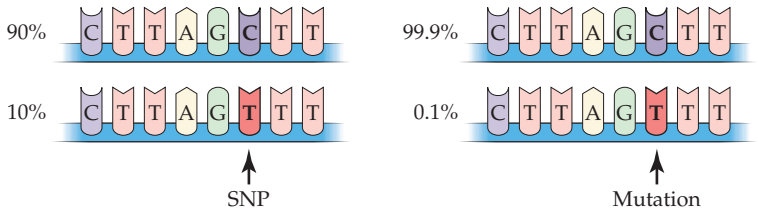


FIGURE 9.14 Variation in a single nucleotide may signify either a single-nucleotide polymorphism (SNP) or a mutation
Shown is the nucleotide sequence of a small region of a hypothetical gene in which two different alleles have been discovered by genotyping a large number of people. The results depicted on the left would be interpreted as a SNP in this region of the gene, because the alternate allele (with T in place of C) was found in over 1% of the sample. In contrast, the results depicted on the right would be interpreted as resulting from a mutation, because the alternate allele was only found in a very small percentage of the sample and, therefore, is very rare.

With this background information in mind, what have we learned, thus far, about the genetics of addiction? The short answer is that some progress has been made, but we have much farther to go. Despite the large amount of research looking for SNPs that may confer vulnerability to substance misuse, only a small number of alleles have reliably showed up across multiple studies. These include several SNPs in the opioid receptor genes associated with vulnerability to opiate addiction (Reed et al., 2014), as well as SNPs in alcohol-metabolizing genes, the DA D_2 receptor gene, and a cluster of genes that code for cholinergic proteins that have been consistently related to alcohol and/or nicotine misuse (Bühler et al., 2015). Other studies have identified SNPs that influence the degree of effectiveness of pharmacotherapies for addicted patients (Nielsen et al., 2014; Bauer et al., 2015). Finally, recent reviews have presented findings from GWAS in relation to alcohol, stimulant, or opiate addiction (Jensen, 2016; Reilly et al., 2017). This approach has revealed many more prospective genetic contributors to addiction vulnerability, but the genes are mostly different from those identified through candidate gene or linkage analyses (Hall et al., 2013). Indeed, many of these genes are quite unexpected, such as genes that

[7]The word *polymorphism* stems from *poly*, meaning "many," and *morphe*, meaning "form."

code for cell adhesion molecules, which are cell surface proteins that help cells bind to each other. Continued research will undoubtedly identify still more genetic contributors to addiction and will hopefully also begin to elucidate the mechanisms underlying the involvement of novel factors like cell adhesion molecules. It will additionally be important to resolve the question of why the various screening approaches have found different gene associations, and which approaches are providing the answers we seek.

Psychosocial variables also contribute to addiction risk

It is important to consider individual differences in susceptibility to drug addiction, not only at a biological level, but also with respect to psychosocial variables (Kalant, 2009; Swendsen and Le Moal, 2011). We can categorize these variables as either increasing addiction risk or having a protective effect. Each category will be discussed in turn.

RISK FACTORS Many modulating factors can influence either the likelihood of someone becoming a drug addict or the probability that they will be able to achieve stable abstinence once addicted. For example, one survey relating sociodemographic variables to later development of a substance use disorder found increased risk to be associated with younger age, less education, nonwhite ethnicity, and lack of employment (Swendsen et al., 2009). Additional risk factors include exhibiting conduct problems during childhood and having substance-using friends. Furthermore, the occurrence of stressful life events and an inability to cope with stress are important contributors to the risk for addiction. The life histories of drug addicts often show instances in which stressful events either promoted increased drug use or precipitated relapse from a previous period of abstinence. Numerous animal studies have confirmed that stress can increase the self-administration of abused drugs and can trigger renewed drug-taking behavior in models of relapse (Sinha, 2008; Stewart, 2000). For this reason, many treatment providers teach their clients new coping skills to deal with life stresses without relapsing.

Addicts and alcoholics are often diagnosed with an anxiety, mood, or personality disorder in addition to their drug problem (Swendsen and Le Moal, 2011). Such co-occurrences are called **comorbidity** in the medical literature. Some investigators have proposed that comorbid psychiatric disorders are causally related to addiction. For example, stressful life events could trigger anxiety and mood disorders such as depression, which in turn could lead to substance use in an attempt at self-medication. Indeed, this idea has sometimes been called the **self-medication hypothesis**. It predicts that individuals suffering from elevated anxiety should prefer alcohol and other sedative–anxiolytic drugs (**FIGURE 9.15**), whereas depressed individuals should seek out stimulant drugs such as cocaine or amphetamine. Also possible is a reverse direction of causality in which a substance use disorder gives rise to symptoms of anxiety and/or depressed mood. An alternative to these causal models is the hypothesis of **shared etiology**, which proposes that certain factors (genetic and/or environmental) contribute to an elevated risk of *both* addiction and other psychiatric disorders. Current evidence does not rule out any of these competing hypotheses.

At the psychological level, there is an abundance of research on the relationship between personality variables and alcoholism or other types of drug addiction. Verheul and van den Brink (2000) proposed three different personality-related pathways to addiction: (1) behavioral disinhibition; (2) stress reduction; and (3) reward sensitivity. The behavioral disinhibition pathway hypothesizes that deviant behaviors such as substance abuse are linked to a trait cluster of impulsivity, antisociality, unconventionality, and aggressiveness, combined with low levels of constraint and harm avoidance. This pathway may be particularly relevant for drug abusers suffering from antisocial or borderline personality disorder. According to the stress reduction pathway, high scores on traits such as stress reactivity, anxiety, and neuroticism are indicative of heightened vulnerability to stressful life events. This pathway fits with the self-medication hypothesis mentioned above. The third pathway, termed reward sensitivity, relates drug abuse to the personality traits of sensation seeking, reward seeking, extraversion, and gregariousness. It suggests that individuals scoring high on these traits seek out drugs for their positively reinforcing qualities.

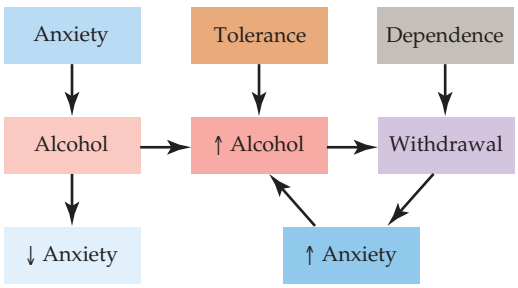


FIGURE 9.15 Self-medication hypothesis applied to the development of alcohol dependence Some people drink alcohol to alleviate anxiety. If anxiety is persistent and alcohol use continues, the onset of tolerance leads to increased alcohol consumption and potentially to the development of alcohol dependence. Attempts at abstinence then lead to withdrawal symptoms that include heightened anxiety, thereby provoking relapse to drinking. (After Lingford-Hughes et al., 2002.)

Finally, familial and sociocultural influences can also influence the risk of developing a pattern of drug abuse or addiction (Whitesell et al., 2013). Familial factors have been studied most extensively in conjunction with the risk of alcoholism. Chartier and colleagues (2010) reviewed evidence implicating childhood maltreatment, presence of violence within the family, and being subjected to less parental monitoring as risk factors for developing problematic alcohol use during adolescence. Other research has found that adult children of alcoholics are at increased risk for having alcohol or other substance abuse problems (Windle and Davies, 1999). In the case of alcohol itself, this may be related in part to modeling (imitation) of the parent's drinking behavior or to a heightened expectancy that drinking will lead to positive mood changes. Sociocultural studies have identified at least four different functions served by drug abuse (Thombs, 1999). The first involves social facilitation. Alcohol and other drugs are often consumed in a group setting, where the substance may enhance social bonds between participants. The second function is to remove the user from normal social roles and responsibilities, thereby allowing an escape from the burdens that may be associated with these responsibilities. Third, substance use may promote group solidarity within a particular ethnic group. An example of this phenomenon is the association of Irish culture with heavy alcohol use and a high rate of alcoholism. As a result, the government of Ireland has taken steps to publicize the damaging health and economic consequences of excessive alcohol use in that country (HRB National Drugs Library, 2017). Finally, substance abuse sometimes occurs within a "drug subculture" that embraces social rituals surrounding a particular subculture and rejects conventional social norms and lifestyles. Sociological studies have identified distinct subcultures for many different substances, including heroin, cocaine, alcohol, marijuana, methamphetamine, and PCP. This is not to say that all users of a particular substance participate in the rituals of a subculture, or that users necessarily limit themselves to just one substance. Nevertheless, one can find groups of individuals who share their common experiences with a specific drug of abuse and who have a similar disdain for the "straight" lifestyle.

PROTECTIVE FACTORS We can think about protective factors in drug addiction in two different ways. First, an absence of the various risk factors described in the previous section should be relatively protective with respect to drug abuse or addiction. Put another way, individuals who do not suffer from a preexisting personality or mood disorder, who do not exhibit the trait clusters mentioned earlier, who come from stable families with no substance abuse, who do not belong to ethnic groups that promote substance use, and who do not become involved in the social rituals surrounding drug use are at reduced risk for becoming addicted.

The second way that protective factors can operate is to help maintain a stable abstinence in previously drug-abusing or addicted individuals. Drug addicts who seek treatment tend to be the most heavily dependent and seriously affected individuals. Many will be able to overcome their dependence, but some will struggle with their drug problems for much of their remaining life. It may be surprising to learn that alcoholics and drug addicts can often achieve long-term abstinence with little or no treatment (Bischof et al., 2001; Klingemann et al., 2009; Humphreys, 2015). This has been termed **natural recovery** or spontaneous recovery. Before providing specific evidence for natural recovery, it is useful to consider the overall trajectory to recover from drug dependence over time. For several widely abused substances, this trajectory is illustrated in the results from the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC), a large survey of alcohol, nicotine, cannabis, and cocaine users conducted in 2001 to 2002 (Lopez-Quintero et al., 2011). Dependence on each substance was defined using the criteria from the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition, which were similar to the substance use disorder criteria in *DSM-5*. Over a period of 10 years from the onset of dependence, 37% of alcohol-dependent respondents experienced remission, meaning that they no longer qualified for a diagnosis of dependence. The corresponding percentages for the other substances were 18% for nicotine, 66% for cannabis, and 75% for cocaine.[8] **FIGURE 9.16** uses the NESARC data to depict the cumulative probability of remission from dependence on alcohol, cannabis, and cocaine over 30 years or more since the beginning of dependence (Heyman, 2013). We can see that the vast majority of individuals who become dependent on alcohol, cannabis, cocaine, or nicotine eventually achieves remission. To determine the role of natural recovery in the remission process, researchers obtained additional information about the alcohol-dependent respondents from NESARC. The results show that at 20 years since the beginning of dependence, full or partial remission was achieved by 96% of individuals who were never treated but only 86% of individuals who had received some type of treatment (Dawson et al., 2005). While these statistics provide strong evidence for natural recovery, they do *not* mean that people who are dependent on alcohol or other substances should not seek treatment. Indeed, among the alcohol-dependent NESARC respondents, full abstinence from drinking was more likely to occur if the person had received treatment

[8] As an interesting side note, by the criterion of remission, cocaine is the least addictive of the four substances surveyed in NESARC.

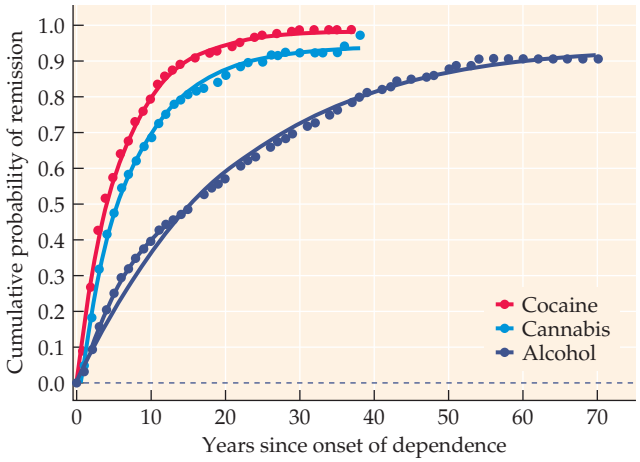


FIGURE 9.16 Survey results showing the cumulative probability over time of remission from dependence on cocaine, cannabis, or alcohol
The time course of remission from drug dependence was assessed using data from the 2001-to-2002 NESARC study. The symbols in the figure represent data from the study, and the curved lines were produced by mathematical equations fitted to the data that permit a quantitative analysis of the results. The upward slope of each curve depicts how rapidly the group of individuals who had become dependent on each substance were able to achieve remission. For the surveyed population, remission from cocaine dependence was the most rapid, with a high probability of remission within 10 years and nearly 100% (probability of 1.0) remission by 30 years after the onset of dependence. Achievement of cannabis remission was a little slower, whereas remission from alcohol dependence was clearly the slowest. Note the flatter slope of the alcohol remission curve and that fewer than 90% of dependent users had remitted even after 60 years from dependence onset. (After Heyman, 2013, CC-BY 3.0; data from Lopez-Quintero et al., 2011.)

than if not. More generally, individuals who recover without the use of professional treatment or even self-help groups like Alcoholics Anonymous tend to have less severe substance use problems than those who seek help for their problem (Cunningham, 1999).

Recovered drug addicts and abusers recount many different tales of how they made and kept the decision to quit using drugs. It is often thought that the addict must hit "rock bottom" or go through an "existential crisis" before he'll be sufficiently motivated to stop using. One such example was recently recounted by Luana Ferreira, a business reporter for the BBC (Ferreira, 2017). Her article describes the story of Khalil Rafati, a homeless heroin and crack addict weighing only 109 lbs and with severely ulcerated skin when he was found unconscious by Los Angeles paramedics in 2003. At the age of 33, Rafati had just suffered his ninth heroin overdose, one that nearly killed him. Realizing that if he didn't overcome his addiction he would die, Rafati spent 4 months in a rehab facility and has been "clean" since then. Indeed, he went from being a drug addict to being a successful entrepreneur, and he is now the millionaire owner of Sunlife Organics, a California health food business. Although this kind of traumatic experience is reported in some cases, many individuals find the means to abstain without reaching such a crisis situation. Spontaneous recovery from drug abuse or addiction may be triggered by a variety of major life changes. Some of these are transitional life changes like getting married, becoming pregnant, or obtaining a good job. Another kind of positive change would be having a spiritual/religious experience. Other motivations for voluntarily stopping drug use are related to the negative consequences of such use, including health problems, financial problems, loss of one's job, social pressures, fear of imprisonment, or death of a drug-abusing friend. Once the decision has been made, the risk of relapse is reduced by such actions as moving to a new area, developing new social relationships with nonusers, obtaining employment, and engaging in substitute activities like physical exercise or meditation. The relative importance of different factors varies somewhat with different drugs of abuse. For example, health concerns are particularly important in motivating tobacco smokers to stop smoking, and, much more than other drug users, smokers cite simple willpower as a critical factor in maintaining abstinence (Walters, 2000).

In conclusion, achievement of stable abstinence either spontaneously or with the aid of treatment is greatly facilitated by certain behavioral changes that help protect the drug addict from relapse. Some of these changes involve avoidance of drug-associated cues (e.g., moving to a new location and shunning drug-using acquaintances), whereas other changes serve to provide substitutes for the former substance use, new sources of reinforcement, a new social support network, financial stability, and general structure to the individual's life.

The factors contributing to drug addiction can be combined into a biopsychosocial model

FIGURE 9.17 summarizes the effects of the factors discussed in this section on the development and maintenance of compulsive drug use (i.e., addiction). You can see that some of these factors promote the likelihood that addiction may occur, whereas others reduce this likelihood. A model that includes the full range of pharmacological, biological, and psychological/sociocultural factors that influence addiction risk can be called a **biopsychosocial model** of addiction. Although it is beyond the scope of this text to propose a detailed model of this kind, we believe that consideration of all such factors, crossing multiple levels of analysis, is necessary for achieving an adequate understanding of the addiction process.

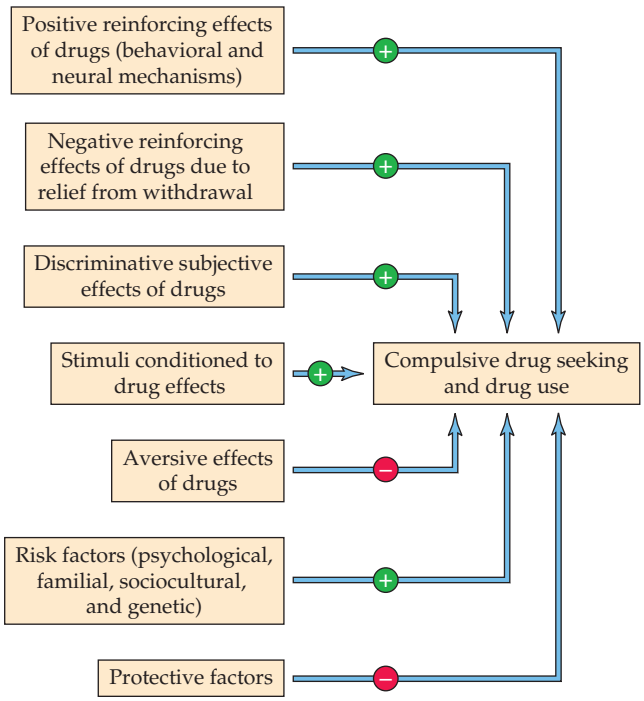


FIGURE 9.17 A biopsychosocial model of drug addiction (After Stolerman, 1992.)

Section Summary

- Several types of factors contribute to the development and maintenance of compulsive drug seeking and drug use.
- Most abused drugs produce positive reinforcing effects in humans and experimental animals, as shown by IV self-administration. The reinforcing efficacy of a drug can be investigated by means of a dose–response function (which typically yields an inverted U-shaped curve) or preferably by means of breaking point determination using a progressive-ratio schedule.
- In animals that have been trained to self-administer a drug by performing an operant response (e.g., a lever press) and then have been extinguished on that response, renewed responding can be provoked by administering a priming dose of the drug, presenting a stimulus cue that was previously paired with drug delivery, or stressing the animal. This procedure is called reinstatement of drug-seeking behavior and is considered to be an experimental model of relapse in an abstinent addict.
- The rewarding effects of drugs are studied using place-conditioning procedures, as well as the effects of drugs on the threshold for electrical self-stimulation of the brain. Acute administration of most abused drugs leads to a reduction in the self-stimulation threshold, whereas withdrawal from drug exposure in dependent animals leads to an increase in the threshold.
- Drugs also sometimes produce aversive effects, although these may not be sufficiently strong to outweigh other factors that promote compulsive drug use.
- Repeated use of some drugs leads to physical dependence. Once this has occurred, attempts at stopping drug use give rise to a highly unpleasant withdrawal, or abstinence, syndrome. Koob and Le Moal proposed that in the development of addiction, drug-taking behavior progresses from an impulsive stage (primary motivation for drug use is the positive reinforcing effect) to a compulsive stage (primary motivation is negative reinforcement from alleviation of these unpleasant withdrawal symptoms). This shift is hypothesized to involve the recruitment of an antireward system in the brain.
- Through conditioning, environmental stimuli previously associated with drug use can elicit withdrawal symptoms such as drug craving in an abstinent individual. This response is accompanied by activation of brain areas that have been implicated in the addictive process.
- Psychoactive drugs, including drugs of abuse, can produce discriminative stimulus effects in animals that are thought to correspond to the subjective effects produced by these same compounds in human users. Experienced users come to expect these subjective effects, and such expectations are thought to contribute to the persistence of drug use.
- Addiction is a heritable disorder, but there is no addiction gene per se. Rather, specific alleles of many different genes are believed to each contribute a small increase in the risk for developing addiction. Different alleles of a gene may vary in just a single nucleotide, thus giving rise to single-nucleotide polymorphisms, or SNPs.
- The common disease–common variant hypothesis and the common disease–rare variant hypothesis are alternative hypotheses to account for the genetic contribution to neuropsychiatric disorders like addiction. These hypotheses differ with respect to whether the risk alleles for a disorder are relatively common or rare within the affected population.
- The genetics of neuropsychiatric disorders are studied using candidate gene analysis, linkage analysis, and genome-wide association studies (GWAS). For reasons yet to be determined, the GWAS approach has identified different addiction vulnerability genes than the other two.

- Psychosocial variables may either increase addiction risk or have a protective effect. One risk factor is suffering from an anxiety, mood, or personality disorder. Some investigators have proposed a self-medication hypothesis that addicts are using drugs to treat the symptoms of a preexisting psychiatric disorder. However, alternate hypotheses such as shared etiology have been put forth to explain the high comorbidity of such disorders with addiction.
- Personality variables such as impulsivity, antisociality, anxiety, high stress reactivity, sensation seeking, and extraversion have been associated with various pathways to drug addiction. The risk of becoming an addict is also affected by familial and sociocultural influences. For example, drugs may promote social facilitation, remove the user from normal social roles and responsibilities, promote solidarity within a particular ethnic group, or lead to association with a specific drug subculture.
- Finally, various protective factors can reduce the likelihood of an individual's becoming addicted or can help prevent relapse in drug users attempting to maintain stable abstinence. These factors encompass the person's personality structure, social (including family) life, and environment.
- Many individuals who at one time had a substance use disorder are able to achieve remission over time. This is often achieved through a process of natural recovery, which may be facilitated by transitional life events and/or by the negative consequences of continuing drug use. However, the likelihood of achieving natural recovery is lower for more severely affected substance users. These individuals may require formal treatment or a self-help group like Alcoholics Anonymous to have the best chance of recovery from their addiction.
- Combining the full range of pharmacological, biological, and psychological/sociocultural factors that influence addiction risk can be called a biopsychosocial model of addiction.

The Neurobiology of Drug Addiction

Research conducted over the past few decades has made tremendous progress in elucidating the neurobiological mechanisms that underlie the development of addiction. Much of this progress has been driven by technological advances in areas such as molecular pharmacology and neuroimaging. There is still much to learn; however, researchers are coming ever closer to understanding how drugs of abuse acutely alter brain function and, more importantly, how repeated exposure to these same substances can produce long-lasting changes in the brain that may lead to compulsive drug use, even in the presence of adverse consequences to the user (i.e., substance use disorder in the *DSM-5*).

The development of addiction has been conceptualized as a repeating spiral of three stages associated with substance use: (1) preoccupation with and anticipation of obtaining and using the substance, (2) binge use and intoxication from the substance, and (3) withdrawal and the associated negative affect (i.e., mood state) when coming down from the drug "high" that motivates further use (Koob and Le Moal, 2001; see Figure 9.4). That framework has been used to help understand the neurobiology of addiction, including the neural circuits and neurotransmitters implicated most directly in each stage (Koob and Volkow, 2016). Accordingly, we will first discuss addiction neurobiology within this three-stage framework. Next will be a section on the role of molecular adaptations in addiction. Last, we will address the important question of whether addiction should be considered a brain disease.

Drug reward and incentive salience drive the binge–intoxication stage of drug use

DRUG REWARD Studies conducted primarily using laboratory rats and mice have allowed researchers to construct a **reward circuit** that mediates the acute rewarding and reinforcing effects (defined operationally as described previously) of most abused drugs. **FIGURE 9.18** depicts this circuit superimposed on a sagittal section of a rodent brain. Anatomical pathways contained within this circuit include the mesolimbic and mesocortical DA pathways (shown in red) that originate in the ventral tegmental area (VTA) of the midbrain and terminate in the nucleus accumbens (NAcc), amygdala (AMG), and frontal cortex (FC). Other important connections are those within the extended amygdala system (shown in blue)—a collection of anatomically and functionally linked structures that includes the central nucleus of the amygdala, the shell of the NAcc, and the bed nucleus of the stria terminalis (BNST). Two additional pathways within the reward circuit are not shown in the figure for the purpose of clarity: the medial forebrain bundle, a collection of ascending and descending fibers that connects the VTA with areas of the ventral forebrain including the NAcc, septal area, and olfactory tubercle; and an output pathway from the NAcc to the ventral pallidum. It is important to note that neuroimaging studies have confirmed that the human brain possesses a reward circuit similar to that identified in animals and that this circuit is activated by both drug and nondrug rewards (Haber and Knutson, 2010) (**FIGURE 9.19**).

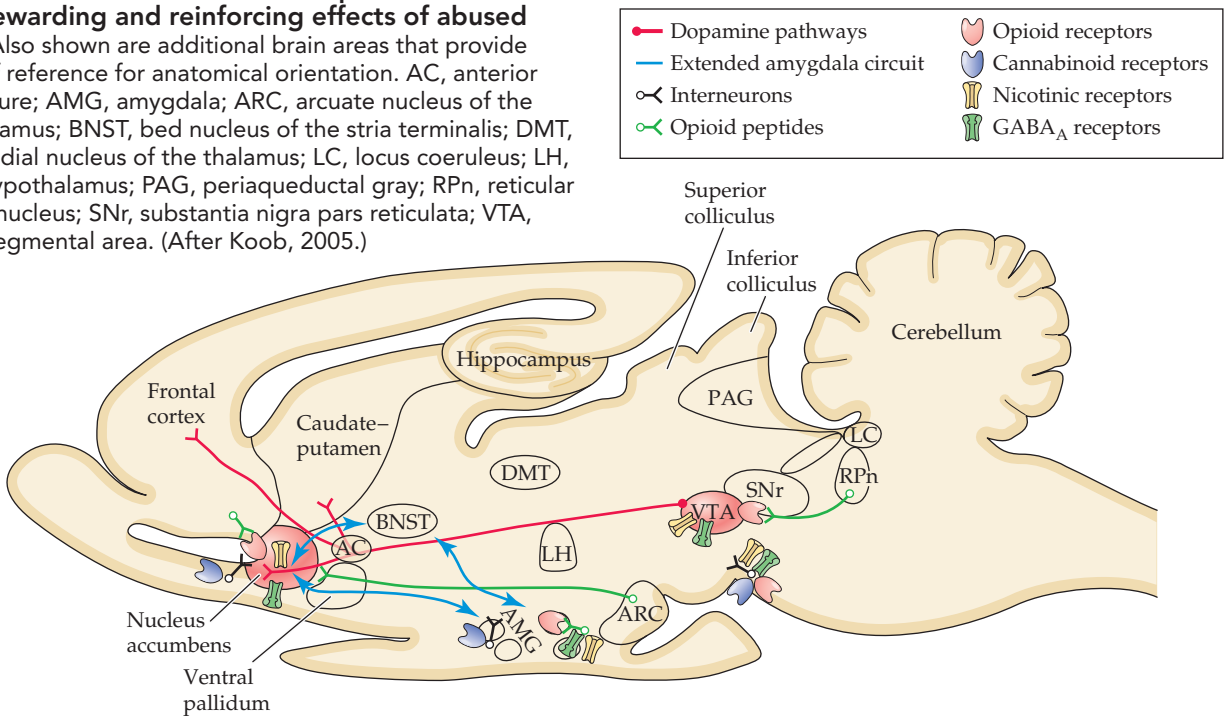


FIGURE 9.18 The neural circuit responsible for the acute rewarding and reinforcing effects of abused drugs Also shown are additional brain areas that provide points of reference for anatomical orientation. AC, anterior commissure; AMG, amygdala; ARC, arcuate nucleus of the hypothalamus; BNST, bed nucleus of the stria terminalis; DMT, dorsomedial nucleus of the thalamus; LC, locus coeruleus; LH, lateral hypothalamus; PAG, periaqueductal gray; RPn, reticular pontine nucleus; SNr, substantia nigra pars reticulata; VTA, ventral tegmental area. (After Koob, 2005.)

The reward circuit can also be characterized neurochemically by considering the locations within the circuit that contain the specific molecular targets for each type of abused drug. For example, psychostimulants such as cocaine and amphetamine exert their rewarding effects by directly releasing DA from dopaminergic terminals in the NAcc and/or by blocking DA reuptake after its release (see Chapter 12). Opioid drugs (e.g., morphine and heroin) and endogenous opioid peptides (endorphins and enkephalins) activate the reward circuit by stimulating opioid receptors in the VTA, NAcc, and AMG (see Chapter 11). Opioid reward is mediated by a combination of DA-dependent (through increased DA release) and DA-independent mechanisms. Alcohol enhances the action of γ-aminobutyric acid (GABA) on GABA$_A$ receptors, and this enhances DA release in the

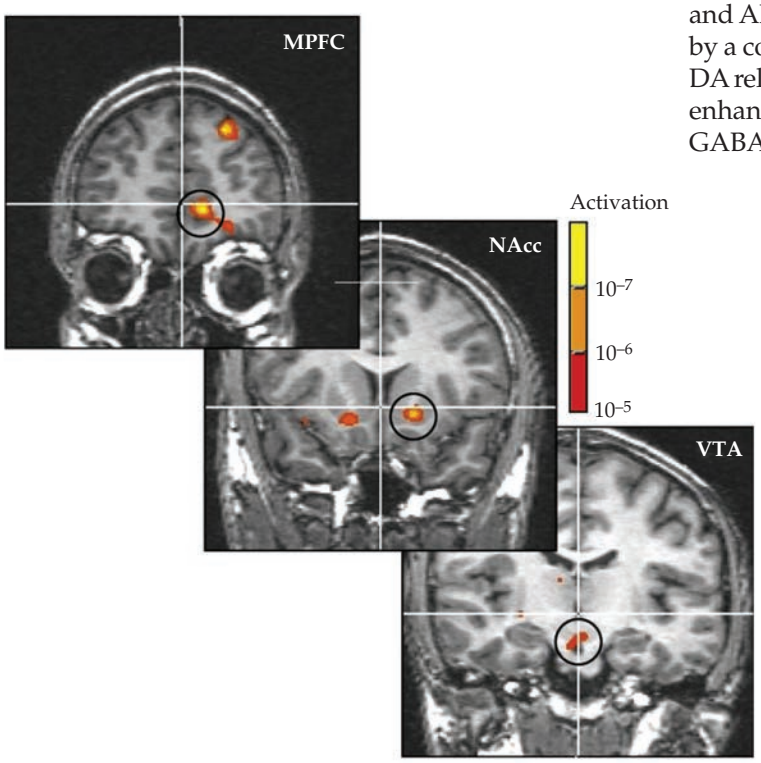


FIGURE 9.19 Activation of the human reward circuit by expectation of monetary rewards Study participants were given a task in which presentation of different visual cues signaled a low, medium, or high probability of either losing or winning a variable amount of money ($0.0, $1.00, or $5.00) on that trial. Regional brain activation was assessed on each trial type using functional magnetic resonance imaging (fMRI). The circled regions on the images presented here demonstrate that the medial prefrontal cortex (MPFC), nucleus accumbens (NAcc), and ventral tegmental area (VTA) all showed activation that was positively correlated with the expected value of the reward. The color scale is coded so that yellow represents the greatest degree of activation, followed by orange and then red. (From Haber and Knutson, 2010; original data from Knutson et al., 2005.)

NAcc and opioid peptide release in the VTA, NAcc, and AMG (see Chapter 10). Nicotine derived from tobacco activates the reward circuit by stimulating nicotinic cholinergic receptors in the VTA, NAcc, and AMG (see Chapter 13), whereas endogenous cannabinoids and Δ^9-tetrahydrocannabinol (THC) derived from marijuana are rewarding because of stimulation of cannabinoid receptors in the same brain areas (see Chapter 14). Both nicotine and THC enhance DA release in the NAcc through local mechanisms and/or by acting within the VTA.

The mesolimbic DA pathway from the VTA to the NAcc has been accorded a central role in drug reward and reinforcement. Animal studies have shown that almost all drugs of abuse activate this pathway either by enhancing VTA cell firing or by increasing extracellular DA levels in the NAcc, as in the case of psychostimulants. In Chapter 5, we saw that burst firing of midbrain dopaminergic neurons causes much greater DA release than single-spiking firing mode. In essence, we can think of abused drugs as mimicking this effect of burst firing, either directly or indirectly.

We also noted that D_1 DA receptors have a lower affinity than D_2 receptors for the transmitter, as a result of which elevated DA levels are required for significant D_1 receptor activation. Indeed, the D_1 receptor subtype is more important than D_2 for drug reward, although activation of both subtypes seems to be necessary to achieve a maximum effect (Volkow and Morales, 2015).

INCENTIVE SALIENCE As individuals progress repeatedly through the cycle depicted in Figure 9.4, drug reward often declines because of a process of tolerance. Consequently, other factors become increasingly important in motivating continued substance use. One such factor is **incentive salience**. The concept of incentive salience is a key component of the **incentive sensitization theory** of addiction first proposed by Robinson and Berridge in 1993 and updated in subsequent reviews (e.g., Robinson and Berridge, 2000; 2008). A key feature of this theory is the distinction between drug *liking* (i.e., the euphoric feeling or "high") and drug *wanting* (i.e., craving). The psychological process leading to "wanting" is called incentive salience because it is a process whereby certain stimuli (in this case, internal and external stimuli associated with drug use) become extremely salient (attention getting) and acquire incentive (attractive and desirable) properties. The incentive sensitization theory proposes that over the course of developing a drug addiction, the user experiences a marked increase in "wanting" the drug even though there is no change or even a decrease in drug "liking" (**FIGURE 9.20**). This disparity is thought to occur because different brain mechanisms are responsible for these two components of drug use motivation,

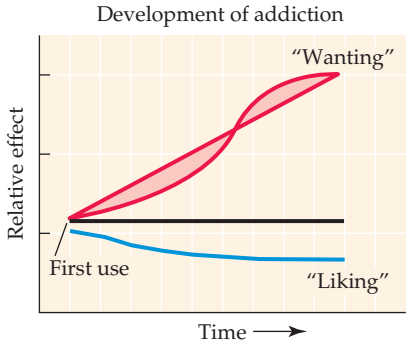


FIGURE 9.20 Sensitization of drug "wanting" but not drug "liking" Incentive sensitization is proposed to contribute to the development of addiction. The horizontal line represents the initial relative levels of liking and wanting during first use of the drug. (After Berridge et al., 2009.)

and repeated drug use causes sensitization of the "wanting" system but no sensitization or even tolerance in the "liking" system. Berridge and Kringelbach (2008) argue that the "liking" system is focused on specific "hedonic hot spots" in the brain where local stimulation produces a pleasurable effect, whereas the "wanting" system is a more widespread circuit that includes the mesolimbic DA pathway. Evidence for a relationship between DA and drug wanting comes from a variety of animal and human studies, such as a study by Leyton and coworkers (2005) that found a reduction in cocaine craving (wanting) after catecholamine depletion despite no effect on cocaine-induced euphoria (liking).

UNDERSTANDING THE ROLE OF DA IN REWARD AND MOTIVATION Despite the widespread agreement that DA plays a role in the rewarding effects of both drugs and natural rewards like food, the motivational properties of this neurotransmitter are not universal, nor do they consist simply of encoding the hedonic (pleasurable) aspects of stimuli. First, the dopaminergic system is *essential* for drug reward for some substances (e.g., cocaine or amphetamine) but not for others (e.g., heroin or alcohol) (Koob and Volkow, 2010; Badiani et al., 2011). Second, human brain imaging studies seem inconsistent with animal findings in that certain drugs such as opiates or THC provoke very little DA release in the ventral striatum (Nutt et al., 2015). Together, these findings suggest that neurotransmitter systems besides DA must also be involved in drug reward. Two such systems are the endogenous opioid and cannabinoid systems, based on findings that drug reward often can be blunted or even blocked by interfering with either of those systems (Solinas et al., 2008; Trigo et al., 2010). Current information on the role of DA in reward and motivation is presented in Web Box 9.3.

The withdrawal/negative affect stage is characterized by stress and by the recruitment of an antireward circuit

During the withdrawal/negative affect stage of repeated drug use, the user experiences stress and a dysphoric mood, often characterized by irritability, anxiety, and depression. Drug reward is reduced, as mentioned above with respect to the incentive sensitization theory of addiction. We can conceptualize this process as a type of tolerance such that the user experiences less and less pleasure from each episode of drug use. Indeed, addicts often report that they need to take drugs just to feel "normal" instead of feeling "high," as they did before becoming addicted. Moreover, laboratory animals withdrawing from chronic drug administration exhibit elevated thresholds for rewarding electrical stimulation of the brain (previously summarized in Table 9.4). Those findings are consistent with a general impairment of the reward circuit during the withdrawal/negative affect stage.

Decreased activity of the reward circuit is just one of many **neuroadaptations** that occur in response to repeated exposure to drugs of abuse and that play a key role in the development of addiction. The term *neuroadaptation* pertains to persistent neurobiological changes that can encompass neurotransmitter activity, gene expression, cellular structure (e.g., altered dendritic branching), and neural circuitry (e.g., synaptic plasticity). Koob and Le Moal have proposed that the transition to addiction involves two different kinds of neuroadaptations (see Koob and Le Moal, 2008a, 2008b; Koob, 2009, 2015). They consider the progressive down-regulation of the reward circuit to be a *within-system* neuroadaptation, because it is a direct attempt by the brain to counteract the repeated drug-induced activation of that circuit. In contrast, a key *between-systems* neuroadaptation is the gradual recruitment of a neural circuit that Koob and Le Moal call the **antireward system**. The neuroanatomical substrate of the antireward system is the previously mentioned extended amygdala, which includes the bed nucleus of the stria terminalis, the central nucleus of the amygdala, and part of the nucleus accumbens. However, the neurotransmitters involved in this system are very different from those that mediate reward. Instead, activation of the antireward system leads to increased release of norepinephrine (NE) and two neuropeptides, corticotropin-releasing factor (CRF) and dynorphin. This system has two major functions: it puts a limit or brake on reward (imagine a situation in which the hedonic effects of natural rewards like food and sex were unlimited), and it mediates some of the aversive effects of stress such as increased anxiety. In a drug-dependent person or experimental animal, the antireward system is activated during drug withdrawal and plays a major role in the aversive effects of withdrawal and

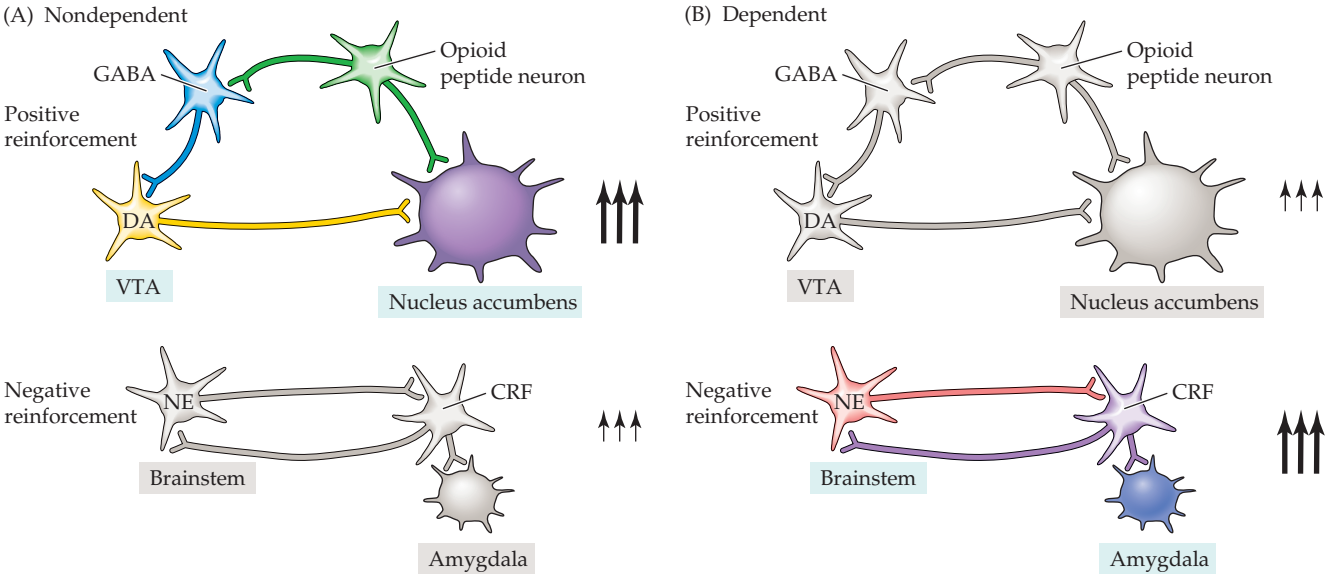


FIGURE 9.21 Neurochemical changes underlying the transition from positive to negative reinforcement in addiction (A) In a nondependent individual, drug use is supported mainly by positive reinforcement mediated by the reward system (large arrows) and relatively little by the antireward system (small arrows). The part of the reward system involving dopamine (DA) and opioid peptides in the nucleus accumbens is illustrated. (B) In a dependent individual, the reward system has been down-regulated and the antireward system has been recruited. As a result, drug use is now supported mainly by negative reinforcement (alleviation of withdrawal symptoms). The part of the antireward system involving norepinephrine (NE) and corticotropin-releasing factor (CRF) acting in the central amygdala is illustrated. VTA, ventral tegmental area. (After Koob and Le Moal, 2008.)

the negative reinforcement produced by renewed drug taking. **FIGURE 9.21** illustrates some of the key neurochemical components involved in drug reward before dependence (addiction) has occurred (i.e., dominance of positive reinforcement mediated by the reward system) compared with the components involved after dependence has taken place (i.e., dominance of negative reinforcement mediated by the antireward system). This model is particularly applicable to drugs that produce strong physical dependence, such as alcohol and opiates, although increased CRF in the central nucleus of the amygdala has also been implicated in animal models of cocaine and nicotine dependence (Koob and Le Moal, 2008a).

The opposing actions of the reward and antireward systems have been conceptualized by Koob and Le Moal as a modern updating of the classical **opponent-process model** of motivation proposed many years ago by Solomon and Corbit (Solomon and Corbit, 1974; Solomon, 1977). The opponent-process model hypothesized that the neural mechanisms responsible for affect (mood and emotion) were organized such that any stimulus that provokes an initial strong affective reaction (e.g., a strong feeling of either pleasure or discomfort) automatically sets in motion an opposing affective response that is experienced after the initial stimulus ends. Think, for example, of the sequence of feelings produced by performing a dangerous physical activity such as skydiving for the first time: initial fear or anxiety followed by a pleasurable sense of relief after completion of the action. In the case of an abused drug, the primary affective response might be the "high," and the subsequent opposing response would be the dysphoric withdrawal reaction after the drug wears off (**FIGURE 9.22**). Solomon and Corbit (1974) further proposed that over time, repeated presentations of the stimulus altered the opponent process by strengthening its magnitude, reducing its latency to onset, and increasing its duration.

In applying the opponent-process model to addiction, Koob and Le Moal (2008a; 2008b) additionally hypothesized that a phenomenon called **allostasis** gradually changes the baseline hedonic state (i.e., mood) of the drug user. Allostasis is a biological concept introduced by Sterling and Eyer (1988) in which a physiological, behavioral, or psychological variable (in this case, hedonic state) that is repeatedly challenged (in this case, by drug exposure) maintains stability by changing its set point (its normal baseline level in the absence of the challenge). Figure 9.23 illustrates the opponent-process view of hedonic affective responses to a drug under different conditions, including an allostatic change in baseline hedonic state produced by repeated drug exposure. In a nondependent organism, drug taking leads to an initial positive hedonic response (upward deflection of the line) that gradually wanes

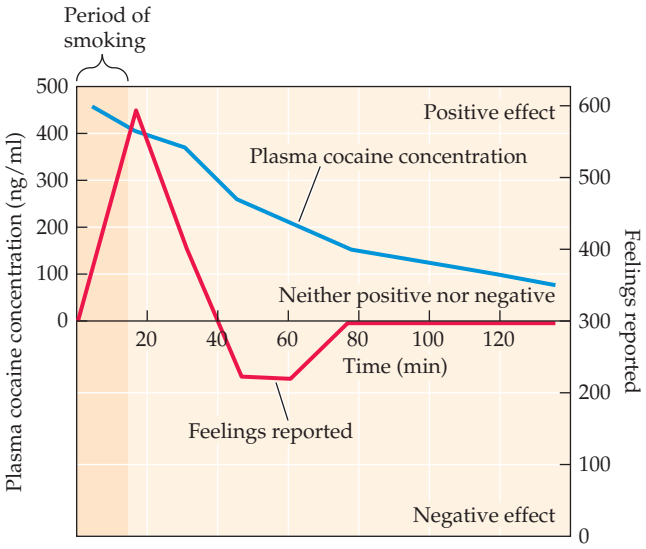


FIGURE 9.22 Euphoric followed by dysphoric feelings reported by individuals smoking cocaine paste Cocaine paste is a crude extract of coca leaves containing 30% to 90% cocaine base. The graph shows the time course of plasma cocaine concentrations and reported positive or negative effect on mood from a representative individual who smoked cocaine paste (amount not reported) during the time shown in the figure. Note that even though cocaine was still present in this person's system, the positive hedonic effect of the drug ended by 40 minutes and was replaced by a negative hedonic state (the "crash") characterized by feelings of fatigue, depression, anxiety, and cocaine craving that lasted for the next 35 minutes. (After Van Dyke and Byck, 1982.)

over time and is replaced by a negative (unpleasant) response (downward deflection) during drug withdrawal (**FIGURE 9.23A**). Finally, the organism returns to its baseline hedonic state. Once the organism has become dependent (addicted), the allostatic set point has shifted downward (0′) and the baseline mood in the absence of the drug is negative (i.e., depressed mood, irritability, or anxiety is experienced) (**FIGURE 9.23B**). The positive hedonic response to the drug now barely crosses the neutral point (0), and thus the organism experiences only a small rewarding effect of the drug. Note the severe and protracted negative hedonic effect of drug withdrawal (strengthening of the opponent process), which produces powerful motivation to take the drug again. **FIGURE 9.23C** depicts drug-taking binges during withdrawal, during which the starting hedonic point (0′) is even lower and the drug response only reaches neutrality (i.e., the addict takes the drug to feel "normal"). Finally, **FIGURE 9.23D** depicts relapse after prolonged abstinence from drug use. Because of long-lasting drug-induced neuroadaptations, the baseline mood remains low (0′) but the positive hedonic response to the drug is actually strengthened. This hypothesized strengthening is based on the finding that

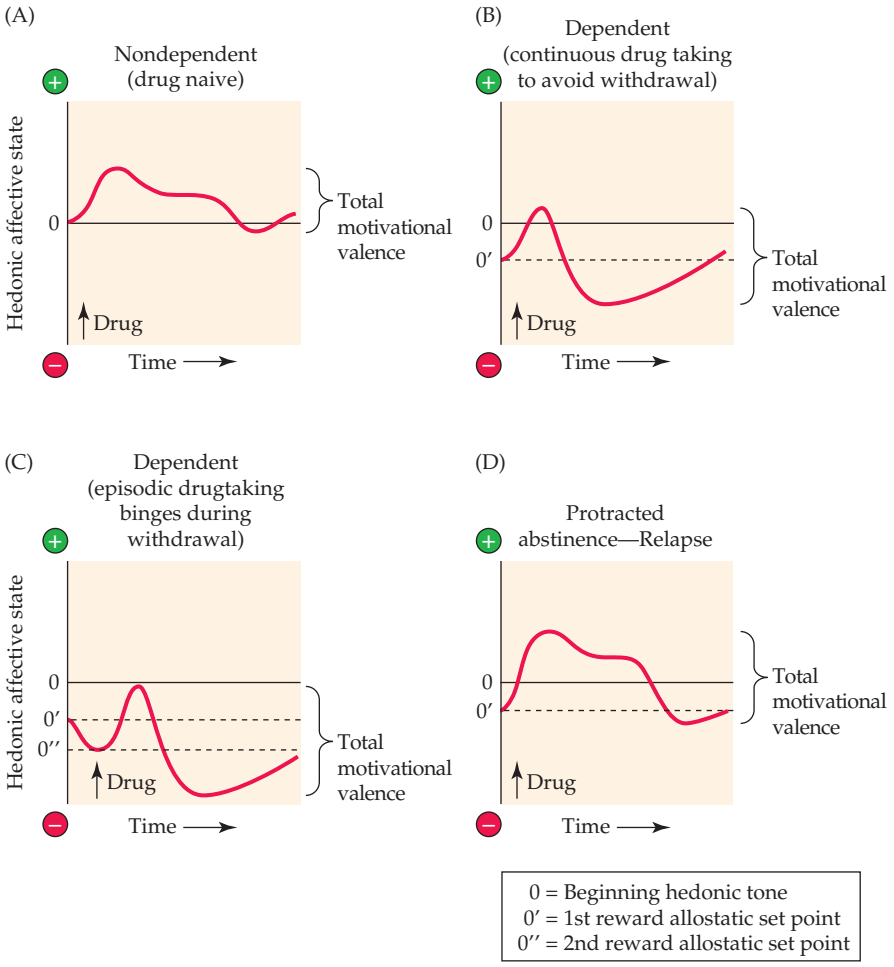


FIGURE 9.23 Hedonic responses to drug use during different stages of the addiction cycle (A) Drug use in a nondependent individual produces a strong positive hedonic response (mood elevation) with a relatively weak opponent process (negative hedonic response) during withdrawal. (B) Development of a dependent (addicted) state leads to an allostatic reduction in hedonic set point, a shorter positive hedonic response that reaches only a small elevation above neutrality, and a stronger and prolonged opponent process during withdrawal. (C) In a dependent individual undergoing episodic drug-taking binges to alleviate withdrawal, the allostatic set point is even lower because of lingering withdrawal symptoms, and the positive hedonic response to the drug reaches only the original baseline of neutral hedonic tone. (D) After protracted abstinence, the hedonic set point remains lower but the positive hedonic response is enhanced for reasons explained in the text. Note that the "total motivational valence," which represents the full range from positive to negative hedonic tone under each state and the motivational impulses driven by these changes, is expanded once the individual has become drug dependent. (After Koob and Le Moal, 2006.)

in animals that are trained to self-administer a drug like cocaine and then are subjected to forced abstinence for a long time, re-exposure to the drug leads to a powerful enhancement of drug-seeking behavior.

The preoccupation/anticipation stage involves dysregulation of prefrontal cortical function and corticostriatal circuitry

Brain imaging studies comparing addicted subjects with healthy controls have revealed both structural and functional abnormalities in the prefrontal cortex (PFC) in the addict group (Goldstein and Volkow, 2011; Volkow et al., 2009, 2012). To understand the relevance of the PFC for addiction, it is useful first to summarize some of the major behavioral functions of the PFC and the major subregions that are believed to mediate these functions. The PFC is best known for its role in **executive function**, which consists of higher-order cognitive abilities, including planning, organization, problem solving, mental flexibility, and valuation of incentives. Damage either to the PFC or to certain parts of the circuitry that connects the PFC with the rest of the brain can cause major impairments in executive function. It is important to note, however, that the PFC contributes not only to executive function, but also to the regulation of emotional and motivational processes. All three of these aspects of PFC functioning could be important for understanding the psychological and behavioral differences between addicted and nonaddicted individuals. The PFC has massive excitatory (glutamatergic) projections to various subcortical areas, and some researchers have proposed an organization of these projections into three main circuits: (1) a dorsolateral circuit that projects from the dorsolateral PFC (DLPFC) to the dorsolateral caudate nucleus and is particularly important for executive function, (2) a ventromedial circuit that connects the anterior cingulate cortex (ACC) with the NAcc and is involved in drive and motivation, and (3) an orbitofrontal circuit that projects from the orbitofrontal cortex (OFC) to the ventromedial caudate and has been associated with behavioral inhibition and impulse control (Alvarez and Emory, 2006).

Dysfunction within the PFC and its associated circuitry is hypothesized to play a key role in the preoccupation/anticipation stage of the addiction cycle. This stage is characterized by intrusive thinking, drug craving, and lack of impulse control. Intrusive thinking, in this case, consists of persistent, uncontrollable

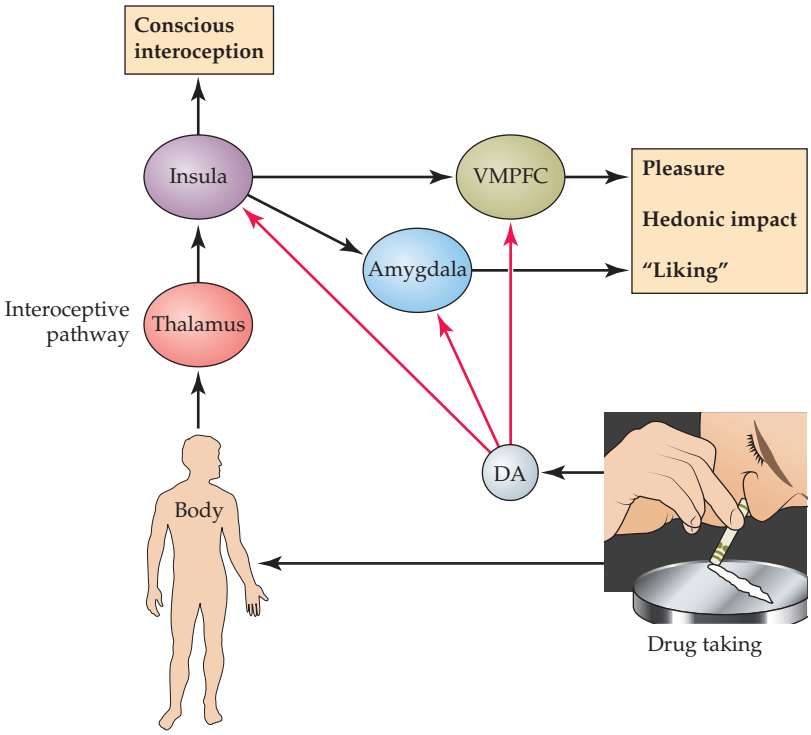


FIGURE 9.24 Hypothesized role of the insula in deriving conscious pleasure from drug-induced interoceptive cues According to this model, the insula mediates conscious awareness of drug-induced interoceptive stimuli upon receiving such stimuli through a pathway from the thalamus. Projections of the insula to the ventromedial prefrontal cortex (VMPFC) and amygdala, modulated by input from VTA dopaminergic neurons, transform the interoceptive information into feelings of pleasure and desire for the drug. (After Naqvi and Bechara, 2010.)

rumination on obtaining and using the drug again. Craving provides a strong motivation to act on the intrusive thoughts. And finally, failure of impulse control means that the individual has difficulty resisting the impulse to use the drug, despite the adverse consequences of such use. Researchers have identified the neural circuitry underlying each of these processes. First, intrusive thinking, whether associated with addiction or with certain other disorders such as obsessive-compulsive disorder, has been linked to abnormal activity within pathways from the PFC, hippocampus, and amygdala to the ventral striatum (Kalivas and Kalivas, 2016). Second, brain imaging studies of subjects exposed to drug-associated cues have found that cue-induced craving correlates with activation of the DLPFC, OFC, ACC, dorsal and ventral striata, and the insula (Jasinska et al., 2014). The insula is a deep cortical area that has increasingly been implicated in motivational regulation, including drug craving and control over drug use (Naqvi et al., 2014). This was demonstrated several years ago by the finding that cigarette smokers who sustained damage to the insula had reduced tobacco craving and found it much easier to quit than smokers with non-insula brain damage (Naqvi et al., 2007). According to Naqvi and Bechara (2010), the thalamus relays drug-induced interoceptive stimuli (i.e., internal cues produced by drug taking) to the insula, which then mediates the conscious awareness of these stimuli. Projections of the insula to the ventromedial prefrontal cortex (VMPFC) and amygdala, modulated by DA from the VTA, transform the interoceptive information into feelings of pleasure and desire for the drug (**FIGURE 9.24**).

Finally, loss of control over drug use is a complex process involving several brain areas and their associated circuits. One part of this process is a transition from the ventral striatum (particularly the NAcc) to the dorsal striatum (caudate–putamen) as a key control area for drug-taking behavior (Everitt et al., 2008; Everitt and Robbins, 2013). Because of strong evidence implicating the dorsal striatum in stimulus–response habit learning, this transition is proposed to play a significant role in the progression of drug taking from a reward-motivated behavior to a behavior that is automatic, habitual, and even compulsive. A second component results from a blunting of striatal DA transmission, which, in turn, has been linked to increased impulsivity. This decrease in DA neurotransmission has been found in both human and animal studies and consists of reduced striatal DA release and lower D_2 receptor binding in substance-dependent subjects (Trifilieff and Martinez, 2014; Trifilieff et al., 2017). Changes in the dopaminergic system could precede drug use and act as a vulnerability factor for addiction, or such changes could be a consequence of repeated drug exposure. Current evidence favors a combination of both.

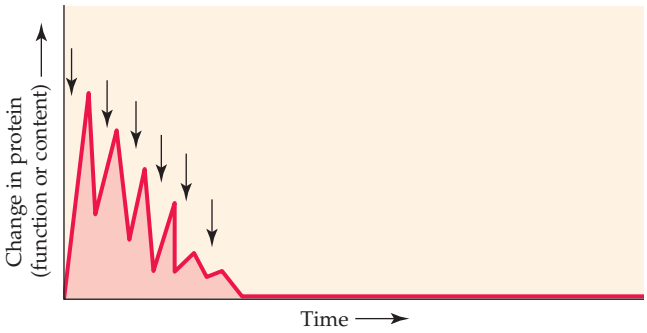
The information presented above can usefully be conceptualized as representing two opposing behavioral control systems: a "go" system that motivates and activates learned responses, and a "stop" system that pulls back on the reins (Koob, 2015). In this scheme, the spiraling pathway to addiction invokes an enhancement of the "go" system, as seen in the process of incentive sensitization and the transition of drug use to a dorsal striatum–driven behavioral habit. Simultaneously there is a progressive dysfunction of the "stop" system, which includes the PFC and the wider corticostriatal circuitry. This dysfunction results in intrusive thinking, drug craving, and loss of impulse control. The importance of PFC control over drug-seeking behavior was illustrated in a recent study of laboratory rats trained to self-administer cocaine by IV injection (Chen et al., 2013). The experimental paradigm used in the study caused some of the rats to seek cocaine

so compulsively that they were willing to endure noxious electrical foot shocks to obtain access to the drug. These compulsively behaving rats, but not rats that reduced their cocaine seeking in response to the foot shock, exhibited diminished neural activity in the PFC. When this hypoactivity was reversed by stimulating the neurons using optogenetics, the animals showed a reduction in their compulsive cocaine-seeking behavior. Findings such as these suggest that the PFC could be an important therapeutic target in future treatment of people with substance use disorders.

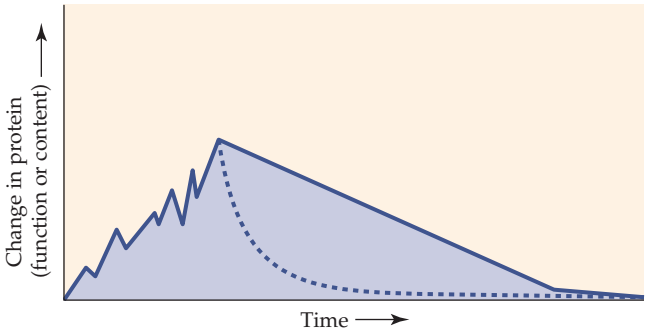
Molecular neuroadaptations play a key role in the transition to an addicted state

Drugs of abuse exert powerful effects on the expression of many genes and, therefore, on the levels of the proteins encoded by those genes. A general conception of drug effects on gene/protein expression is shown in **FIGURE 9.25**. The pattern shown in Figure 9.25A represents changes in neural proteins that are rapidly increased (or decreased in some cases) by initial drug exposure but exhibit tolerance to subsequent exposures. The pattern in Figure 9.25B is representative of other proteins that may accumulate with repeated drug exposures but then return to baseline either rapidly or more slowly during prolonged abstinence. Finally, the pattern shown in Figure 9.25C occurs for a small number of proteins that may accumulate and remain persistently elevated for a long time in the absence of further drug-taking behavior. It is reasonable to assume that proteins in category A help to mediate acute drug-induced behavioral and physiological responses that undergo tolerance when episodes of use occur frequently. Category B proteins could, over time, play a role in the transition from recreational use to addiction, and proteins in category C might be important for maintaining the "addicted" state in the sense of persistent vulnerability to relapse even after a long period of abstinence.

Here we will focus on the transitional proteins of category B, because category A proteins (which include many molecules involved in cell signaling and second-messenger systems) by themselves cannot mediate the development of addiction, and some of the major category C proteins identified thus far are structural proteins necessary for long-lasting drug-induced synaptic changes (e.g., increased growth of dendritic spines in the NAcc). Within category B, one particular protein stands out, namely, a transcription factor called **ΔFosB**. Transcription factors are proteins located in the cell nucleus that either stimulate or inhibit expression of a target gene (i.e., the rate of mRNA transcription from that gene) and accordingly up- or down-regulate levels of the protein encoded by the gene (see Chapter 2). ΔFosB is a member of the Fos family of transcription factors that are rapidly induced in areas such as the NAcc and the dorsal striatum after administration of many different drugs of abuse, including cocaine, amphetamine, methamphetamine, alcohol, opiates, and cannabinoids. Whereas most Fos proteins show only a transient response to drug administration and tolerance during repeated drug use episodes (i.e., they belong to category A), ΔFosB gradually accumulates in certain



(B) Accumulating and temporary abstinence



(C) Enduring in abstinence

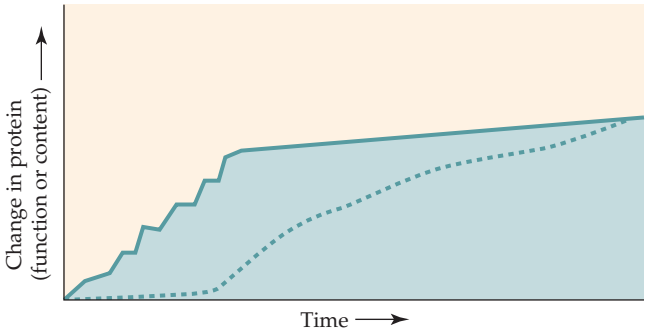


FIGURE 9.25 Patterns of neuroadaptive protein expression with repeated drug exposure
(A) Some proteins respond to drug administration (downward arrows) with an increase (or decrease; not shown) in expression, a rapid return to baseline levels, and tolerance to repeated episodes of use. (B) Proteins in this category accumulate with repeated drug use episodes and then return to baseline either slowly (solid line) or quickly (dashed line) during temporary abstinence before drug use recurs. (C) Proteins in this category show a persistent accumulation, usually following repeated drug use episodes (solid line) or alternatively during abstinence (dashed line). (After Kalivas and O'Brien, 2008.)

cells including a subset of neurons within the NAcc (Nestler, 2008). This accumulation, which lasts for at least several weeks after the last drug administration, occurs because the protein has a very long half-life once it has been synthesized by the cell.

The behavioral relevance of ΔFosB accumulation has been investigated using mice that have been genetically engineered to overexpress this protein selectively in the same NAcc and dorsal striatum cells that normally show ΔFosB induction in response to drug administration. Compared with wild-type mice, ΔFosB-overexpressing animals show enhanced sensitivity to a variety of drug effects with the exception of morphine-induced analgesia (Nestler, 2008; **TABLE 9.5**). These findings support the idea that ΔFosB may play a significant role in the transition from controlled drug use to addiction.

The behavioral effects produced by elevated ΔFosB are mediated by long-term changes in the synthesis of proteins whose genes are regulated by this transcription factor. These proteins include other transcription factors, protein kinases, and proteins involved in glutamate transmission and structural plasticity (e.g., increased dendritic branching and dendritic spine density) within the NAcc (Nestler, 2008; Ruffle, 2014). What are the mechanisms by which this occurs? The answer seems to be that ΔFosB modulates gene (and ultimately protein) expression through epigenetic mechanisms. As you learned in Chapter 2, epigenetics involves chromatin modifications that alter gene expression without changing the nucleotide sequence of the DNA strand. These modifications are of two types: methylation of the DNA at specific cytosines (the C in the genetic alphabet AGCT) in the promoter region of the gene, or various modifications of the histone proteins that form complexes around which the DNA double helix is wrapped. The best-studied type of histone modification is acetylation, or the attachment of acetyl groups to specific sites along the tail of the protein (see Figure 2.6). DNA methylation usually promotes compaction of the chromatin, thereby repressing gene expression, whereas histone acetylation facilitates gene expression by opening the chromatin and allowing the transcriptional machinery to bind to DNA (Wong et al., 2011). ΔFosB exerts both types of effects, thus up-regulating some proteins and down-regulating others. This remarkable specificity is illustrative of the way that drugs, working through transcription factors such as ΔFosB, can produce widespread and long-lasting neuroadaptive changes that underlie not only addiction but also the therapeutic actions of drugs used to treat psychiatric disorders such as major depression and schizophrenia (see Chapters 18 and 19).

Beyond ΔFosB, epigenetic regulation of gene expression is now seen as playing an important role in substance use and the vulnerability to addiction (Cadet, 2016; Berkel and Pandey, 2017). The general notion of how epigenetic mechanisms might interact with other factors to promote and maintain the addicted state is shown in **FIGURE 9.26A**. According to this model, inherited predispositions toward drug use and environmental stimuli such as stress promote initial drug-seeking behavior and drug use. Acute drug exposure, in turn, produces epigenetic changes in the expression of certain critical genes, and this subsequently facilitates repeated drug use, triggers additional epigenetic processes, and so forth, until the individual has become addicted (Wong et al., 2011). Depending on the drug and the particular gene of interest, chronic drug exposure can either prime the gene to be more strongly expressed (activated) in response to later drug administration or desensitize the gene such that its expression is repressed when drug administration occurs at a later time (Nestler, 2014; **FIGURE 9.26B**). Such changes in gene expression could, in turn, affect the subjective or behavioral responses to the drug. For example, if the subjective response to a drug (i.e., the drug-induced "high") is reduced because of epigenetically mediated receptor down-regulation or accelerated drug metabolism, then the individual has become tolerant to the drug and must take higher doses in order to obtain the same desired effect.

There are at least two other ways in which epigenetic processes may influence either the propensity to use drugs of abuse

TABLE 9.5 Behavioral Phenotype of Drug Responses in Mutant Mice[a] Compared with Wild-type Mice

Drug	Phenotype
Cocaine	Increased locomotor response to acute administration
	Increased locomotor sensitization to repeated administration
	Conditioned place preference at lower doses
	Acquisition of self-administration at lower doses
	Higher breaking point of self-administration under a progressive-ratio schedule
Morphine	Conditioned place preference at lower doses
	Increased development of physical dependence and withdrawal
	Decreased analgesia to initial exposure and more rapid tolerance
Alcohol	Increased anxiolytic (anxiety-reducing) responses

Source: Nestler, 2008.

[a]The mutant mice overexpress ΔFosB in the NAcc and dorsal striatum.

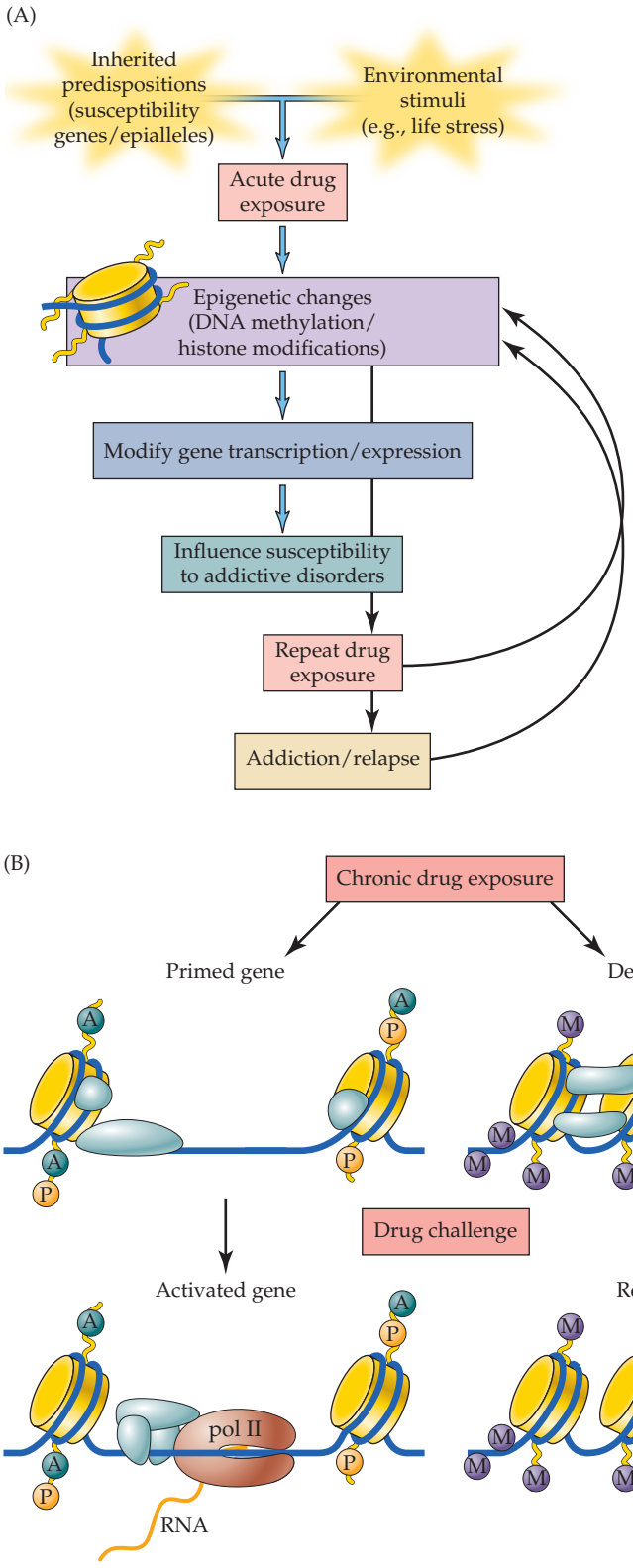


FIGURE 9.26 Epigenetic mechanisms in addiction
(A) This model shows how genetically coded predispositions and environmental stimuli may interact with drug exposure to produce epigenetic changes in gene expression that promote the development of an addicted state. Note: epialleles are inherited alleles of a gene that possess the same DNA sequence but differ epigenetically (e.g., one allele might be transcriptionally active, whereas another is silenced by prior DNA methylation). (B) This model illustrates that chronic drug exposure can result in either an epigenetically primed or a desensitized target gene, thereby resulting in activated or repressed transcription of that gene when the organism is subsequently challenged with the same drug. M, methyl groups attached to DNA that tend to repress gene transcription by compacting the chromatin structure; A and P, acetyl and phosphate groups, respectively, that are attached to histone proteins and that tend to activate gene transcription by opening up the chromatin structure; pol II, RNA polymerase II, which is responsible for transcribing DNA to RNA. (A after Wong et al., 2011; B after Robison and Nestler, 2011.)

or the consequences of such use. First, early adversity in the form of childhood maltreatment or other stressful events is known to increase the risk for later substance use problems. Epigenetic-mediated changes in gene expression have been implicated in this increased risk (Babenko et al., 2015; Gröger et al., 2016). Second, researchers have discovered that stable epigenetic modifications can occur in the germ line, which refers to the cells that give rise to eggs and sperm. This is a mechanism by which parental experiences such as drug exposures that occur *before* mating can be transferred to the offspring (Vassoler et al., 2014; Finegersh et al., 2015). An example of this phenomenon can be seen in recent work by Wimmer and colleagues (2017). These investigators trained male rats to self-administer cocaine by IV injection. Following this exposure, the cocaine-exposed and control males were bred with drug-naive females. Compared with the male offspring of control males, male offspring of the cocaine-exposed males exhibited poorer memory in a hippocampal-dependent task, deficits in hippocampal long-term potentiation, and epigenetic changes in the hippocampus that presumably contributed to these behavioral and electrophysiological effects. It is worth emphasizing that these impairments were produced by a *transgenerational* mechanism, since they occurred in animals that never received any drug exposure themselves. If the results translate to humans, they suggest that male offspring of cocaine-using men may be vulnerable to later cognitive impairment due to their fathers' drug exposure. Appropriate studies are needed to test this

hypothesis, and of course researchers must also consider the potential contribution of many other variables, including the mothers' drug history, other prenatal risk factors, and the postnatal environment.

Although many studies with experimental animals suggest an important role for epigenetic mechanisms in the development of addiction (McQuown and Wood, 2010; Robison and Nestler, 2011), evidence in humans is much more limited thus far. Nevertheless, just as epigenetic therapies are being introduced to fight various cancers (Cherblanc et al., 2012), researchers have become hopeful that similar approaches might also prove therapeutically beneficial in the treatment of drug addiction.

Is addiction a disease?

It is likely that all of the neurobiological mechanisms described in the previous sections play a significant role in drug abuse and addiction. The reward system mediates the acute rewarding and reinforcing effects of abused drugs, which are particularly relevant for the initial stages of drug use and abuse. Several types of drug-induced neuroadaptations underlie the transition from controlled to uncontrolled drug use in addiction. Impaired functioning of the PFC and corticostriatal pathways also contributes to the development and maintenance of the addicted state. But having all of this information does not, in itself, provide a simple answer to an important question, namely, *whether addiction is a disease*.

The most widely accepted model of addiction in our society is the **disease model**. Not only has this view been popularized in the mass media, but addicts themselves and their treatment providers often ascribe to this model. The disease model of addiction arose from early work with alcoholics and was only later applied to cocaine and opioid addiction (Meyer, 1996). Benjamin Rush, the Philadelphia physician who founded the alcohol temperance movement, was also the first to consider alcoholism a disease. This view was later expanded and promoted by E. M. Jellinek in his influential book, *The Disease Concept of Alcoholism* (Jellinek, 1960). For many years now, alcoholism has been formally considered a disease by medical organizations such as the World Health Organization and the American Medical Association. Indeed, the disease model is sometimes called a **medical model**. Not surprisingly, it is the leading model used both in the professional treatment of alcoholics and other drug addicts (e.g., in 12-step programs) and in self-help groups such as Alcoholics Anonymous (AA) and Narcotics Anonymous. It should be noted that the disease model is not an alternative to the various neurobiological models or even the more inclusive biopsychosocial model presented earlier. Rather, it is a way of thinking about the fundamental nature of addiction and the best approach to treating it.

The current disease model of addiction is based largely on evidence of dysregulation of brain function in addiction and on the idea that such dysfunction is, at least partly, caused by repeated drug exposure (i.e., the neuroadaptations described above). This notion was presented forcefully in an article entitled "Addiction Is a Brain Disease, and It Matters" by Alan Leshner, former director of the National Institute on Drug Abuse (NIDA). Leshner said, "That addiction is tied to changes in brain structure and function is what makes it, fundamentally, a brain disease. A metaphorical switch in the brain seems to be thrown as a result of prolonged drug use. Initially, drug use is a voluntary behavior, but when the switch is thrown, the individual moves into the state of addiction, characterized by compulsive drug seeking and use" (Leshner, 1997, p. 46). Almost all neurobiologists who study addiction advocate some version of the brain disease model, most notably Nora Volkow, current director of NIDA, and George Koob, current director of the National Institute on Alcohol Abuse and Alcoholism (Volkow and Koob, 2015; Volkow and Morales, 2015; Koob and Volkow, 2016; Volkow et al., 2016).

The disease model has had a tremendously valuable impact on society's reaction toward drug abuse and addiction. For a long time, excessive drug use and addiction were seen primarily as signs of personal and moral weakness (Nathan et al., 2016). Indeed, this earlier view has sometimes been termed a **moral model** of addiction. Initial development of the disease model sought to remove the social stigma of addiction (after all, no one *blames* you for coming down with a disease) and to involve the medical profession in helping addicts deal with their problem through treatment programs. It is important to note, though, that our society is still ambivalent about disease versus moral conceptions of drug use. Although you can get treatment for alcohol or tobacco abuse without fear of prosecution, because these substances are legal, abuse of heroin, cocaine, or (in some states) marijuana often leads to a jail sentence instead of medical help.

Despite its wide acceptance, however, the disease model of addiction has been criticized by a number of writers, some of whom have experience working with drug-addicted patients. Books that offer alternative theories of addiction include *Addiction Is a Choice*, by Jeffrey Schaler; *Addiction: A Disorder of Choice*, by Gene Heyman; and most recently, *The Biology of Desire: Why Addiction Is Not a Disease*, by Marc Lewis. Other critiques may be found in articles such as "The Brain Disease Model of Addiction: Is It Supported by the Evidence and Has It Delivered on Its Promises?" (W. Hall et al., 2015), "Addiction and the Brain-Disease Fallacy" (Satel and Lillienfeld, 2014), "Addiction Is Not a Brain Disease (and It Matters)" (Levy, 2013), "Addiction: Choice or Compulsion" (Henden et al., 2013), and "The Purpose in Chronic Addiction" (Pickard, 2012).

Space limitations preclude our discussing all of the issues raised in the abovementioned books and

articles; however, a number of key points are worth noting. First, almost all critiques of the disease model acknowledge the wealth of human and laboratory animal research demonstrating that repeated exposure to drugs of abuse alters the brain in significant ways. However, in and of itself, this does not prove that addiction is a disease. After all, *every* set of experiences we encounter, whether learning to ride a bike, memorizing molecular formulas in a chemistry class, or falling in love, affects the structure and functioning of our brain, often permanently. The proposition that the brain changes observed in addicted people are *pathological*, and therefore constitute a disease state, is a matter of debate, since some of these same changes may occur under other conditions.

Second, there are disorders of the brain that unequivocally constitute disease but that differ importantly from addiction. A few clear-cut examples include Alzheimer's and Parkinson's diseases, multiple sclerosis, ALS (Lou Gehrig's disease), epilepsy, and brain cancers such as neuroblastomas. All of these disorders can be diagnosed conclusively by specific laboratory tests either in the living patient or, if necessary, using postmortem brain tissue. This is not true for addiction; there is not a single diagnostic test to confirm that someone is addicted to alcohol or any other abused substance. Instead, the diagnosis of a substance use disorder in the *DSM-5* is made entirely on the basis of behavioral symptomatology. Moreover, none of the above mentioned brain diseases can be cured by changes in the person's behavior. Yet, that is exactly what occurs when people recover from addiction, either of their own accord or with the assistance of medication, a therapist, or a self-help group.

Third, substantial empirical evidence exists that argues against the disease conception of addiction as a chronic (often implying life-long) disorder characterized by loss of control over drug use. In an earlier section we presented data from NESARC showing the extent to which people are able to recover from addiction, often without treatment. Drug abuse researchers also know that of the many American soldiers who abused, and even became addicted to, heroin during the Vietnam War, only a small percentage maintained their addiction upon returning to the United States (Hall and Weier, 2017). Laboratory studies have even shown that regular drug users can cognitively control their cravings and the associated regional brain activations when instructed to do so in an experimental setting (Kober et al., 2010; Volkow et al., 2010). Taken together, these findings raise serious questions about whether heavy drug use is inevitably outside the control of the user.

Fourth, nondisease theories of addiction argue that addicts choose to use drugs because such use serves a purpose such as alleviating emotional pain, because other positive reinforcers are lacking in their lives, or because the available reinforcers fail to provide sufficient motivation to be chosen over the drug. Such a framework suggests that treatments for addiction should aim for a reallocation of behavior away from using drugs for reinforcement toward healthier reinforcers (**FIGURE 9.27**; Banks and Negus, 2017). Such reallocation can be aided by an existing type of program called **contingency management**. Contingency management is a behavioral intervention in which the user is regularly subjected to urine testing and receives reinforcement, typically in the form of vouchers redeemable for retail goods or services, for each negative test (Stitzer and Petry, 2006; Petry, 2010). Even the lowly lab rat adjusts its drug-taking behavior based on the availability of alternative reinforcers. This was shown many years ago in the "Rat Park" studies of Bruce Alexander (Alexander, 2010). During the 1970s, Alexander and colleagues at Simon Fraser University set out to test whether drugs, as he put it, are "irresistibly addictive." The reason for their skepticism stemmed from the realization that in a typical drug addiction experiment, the rats, which in the wild are highly social, are housed by themselves in small cages with few or no sources of stimulation outside of the drug being tested. Consequently, the researchers set up a large enclosure containing objects to interact with, running wheels, and many animals living together, including both males and females that were allowed to breed (photos of Rat Park are available at Alexander, 2010). When given the opportunity to voluntarily consume a morphine-laced solution, the Rat Park animals consumed much less than the animals housed in the usual barren and socially isolated environment.[9] More recent studies described in **Web Box 12.1** have shown that many rats choose to consume a sweetened water solution over receiving an IV injection of cocaine, even after considerable experience with the drug. However, some rats prefer the cocaine, which arguably are the ones that most closely model humans who prefer drugs even when other reinforcers are available.

Finally, researchers and clinicians who question the disease model of addiction argue that a brain-centric view of this problem pays too little attention to the whole *person* in whose head the brain resides. This is not to deny the vital role that neuroscience has played and will continue to play in helping us unravel the problem of addiction. But it's also true that despite many years of research and millions of dollars spent in the pursuit of new medications to combat addiction,

[9]It is worth noting that since the 1970s, increasing concern for the welfare of laboratory animals, including rodents, has led to improved living conditions; however, even now the requirements of some experimental procedures make it necessary to house animals individually, and they are rarely allowed to breed unless it is specifically required for the study.

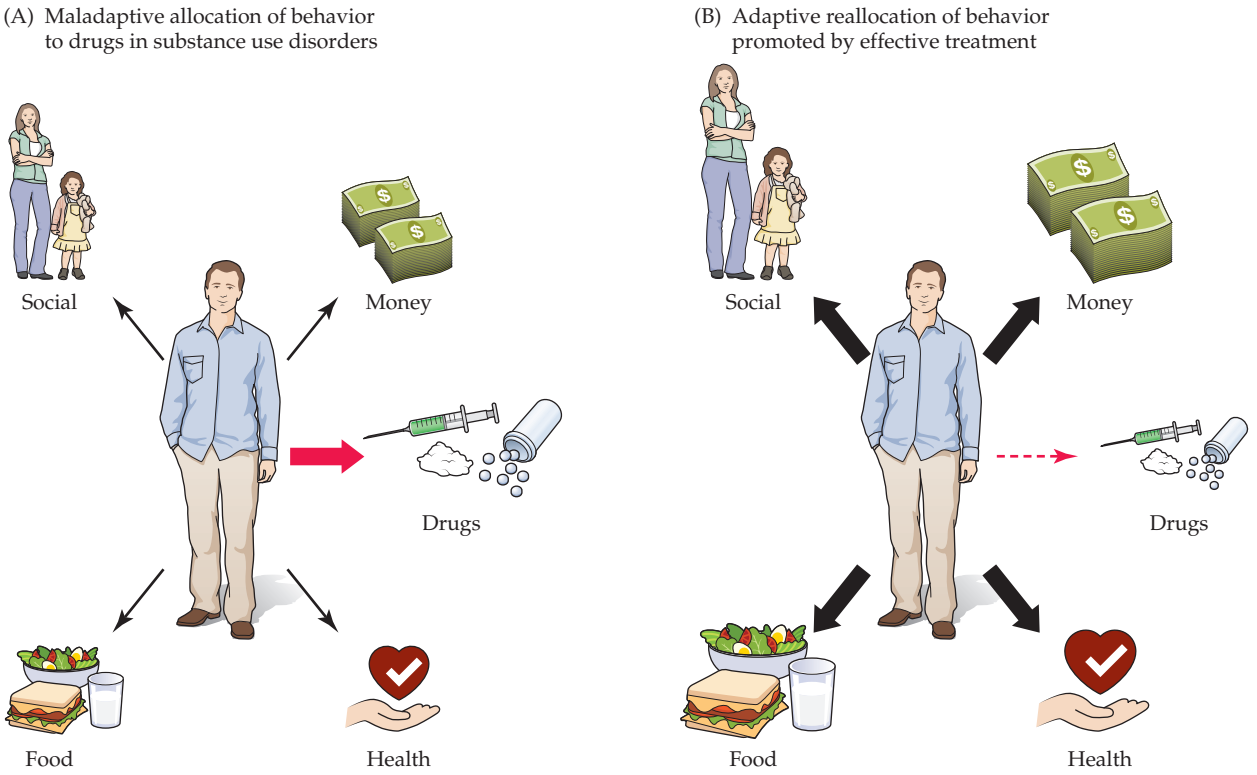


FIGURE 9.27 Drug addiction conceptualized as a maladaptive allocation of behavior (A) People suffering from a substance use disorder (addiction) maladaptively allocate their behavior toward procuring and using drugs of abuse, to the detriment of other choices. (B) For maximum effectiveness, treatments for substance use disorders should aim not only to reduce drug-related behaviors, but also to promote behavioral allocation to more adaptive, healthier reinforcers. (After Banks and Negus, 2017.)

very few have yet emerged. Taken together with the lessons from Rat Park, heroin-using Vietnam War veterans, and the many drug users who have achieved a natural recovery, the relative lack of progress from research based on the disease model suggests that we need to pay more attention to the psychosocial roots of addiction and allow that addictive behaviors may fall within a wide range of compulsivity versus controllability.

Whether society conceptualizes addiction as a disease or not has a few important implications besides the obvious ones regarding addiction research and treatment. First, it has an effect on the addicts themselves. Some addicts and therapists contend that acceptance of the disease model helps reduce the sense of guilt experienced by the recovering addict. For example, there may be strong feelings of remorse for past problems caused by the person's drug use. If such problems are viewed as stemming from a disease, then the therapeutic process may benefit. As one alcoholic put it, "Calling [alcoholism] a disease allows us to put the guilt aside so that we can do the work that we need to do" (Thombs, 1999). Believing that one has a disease may also help the individual deal with relapses, since these returns to drug use could otherwise be construed as resulting from a moral failing. But there is an alternative view expressed by Marc Lewis, author of *The Biology of Desire: Why Addiction Is Not a Disease*, who is both a neuroscientist and a former addict himself. Based on not only his own experience but also his interactions with many other present and former drug addicts, Lewis states, "While shame and guilt may be softened by the disease definition, many addicts simply don't see themselves as ill, and being coerced into an admission that they have a disease can undermine other—sometimes highly valuable—elements of their self-image and self-esteem. Many recovering addicts find it better *not* to see themselves as helpless victims of a disease, and objective accounts of recovery and relapse suggest that they may be right. Treatment experts and addiction counselors often identify empowerment or self-efficacy as a necessary resource for lasting recovery" (Lewis, 2015). But there is yet another implication that has significant practical consequences. An article published several years ago in the *Journal of the American Medical Association* not only supported the notion that addiction is a chronic disease but also pointed out the importance of providing health insurance coverage for addiction treatment (McLellan et al., 2000). It is difficult to argue against this view, since medically based treatment programs have undoubtedly helped many people overcome their drug abuse problems, and

access to such programs is often out of reach without insurance coverage.

In summary, the brain disease model of addiction is the principal model held by major health organizations, neuroscience researchers who study addiction, many (most?) addiction treatment providers, and many (though not all) addicts themselves. This model is also widely accepted in society, which accounts for health insurance coverage for treating substance use disorders. However, some strong evidence-based arguments have been made against the central tenets of the disease model. Whether or not the reader is convinced by these arguments, we hope to have made clear that people diagnosed as having a substance use disorder vary tremendously, not only in how they came to that stage in their lives, but also in how they perceive their substance use, the extent to which they are motivated to stop using (or not), and their future trajectory, including an eventual recovery that may or may not require outside help.

Section Summary

- The development of addiction has been conceptualized as a repeating spiral of three stages: (1) preoccupation/anticipation, (2) binge/intoxication, and (3) withdrawal/negative affect.
- The binge/intoxication stage is motivated by drug reward and incentive salience. The reward circuit mediates the acute rewarding and reinforcing effects of most abused drugs. One of the key components of this circuit is the DA pathway from the VTA to the NAcc. Virtually all drugs of abuse elevate extracellular DA levels in the NAcc, either by enhancing VTA cell firing or by acting locally to release DA from the dopaminergic nerve terminals and/or blocking DA reuptake. Drug-induced elevations in DA mimic the effect of burst firing of the dopaminergic neurons and cause activation of low-affinity D_1 receptors.
- Over repeated drug exposures, rewarding effects often decline because of drug tolerance. However, the incentive properties of the drug and its related cues become sensitized, thus leading to an increasingly important role for incentive salience as a motivator of continued drug use. The concepts of drug reward versus incentive salience are captured in the difference between drug *liking* and drug *wanting*.
- In addition to its involvement in reward and incentive salience, DA has also been implicated in the regulation of effortful behavior and as encoding signal for reward-prediction error.
- Repeated exposure to drugs of abuse lead to within-system and between-systems neuroadaptations involving persistent neurobiological changes of various kinds. Progressive down-regulation of the reward system is an important within-system neuroadaptation. At the same time, there occurs a between-systems neuroadaptation consisting of recruitment of an antireward circuit that mediates the withdrawal/negative affect stage of addiction.
- The antireward system is centered around the extended amygdala and is activated during stress and drug withdrawal. Neurochemically, activation of this system results in increased release of NE, CRF, and dynorphin.
- The opposing actions of the reward system and the antireward system have been conceptualized by Koob and Le Moal in an opponent-process model of addiction. According to this model, early drug use is motivated primarily by positively reinforcing effects (reward circuit), whereas later drug use (after addiction has taken place) is motivated primarily by negative reinforcement produced by alleviation of aversive withdrawal symptoms (antireward system). The opponent-process model further hypothesizes the development of an allostatic reduction in baseline hedonic tone (mood) that persists even after long-term abstinence from drugs.
- The preoccupation/anticipation stage is characterized by intrusive thinking, drug craving, and lack of impulse control. Additionally, chronic drug abuse and addiction are associated with impaired executive function. Together, these abnormalities are associated with dysregulation of the PFC and of the descending glutamatergic projections from the PFC and other cortical areas to the striatum and other subcortical structures.
- Cue-induced craving activates several brain regions, notably a deep cortical area known as the insula. Loss of control over drug use is associated with a transition of behavioral control from the ventral striatum (especially the NAcc) to the dorsal striatum, a brain area important for stimulus–response habit learning. Increased impulsivity has been linked to blunted striatal DA transmission, consisting of reduced DA release and lower D_2 receptor binding.
- Drugs of abuse produce both transient and longer-lasting changes in gene and protein expression. These changes represent molecular neuroadaptations to drug use. The transcription factor ΔFosB can be induced in the NAcc for relatively long periods by a variety of abused drugs, and this factor acts through epigenetic

mechanisms to regulate other genes that may contribute to the transition from recreational drug use to addiction.

- Beyond ΔFosB, epigenetic regulation of gene expression can influence substance use and the risk for addiction in several different ways. First, chronic exposure to a drug may, through epigenetic mechanisms, either prime a gene to be expressed more strongly upon later drug administration or desensitize the gene so that its later expression is repressed. Second, epigenetic changes resulting from early adverse experiences (e.g., childhood maltreatment) may increase the risk of substance misuse later in life. Third, drug-induced epigenetic modifications in the germ line of the mother or father may be transmitted transgenerationally to the offspring and influence the offspring's brain development and behavior.
- The most influential model of addiction in our society is the disease, or medical, model, which is based on brain dysfunction brought about by repeated drug exposure. Despite its wide acceptance, the disease model has been subject to a number of criticisms, including some criticisms based on evidence against the notion that addiction is a life-long disorder (in the absence of treatment) and that drug use by addicts is always uncontrollable and pathological. Alternative models of addiction focus on psychosocial factors that led to and now maintain excessive drug use, and they advocate for behavioral interventions that focus the person's behavior toward healthier kinds of reinforcers.

STUDY QUESTIONS

1. How did the availability and use of psychoactive drugs in the United States differ 200 years ago compared with the present time?
2. Trace the history of federal drug laws from 1900 to the present. Which of these laws was responsible for instituting the Schedule of Controlled Substances and the Drug Enforcement Agency (DEA)?
3. How does the modern conception of addiction differ from the earlier idea that addiction is determined primarily by the development of physical dependence?
4. Define the terms *relapse* and *remission*.
5. In the fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-5*), what are the meanings of the terms *substance use disorder* and *substance-induced disorder*? Include in your answer a listing of the criteria used to diagnose an alcohol use disorder, which has been provided in the text as an example that reflects the criteria applied to most abused substances.
6. What is the "severity component" in the *DSM-5* criteria for diagnosing a substance use disorder, and why is the inclusion of this component important?
7. What are behavioral addictions? Which behavioral addiction has its own diagnostic classification in the *DSM-5*? What factors are most important in determining whether a proposed behavioral addiction should be considered to be a valid psychiatric disorder for inclusion in the *DSM-5*?
8. Describe the two different kinds of progressions in drug use that are discussed in the text.
9. List the five drug classifications in the Schedule of Controlled Substances, including a description of each class. Provide at least one example of a drug belonging to classes I–IV. What is the status of alcohol and tobacco in the schedule?
10. Discuss how the route of administration influences the addiction potential of abused drugs. Provide at least one example of this influence.
11. Discuss the various experimental procedures used to study the reinforcing and rewarding properties of drugs. If you had to select only one method to test the addiction potential of a newly discovered drug, which method would you choose and why?
12. Define the term *abstinence syndrome*. What is the role of drug craving, either unconditioned or conditioned, in drug abstinence?
13. How are the discriminative stimulus effects of a drug studied experimentally? Consider an experiment in which an animal is trained to discriminate drug A from vehicle and then is tested for its response to administration of a new drug (drug B) to which it has never been

(*Continued*)

STUDY QUESTIONS (*continued*)

exposed previously. If the animal makes the "drug-correct response" when given drug B, what information have we learned about the relationship of drug B to drug A?

14. Define the common disease–common variant and common disease–rare variant hypotheses of neuropsychiatric disorders.
15. Describe the three main approaches used to study the genetics of neuropsychiatric disorders, including addiction.
16. What are single-nucleotide polymorphisms (SNPs), and how do they differ from gene mutations?
17. Discuss the psychosocial risk and protective factors that influence the likelihood of an individual's developing a substance use disorder.
18. What is meant by the term *natural recovery* as applied to addiction? What is the evidence for this phenomenon?
19. Describe the three stages associated with substance use that can lead to a downward spiral resulting in addiction.
20. Describe the components of the reward circuit with respect to both anatomy and neurochemistry.
21. Define the terms *incentive salience* and *incentive sensitization*, and discuss the hypothesized role of these factors in the development of a substance use disorder.
22. Define the term *neuroadaptation*, and discuss the hypothesized role of neuroadaptive recruitment of an antireward system in the development of a substance use disorder.
23. Discuss Koob and Le Moal's application of the opponent-process model of motivation and the process of allostasis to their conceptualization of addiction.
24. Discuss the evidence for dysregulation of prefrontal cortical function in the preoccupation/anticipation stage of the addiction cycle.
25. What is ΔFosB? What is the evidence for an involvement of this molecule in the development of addiction?
26. Describe several ways in which epigenetic processes may play a role in substance use and vulnerability to addiction.
27. Discuss evidence for and against the idea that addiction is a brain disease. If you find either side of the argument to be significantly stronger than the other, give the reasons why.

Go to the *Psychopharmacology* Companion Website at oup-arc.com/access/meyer-3e for animations, web boxes, flashcards, and other study aids.

CHAPTER 10

The tragic outcome of a high-speed chase that took the life of Princess Diana of Britain. (AP Photo/Jerome Delay.)

Alcohol

THE HEART OF BRITAIN WAS BROKEN the day Princess Diana died, August 31, 1997. Many are still asking how it could have happened, how a beautiful young life could be snuffed out in an instant when her chauffeured limousine slammed into a concrete tunnel. The events immediately preceding the disaster included a high-speed chase as the Princess was trying to avoid the harassment of a herd of tabloid photographers who were chasing her in their cars and on motorcycles. Was her professional driver, Henri Paul, to blame? Or was the accident caused by the vicious persistence of the paparazzi?

On face value, the chauffeur's blood level of 0.175% was clearly beyond legal intoxication, so the cause seems clear. Yet those who spoke to Henri Paul before the fateful trip claimed he seemed fine, walked normally, held normal conversations, and showed no external signs of intoxication. That would seem impossible, yet the occurrence may be explained by behavioral tolerance. Since the chauffeur was a chronic heavy drinker, he had had many opportunities to practice walking and talking under the influence of alcohol. He had considerable motivation to learn these behaviors, since his job would be jeopardized by signs of intoxication. Could he also have learned to maneuver his car while intoxicated? We cannot know for sure. But driving is a complex task involving timing, reflexes, coordination, alertness, memory, and judgment. Tolerance does not affect all skills equally. Perhaps simple operation of the vehicle was possible, but when the task became more complex because of the chase and rapidly changing conditions where judgement was critical, the effects of alcohol became tragically fatal. ■

Psychopharmacology of Alcohol

Alcohol, after caffeine, is the most commonly used psychoactive drug in America and is certainly the drug that is most abused. Despite the fact that alcohol has dramatic effects on mood, behavior, and thinking, and that its chronic use is damaging to the individual, the individual's family, and society, most people accept its use. In fact, many people do not consider alcohol to be a drug. How many people do you know who shun taking over-the-counter (OTC) or prescription medicines because they don't want to take "drugs" but will have a beer at a party or a cocktail before dinner? How many books and magazine articles have been titled "Drugs and Alcohol," as though alcohol was not included in the drug category? The popularity of alcohol use means that almost everyone has an idea about its effects. Some of these ideas are based on fact, but frequently people's beliefs about alcohol are misconceptions based on myth and "common" wisdom. Our job is to present the empirical evidence that describes not only the acute effects of the drug and its mechanism of action in the brain but also some of its long-term effects on other organ systems.

Alcohol has a long history of use

Alcohol use in America began with the very first immigrants, but its history is really very much longer than that. Perhaps as early as 8000 BCE, mead was brewed from fermented honey, producing the first alcoholic beverage. Archeological evidence shows that about 3700 BCE, the Egyptians prepared the first very hearty beer, called *hek*, which might have been thick enough to stand up a spoon in, and wine may have first come from Babylonia in 1700 BCE. Later still, the popularity of alcohol may have contributed to the decline of the Roman Empire. Certainly, many historians believe that the civilization was doomed by the corruption of society, alcohol intemperance, and moral decay, but the mental instability of the Roman nobility is an additional factor. Some members of the noble class exhibited signs of confusion and dementia, which may have been due to lead poisoning caused by alcohol prepared with a flavor enhancer that had a high lead content. *Aqua vitae* (meaning "the water of life" in Latin) represents the first distilled conversion of wine into brandy during the Middle Ages in Italy. Production of gin by the Dutch in the early seventeenth century is frequently credited with the start of serious alcohol abuse in Europe. Not only was gin far more potent than wine and very inexpensive to buy, but it was introduced during a time of social upheaval. Gin turned out to be a common method of dealing with the poor living conditions and social instability caused by the newly created urban societies following the feudal period. Gin consumption became associated with the lower class, while the more respectable middle class drank beer (**FIGURE 10.1**).

Colonial Americans brought their habit of heavy drinking from Europe, and alcohol had a large part in their daily lives. The American tavern was not just a place for food and drink and overnight accommodation, it was also the focal point in each town for conducting business and local politics and for mail delivery. The Continental Army supplied each soldier with a daily ration of rum, and employers and farmers supplied their workers with liquor on the job. Students, then as now, had reputations for hard drinking, and Harvard University operated its own brewery. At some point, the celebrations at Harvard's graduation ceremonies became so wild and unrestrained that the administration developed strict rules of behavior. American drinking of alcohol remained at a high level until the 1830s, when the temperance movement began a campaign to educate society about the dangers of long-term alcohol consumption. Although their initial goal was to reduce rather than prevent alcohol consumption, later offshoots of the group used social and religious arguments to convince Americans that alcohol itself was the source of evil in the world and was directly responsible for broken families, poverty, social disorder, and crime. Some of these same arguments are currently being used to regulate the use of other drugs in our society, such as marijuana, heroin, and cocaine.

In 1917, Congress passed a law that in 1920 became the Eighteenth Amendment to the United States

FIGURE 10.1 Engraving of Gin Lane The artist William Hogarth (1697–1764) depicted the popular opinion that the "lower classes" drank gin and got drunk.

Constitution; it prohibited the "manufacture, sale, transportation, and importation" of liquor. Despite its intent, the period of Prohibition increased illegal manufacturing that often produced highly toxic forms of alcohol, increased consumption of distilled spirits rather than beer because they were easier to hide and store, and made drinking in illegal speakeasies a fad. Medicinal "tonics" containing up to 75% alcohol became increasingly popular. Worst of all, Prohibition increased the activity of organized crime mobs that were heavily involved in the sale and distribution of alcohol. By 1933, most Americans realized that the experiment was a failure, and the Eighteenth Amendment was repealed by Congress during the presidency of Franklin D. Roosevelt. (For a brief history of alcohol use in America, see Goode, 1993.) Today, the use of alcohol is restricted by age and circumstance (e.g., prohibited when operating a motor vehicle) and is regulated to some extent by an increased tax on the cost of consumption (the "sin tax"). Such liquor laws vary by state. Phillip Cook's book *Paying the Tab: The Costs and Benefits of Alcohol Control* (2007) provides a history of the attempts to "legislate morality" and discusses the potential to control alcohol use with supply-side economics.

What is an alcohol and where does it come from?

Alcohols come in many forms, and although they have similarities in structure, they have very different uses. Ethyl alcohol is the alcohol with which we are most familiar because it is used as a beverage. An ethyl alcohol molecule has only two carbon atoms, a complement of hydrogens, plus the –OH (hydroxyl group) characteristic of all alcohols (**FIGURE 10.2**). Methyl alcohol, or wood alcohol, has an even simpler chemical structure but is highly toxic if consumed, because the liver metabolites of methyl alcohol include formic acid and formaldehyde. Drinking wood alcohol causes blindness, coma, and death. It is commonly used as a fuel, an antifreeze, and an industrial solvent. Isopropyl alcohol has a small molecular side chain that changes its characteristics and makes it most useful as rubbing alcohol or as a disinfectant. It is also dangerous to consume.

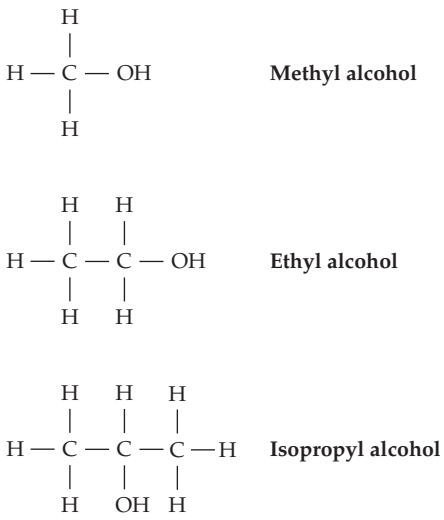


FIGURE 10.2 Chemical structures of three commonly used forms of alcohol

Ethyl alcohol (or ethanol) is the form we focus on in this chapter. It is produced by fermentation—a process that occurs naturally whenever microscopic yeast cells in the air fall on a product containing sugar, such as honey, fruit, sugar cane, or grains like rye, corn, and others. The material that provides the sugar determines the type of alcoholic beverage, for example, grapes (wine), rice (sake), or grains (beer). Yeast converts each sugar molecule into two molecules of alcohol and two molecules of carbon dioxide. This fermentation process is entirely natural and explains why alcohol has been discovered in cultures all over the world. The fermentation process continues until the concentration of alcohol is about 15%, at which point the yeast dies. Most wines have alcohol content in this range. To achieve higher alcohol concentrations, distillation is necessary. Distillation requires heating the fermented mixture to the point where the alcohol boils off in steam (since it has a lower boiling point than water), leaving some of the water behind. The alcohol vapor passes through a series of cooling tubes (called a still) and condenses to be collected as "hard liquor," or distilled spirits, such as whiskey, brandy, rum, tequila, and so forth. The alcohol concentration of these beverages varies from 40% to 50%. A second way to increase alcohol concentrations to above 15% is to add additional alcohol; this procedure is used to make fortified wines such as sherry. Flavoring and sugar may also be added to produce liqueurs such as crème de menthe (mint), amaretto (almond), and ouzo (anise). Regardless of the form, alcohol is high in calories, which means that it provides heat or energy when it is metabolized. However, no nutritional value is associated with those calories, because alcohol provides no proteins, vitamins, or minerals that are necessary components of a normal diet. For this reason, individuals who chronically consume large quantities of alcohol in lieu of food frequently suffer from inadequate nutrition, leading to health problems and brain damage.

Although it would make the most sense to describe alcohol content as a percentage, if you look at a bottle of distilled spirits, you are more likely to see alcohol content described according to "proof." This convention is based on an old British army custom of testing an alcoholic product by pouring it on gunpowder and attempting to light it. If the alcohol content is 50%, the gunpowder burns, but if the alcohol is less concentrated, the remaining water content prevents the burning. Hence, the burning of the sample was 100% proof that it was at least 50% alcohol. The proof number now corresponds to twice the percent of alcohol concentration.

The pharmacokinetics of alcohol determines its bioavailability

To evaluate the effects of alcohol in the central nervous system (CNS), we need to know how much alcohol is freely available to enter the brain from the blood (i.e., its bioavailability). Ethyl alcohol is a unique drug in several respects. Although alcohol is a small, simple molecule that cannot be ionized, it nevertheless readily mixes with water and is not high in lipid solubility. Despite these characteristics, it is easily absorbed from the gastrointestinal (GI) tract and diffuses throughout the body, readily entering most tissues, including the brain. The rates of absorption, distribution, and clearance of alcohol are modified by many factors, all of which contribute to the highly variable blood levels that occur after ingestion of a fixed amount of the drug. For this reason, behavioral effects are described on the basis of **blood alcohol concentration** (**BAC**) rather than the amount ingested. In general, it takes a BAC of 0.02% (i.e., 20 mg of alcohol per 100 ml of blood) to produce measurable behavioral effects. Keep in mind that one "drink" may take the form of one 12-ounce can of beer, one 5-ounce glass of wine, a cocktail with 1.5 ounces of spirits, or a 12-ounce wine cooler, but each will raise blood levels by the equivalent amount (**FIGURE 10.3**).

ABSORPTION AND DISTRIBUTION Since oral administration is about the only way the drug is used recreationally, absorption will necessarily occur from the GI tract: about 10% from the stomach and 90% from the small intestine. The small molecules move across membrane barriers by passive diffusion from the higher concentration on one side (the GI tract) to the lower concentration on the other (blood). Of course, this means that the more alcohol you drink in a short period of time or the more alcohol you drink in an undiluted form (i.e., more concentrated), the more rapid will be the movement from stomach and intestine to blood, producing a higher blood level (**FIGURE 10.4A**). The presence of food in the stomach slows absorption because it delays movement into the small intestine through the pyloric sphincter, a muscle that regulates the movement of material from stomach to intestine (**FIGURE 10.4B**). The delayed absorption means alcohol dehydrogenase has more opportunity to metabolize alcohol in the stomach (see the next section on metabolism). Milk seems to be particularly effective in delaying absorption. In contrast, carbonated alcoholic beverages such as champagne are absorbed more rapidly because carbonation speeds the movement of materials from the stomach into the intestine.

Gender differences also exist in the absorption of alcohol from the stomach, because certain enzymes

	Wine	Beer	Hard liquor	Wine cooler
Volume (ounces)	5	12	1.5	12
× Percent alcohol by volume	12	5	40	5
Ethyl alcohol per serving (ounces)	0.60	0.60	0.60	0.60

FIGURE 10.3 Alcohol content A comparison of alcohol content of various beverages shows an equivalent amount despite differences in volume. To calculate the amount of alcohol in a given beverage, multiply the number of ounces in the container by the percent alcohol content by volume. Note that the alcohol content of beer varies from 3% to well over 10% for some microbrews.

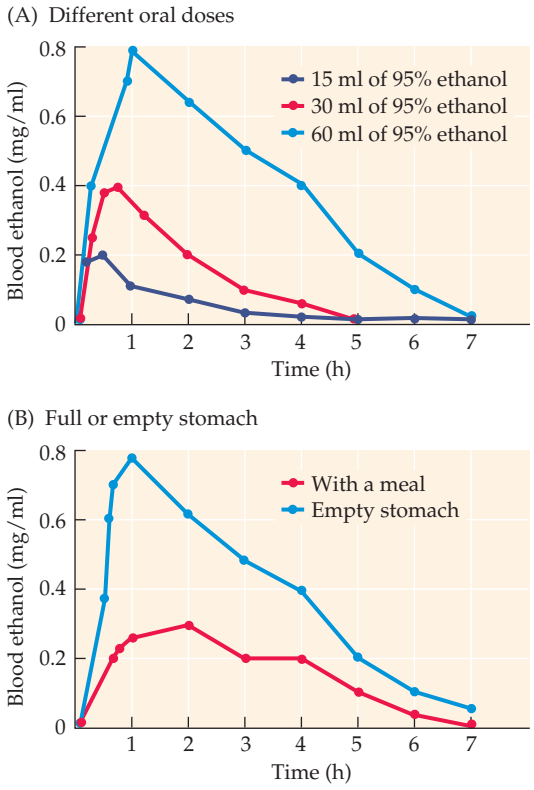


FIGURE 10.4 Blood levels of alcohol after oral administration (A) Larger oral doses of alcohol produce higher concentrations in the stomach, and this causes faster absorption and higher peak blood levels. (B) The presence of food in the stomach slows absorption of alcohol and prevents the sharp peak in blood level.

(particularly alcohol dehydrogenase) that are present in gastric fluid are about 60% more active in men than in women, leaving a higher concentration of alcohol that will be absorbed more rapidly in women (Freeza et al., 1990). Further, taking aspirin generally inhibits gastric alcohol dehydrogenase, but to a greater extent in women than in men. Because women have lower levels of alcohol dehydrogenase to begin with, aspirin use before drinking may essentially eliminate any gastric metabolism of alcohol in women (Roine et al., 1990). Ulcer medications (such as Tagamet or Zantac) also impair gastric metabolism, increasing alcohol concentrations and hence increasing absorption.

Once alcohol is in the blood, it circulates throughout the body. It readily moves, by passive diffusion, from the higher concentration in the blood to all tissues and fluid compartments. Body size and gender play a part in the distribution of alcohol and in the magnitude of its effect. The same amount of alcohol, say one beer, is much more concentrated in the average woman than in the average man, because her fluid volume is much smaller as a result of her size and because women have a higher fat-to-water ratio.

METABOLISM Of the alcohol that reaches the general circulation, approximately 95% is metabolized by the liver before it is excreted as carbon dioxide and water in the urine. The remaining 5% is excreted by the lungs and can be measured in one's breath by using a Breathalyzer, which provides law enforcement officials a means to calculate alcohol levels. Alcohol metabolism is different from that of most other drugs in that the rate of oxidation is constant over time and does not occur more quickly when the drug is more concentrated in the blood. The rate of metabolism is quite variable from one person to another, but the average rate is approximately 1 to 1.5 ounces or 12 to 18 ml of 80-proof alcohol per hour. Because the metabolic rate is constant for an individual, if the rate of consumption is faster than the rate of metabolism, alcohol accumulates in the body, and the individual becomes intoxicated.

Several enzyme systems in the liver are capable of oxidizing alcohol. The most important is **alcohol dehydrogenase**, which we already know is also found in the stomach and reduces the amount of available alcohol for absorption—a good example of the first-pass effect (see Chapter 1). Alcohol dehydrogenase converts alcohol to acetaldehyde, a potentially toxic intermediate, which normally is rapidly modified further by **aldehyde dehydrogenase** (**ALDH**) to form acetic acid. Further oxidation yields carbon dioxide, water, and energy (**FIGURE 10.5A**). ALDH exists in several genetically determined forms with varying activities. About 10% of Asian individuals (e.g., Japanese, Korean, Chinese) have genes that code only for an inactive form of the enzyme (**FIGURE 10.5B**). For these individuals, drinking even small amounts of alcohol produces very high levels of acetaldehyde, causing intense flushing, nausea and vomiting, tachycardia, headache, sweating, dizziness, and confusion. Because these individuals almost always

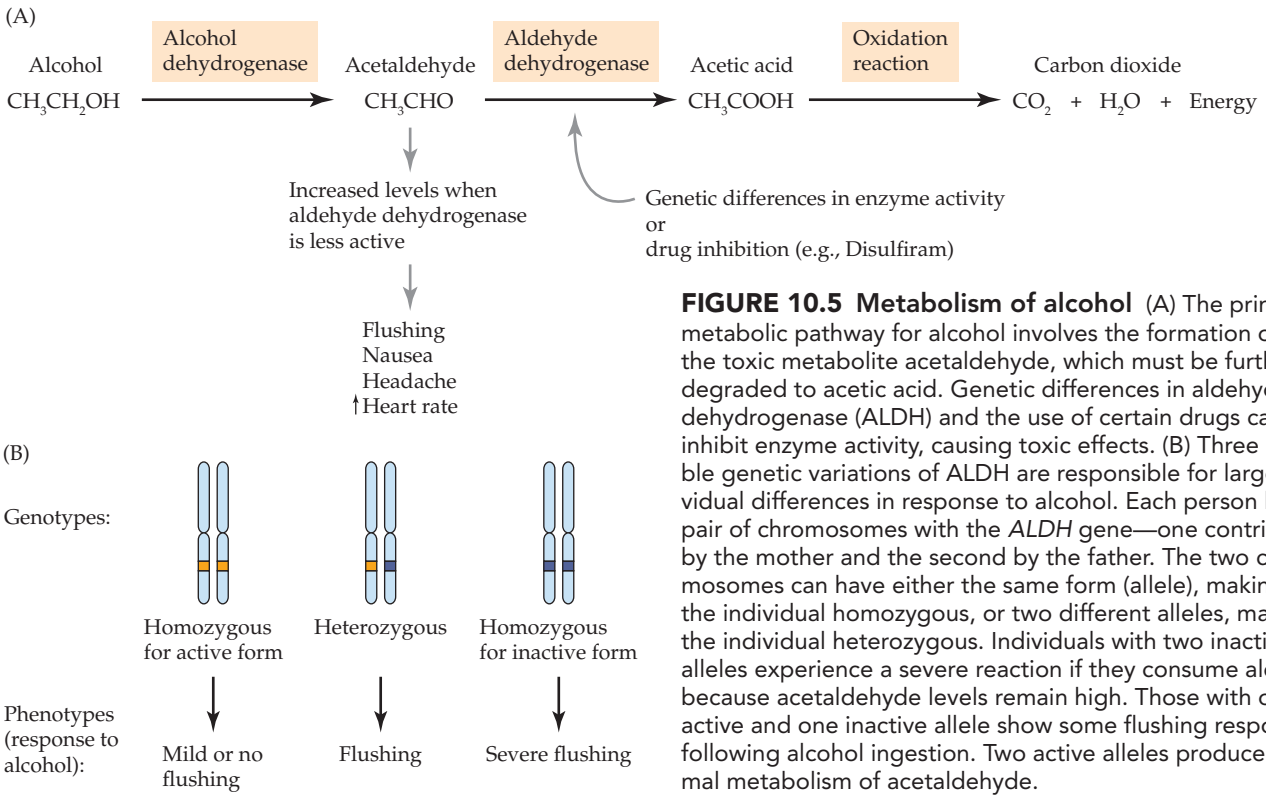


FIGURE 10.5 Metabolism of alcohol (A) The principal metabolic pathway for alcohol involves the formation of the toxic metabolite acetaldehyde, which must be further degraded to acetic acid. Genetic differences in aldehyde dehydrogenase (ALDH) and the use of certain drugs can inhibit enzyme activity, causing toxic effects. (B) Three possible genetic variations of ALDH are responsible for large individual differences in response to alcohol. Each person has a pair of chromosomes with the *ALDH* gene—one contributed by the mother and the second by the father. The two chromosomes can have either the same form (allele), making the individual homozygous, or two different alleles, making the individual heterozygous. Individuals with two inactive alleles experience a severe reaction if they consume alcohol, because acetaldehyde levels remain high. Those with one active and one inactive allele show some flushing response following alcohol ingestion. Two active alleles produce normal metabolism of acetaldehyde.

totally abstain from using alcohol, they are at no risk for alcohol use disorder (AUD). Another 40% of the Asian population has genes that code for both the active and inactive enzyme. These heterozygous individuals exhibit a more intense response to alcohol but not necessarily an unpleasant one. They are partially protected from alcohol dependence and have less vulnerability, making the *ALDH* gene a marker for low risk of AUD.

The second class of liver enzymes that convert alcohol to acetaldehyde are those that belong to the **cytochrome P450** family. The enzyme of importance within this family is CYP2E1, which is sometimes called the **microsomal ethanol oxidizing system** (**MEOS**). These enzymes metabolize many drugs in addition to alcohol. When alcohol is consumed along with these other drugs, they must compete for the same enzyme molecules; therefore, alcohol consumption may lead to high and potentially dangerous levels of the other drugs. If you drink alcohol, be sure to look for warnings on both prescription and OTC medications before consuming alcohol with any other drug. In contrast to the acute effect, when alcohol is consumed on a regular basis, these liver enzymes *increase* in number, which increases the rate of metabolism of alcohol as well as any other drugs normally metabolized by these enzymes. The process, called **induction** of liver enzymes, serves as the basis for metabolic tolerance, which is described in the next section. Finally, prolonged heavy use of alcohol causes liver damage that significantly impairs metabolism of alcohol and many other drugs.

TABLE **10.1** Estimated BAC and Impairment for Men and Women According to Body Weight[a]

	Approximate Blood Alcohol Concentration[b] (Men)							
	Body weight (pounds)							
Drinks	100	120	140	160	180	200	220	240
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.04	0.03	0.03	0.02	0.02	0.02	0.02	0.02
2	0.08	0.06	0.05	0.05	0.04	0.04	0.03	0.03
3	0.11	0.09	0.08	0.07	0.06	0.06	0.05	0.05
4	0.15	0.12	0.11	0.09	0.08	0.08	0.07	0.06
5	0.19	0.16	0.13	0.12	0.11	0.09	0.09	0.08
6	0.23	0.19	0.16	0.14	0.13	0.11	0.10	0.09
7	0.26	0.22	0.19	0.16	0.15	0.13	0.12	0.11
8	0.30	0.25	0.21	0.19	0.17	0.15	0.14	0.13
9	0.34	0.28	0.24	0.21	0.19	0.17	0.15	0.14
10	0.38	0.31	0.27	0.23	0.21	0.19	0.17	0.16
	Approximate Blood Alcohol Concentration[b] (Women)							
	Body weight (pounds)							
Drinks	90	100	120	140	160	180	200	220
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.05	0.05	0.04	0.03	0.03	0.03	0.02	0.02
2	0.10	0.09	0.08	0.07	0.06	0.05	0.05	0.04
3	0.15	0.14	0.11	0.10	0.09	0.08	0.07	0.06
4	0.20	0.18	0.15	0.13	0.11	0.10	0.09	0.08
5	0.25	0.23	0.19	0.16	0.14	0.13	0.11	0.10
6	0.30	0.27	0.23	0.19	0.17	0.15	0.14	0.12
7	0.35	0.32	0.27	0.23	0.20	0.18	0.16	0.14
8	0.40	0.36	0.30	0.26	0.23	0.20	0.18	0.17
9	0.45	0.41	0.34	0.29	0.26	0.23	0.20	0.19
10	0.51	0.45	0.38	0.32	0.28	0.25	0.23	0.21

Source: Pennsylvania Liquor Control Board, 1995.

[a]Your body can get rid of one drink per hour.

[b]At 0.02–0.04, impairment begins; at 0.05–0.07, driving is impaired; 0.08 and greater indicates legal intoxication.

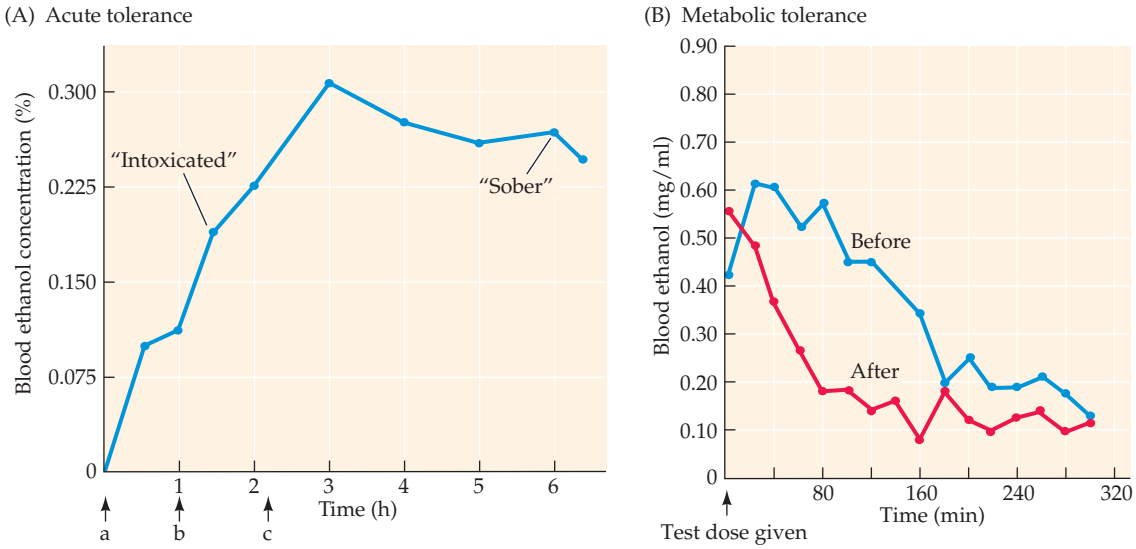


FIGURE 10.6 Tolerance to alcohol (A) In a trial using a human participant given three doses of alcohol (a,b,c), signs of intoxication (such as incoordination in the balance beam test) appeared during the rising phase of blood alcohol levels at about 0.20%. However, as blood alcohol was declining, the person became "sober" at a higher concentration (about 0.265%), showing that acute tolerance had occurred. In this case *sober* does not mean unimpaired in skills other than the balance beam test. Note that the high blood levels for intoxication reflect the fact that the participant was a chronically heavy user of alcohol. (B) Blood alcohol levels were calculated at 20-minute intervals after a test dose was given at time zero. The blue line represents blood levels before a 7-day period of drinking; the red line shows blood levels in the same person after 7 days of drinking (3.2 grams of ethanol per kilogram of body weight per day in individual doses). Tolerance after repeated alcohol consumption is shown by the more rapid decrease in blood alcohol. (A after Mirsky et al., 1941; B after Mendelson et al., 1965.)

Given the pharmacokinetic factors just described, you know that the amount of alcohol in the blood depends on how much an individual has consumed and on rates of absorption and metabolism. **TABLE 10.1** provides a rough estimate of BAC that is based on the number of drinks consumed in 1 hour and the body weight of the individual, assuming the metabolism of approximately 1 ounce per hour.

Chronic alcohol use leads to both tolerance and physical dependence

Several types of tolerance for the biobehavioral effects of alcohol occur with repeated consumption. Prolonged use can also lead to physical dependence and cross dependence with other sedative–hypnotic drugs such as the benzodiazepines.

TOLERANCE The effects of alcohol are significantly reduced when the drug is administered repeatedly; hence, **tolerance** occurs. There is also **cross-tolerance** with a variety of other drugs in the sedative–hypnotic class, including the barbiturates and the benzodiazepines. Each of the four mechanisms that we described in Chapter 1 contributes to alcohol tolerance.

1. **Acute tolerance** occurs within a single exposure to alcohol. Several of the subjective and behavioral drug effects are greater while the blood level of alcohol is increasing and are less while the blood level is falling even if the BAC is the same at both times (**FIGURE 10.6A**). LeBlanc and colleagues (1975) found that alcohol-induced incoordination in rats was 50% less while blood levels were falling, as measured by the amount of time off a minitreadmill during a single exposure to alcohol. More problematic is the finding that interoceptive cues that determine the subjective evaluation of intoxication undergo acute tolerance, particularly in binge drinkers,[1] although in some studies, social drinkers also reported feeling less intoxicated by alcohol on the declining limb of the blood alcohol curve than on the ascending limb. Failure to accurately predict their blood alcohol levels and the amount of impairment they will experience leads individuals to risk driving while legally intoxicated. Unfortunately, although binge drinkers perceived that they were less intoxicated on the descending limb of the blood alcohol curve and were more willing to drive an automobile at that time, their driving performance as measured on a simulated driving test was significantly worse than on the ascending limb. Deterioration of driving skills may have been due to fatigue or to the fact that these individuals failed to compensate for

[1]Binge drinking is generally defined as five or more drinks for men and four or more drinks for women in a single 2-hour session.

their intoxication, which they failed to recognize (Marczinski and Fillmore, 2009). This differential development of acute tolerance for various effects of alcohol may explain why binge drinkers are responsible for about 80% of the alcohol-impaired driving incidents each year. Why acute tolerance occurs is not entirely clear, but some rapid adaptation of neuronal membranes is one possibility.

2. Chronic alcohol use significantly increases the P450 liver microsomal enzymes that metabolize the drug. More rapid metabolism means that blood levels of the drug will be reduced (**FIGURE 10.6B**), producing diminished effects. This is **metabolic tolerance**.
3. Neurons also adapt to the continued presence of alcohol by making compensatory changes in cell function. The mechanism of this **pharmacodynamic tolerance** is described in later sections of this chapter dealing with specific neurotransmitters.
4. Finally, there is also clear evidence of **behavioral tolerance**. Rats, like humans, seem to be able to learn to adjust their behaviors when allowed to practice while under the influence of alcohol (Wenger et al., 1981). Although initially unsuccessful, rats readily learned to run on a treadmill by trial and error despite administration of alcohol. Other rats given the same amount of drug each day *after* their treadmill session showed only minimal improvement when tested on the treadmill under the influence of alcohol. This small amount of improvement may have been due to metabolic tolerance.

Classical conditioning may also contribute to behavioral tolerance. In animal experiments, alcohol initially reduces body temperature, but when the drug is administered repeatedly in the same environment, a compensatory increase in body temperature occurs, and this reduces the initial hypothermia (low body temperature). If these animals are given saline instead of alcohol in this environment, they show *only* the compensatory mechanism and their body temperature rises (hyperthermia). The importance of environment is further demonstrated by evidence that in a novel environment, tolerance is significantly less, because no conditioned hyperthermia is present (Le et al., 1979).

PHYSICAL DEPENDENCE We know that prolonged use of alcohol produces **physical dependence**, because a significant withdrawal syndrome occurs when drinking is terminated. As you already know, the intensity and duration of withdrawal (i.e., abstinence) signs are dependent on the amount and duration of drug taking (**FIGURE 10.7**). In addition, alcohol shows **cross dependence** with other drugs in the sedative–hypnotic class, including barbiturates and benzodiazepines. A quick review of Chapter 1 will remind you that withdrawal signs can be eliminated by taking the drug again or by taking any drug in the same class that shows cross dependence.

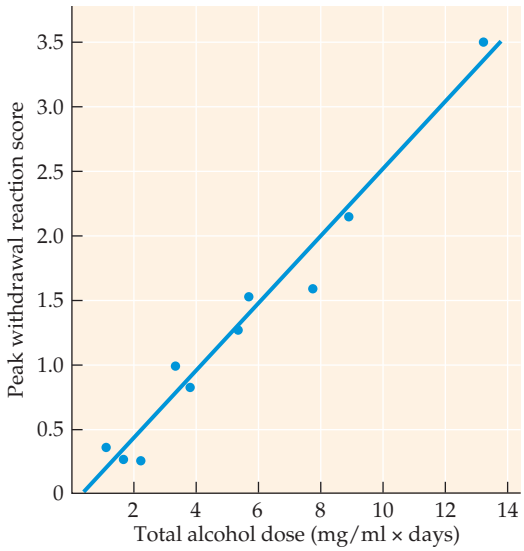


FIGURE 10.7 Relationship between alcohol dose and withdrawal severity A linear relationship exists between the total alcohol dose (amount of alcohol consumed multiplied by number of days of alcohol exposure) and the maximum withdrawal response at abstinence in mice. Withdrawal response was calculated on the basis of standard physiological and behavioral measures. (After Goldstein, 1972.)

Some investigators suggest that the **hangover** that occurs after even a single bout of heavy drinking may in fact be evidence of withdrawal, although others consider it a sign of acute toxicity. Possible explanations for hangover symptoms include residual acetaldehyde in the body; alcohol-induced gastric irritation; rebound drop in blood sugar; excess fluid loss the previous night; and perhaps toxic effects from congeners, which are small quantities of by-products from fermentation and distillation that may accumulate after heavy drinking. The classic symptoms of hangover are recognized by many social drinkers who on occasion consume an excess of alcohol. Among the usual signs are nausea and perhaps vomiting, headache, intense thirst and dry mouth that feels a bit like cotton balls, fatigue, and general malaise.

Withdrawal from repeated heavy drinking over months or years produces an intense abstinence syndrome that develops within a few hours after drinking stops and may continue over 2 to 4 days, depending on the dose previously consumed. Generally, symptoms include tremor (the "shakes") and intense anxiety, high blood pressure and rapid heart rate, excessive sweating, rapid breathing, and nausea and vomiting. A small percentage of alcohol-dependent individuals undergoing withdrawal demonstrate more-severe effects called **delirium tremens**, or **DTs**. Signs of DTs include irritability, headaches, agitation, and confusion.

In addition, convulsions; vivid and frightening hallucinations that include snakes, rats, or insects crawling on their bodies; total disorientation; and delirium may occur. Withdrawal signs such as unstable blood pressure, depression and anxiety including panic attacks, and sleep disturbances may last for several weeks. Because the most extreme symptoms are potentially life threatening, detoxification of an individual with alcohol use disorder (see the section on alcohol use disorder later in the chapter) should always be done under medical supervision. The signs of withdrawal are characteristically a "rebound" phenomenon and represent a hyperexcitable state of the nervous system after the prolonged depressant effects of alcohol. The neuroadaptive mechanisms responsible are described more completely later in this chapter.

Alcohol affects many organ systems

Alcohol, like all drugs, produces dose-dependent effects that are also dependent on the duration of drug taking. Because it is so readily absorbed and widely distributed, alcohol has effects on most organ systems of the body. As you read this section, keep in mind that most individuals who use alcohol drink in ways that do not increase their risk for alcohol use problems, and at low doses alcohol may even have some minor beneficial effects. However, the transition from moderate to heavy drinking that leads to the chronic intoxication associated with alcohol use disorder is a part of the same dose–response curve, and the precise point at which alcohol becomes damaging is not clear for a particular individual.

A second thing to keep in mind is that the environment and expectations have a great influence on many of the behavioral effects of alcohol. A host of well-controlled studies clearly show that an individual's *belief* that alcohol will produce relaxation, sexual desire, or aggression may have a far greater effect on the individual's behavior than the pharmacological effects of the drug, at least at low to moderate doses. Pronounced behavioral effects occur under placebo conditions when the individual believes that alcohol has been ingested. Refer to **BOX 10.1**. As you might expect, the environment plays less of a role in the effects of alcohol as the dose increases.

CNS EFFECTS As is true for all drugs in the sedative–hypnotic class, at the lowest doses an individual feels

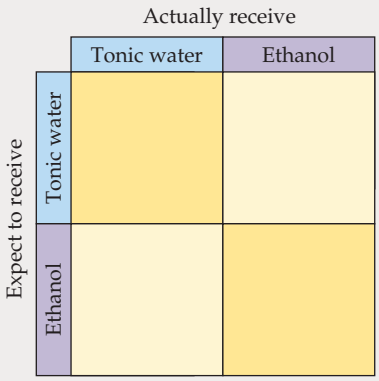
BOX 10.1 Pharmacology in Action

The Role of Expectation in Alcohol-Enhanced Human Sexual Response

One problem in evaluating the effects of alcohol on behavior is that individuals have expectations about how alcohol will affect them. It is frequently believed (in our culture) that alcohol will increase sociability, reduce anxiety and tension, increase aggression, and enhance sexual responses. However, many of the effects of alcohol, especially at low doses, are due more to the individual's expectation of effect than to the drug's pharmacological effect (Marlatt and Rohsenow, 1980).

As you probably recall, experiments measuring drug effects have at least two groups of participants: a drug treatment group and a placebo (nondrug) group, who generally assume they are also receiving the drug. Since both groups expect to receive the drug, any difference in their scores reflects the effect of the drug alone. However, because both groups believe they are getting the drug, there is no direct measure of the extent of **expectancy**. A further elaboration of the research design that more specifically tests the role that expectation plays is a four-block, or 2 × 2, design (see **Figure**). In this design, half the participants are *told* that they will get alcohol and half are *told* that they will get placebo. Half of each of the two groups will actually get alcohol. Thus, two groups get what they are expecting, and two groups are deceived and receive the opposite treatment.

To be effective, these experiments must completely deceive the participants; this includes providing the alcohol in a way that it cannot be detected, such as combining vodka and tonic and at relatively low doses. In addition, participants must be deceived about the purpose of the experiment, for instance, by being told that they are involved in a taste test of different vodkas or different tonic waters.



Results from experiments using the 2 × 2 design support the hypothesis that when participants think they have received alcohol, their behavior reflects their *expectations* of the drug effect. For example, in one study, college students watched erotic videos showing heterosexual and homosexual activities. The low dose of alcohol administered (0.04%) did not have an effect on

(*Continued*)

BOX 10.1 Pharmacology in Action (*continued*)

physiological arousal as measured by penile tumescence, but an expectancy effect occurred. The group *expecting* to receive alcohol showed more physical and subjective arousal than the group that did not expect to receive alcohol, regardless of whether they actually received alcohol or not (Wilson and Lawson, 1976). This seems to be a case in which a drinker's beliefs about the effects of a drug become a self-fulfilling prophecy, and actions match expectations.

Another way to isolate pharmacological effects from expectations is to look at cross-cultural studies. Those of us who drink alcohol or observe others drinking may believe that alcohol induces our amorous nature, makes us more sexually appealing, and enhances our basic sexuality. But does it really? The observations of the anthropologists MacAndrew and Edgerton (1969) say something quite different. They found, for example, that the Camba of Bolivia are a people with strong, almost puritanical taboos regarding sexual activities. When intoxicated, they become extremely gregarious and outgoing, maintaining festivities long into the night. However, regardless of the revelry, they never fail to maintain strict sexual limits. The Tarhumara of Mexico also strictly limit sexual encounters under normal conditions. However, when they are drunk, mate swapping becomes the norm and is not considered inappropriate. For the Lepcha of Sikkim, in India, sex is the primary recreation beginning at age 10 or 11 and continuing through old age. Adultery is expected and generates no ill will. Sex is an open topic for conversation and humor. During harvest festival time, large amounts of homemade liquor are consumed, and the Lepchas' casual sexual customs become wildly promiscuous to enhance the harvest. With adult encouragement, even 4- and 5-year-olds imitate copulation with each other. Nevertheless, their very strict guidelines regarding incest taboos are never broken even when Lepchas are quite drunk.

How can we explain experiences so different from our own? Does alcohol have a predetermined biological effect on sexual activity? Or does it induce disinhibition only within the context and limits of a given culture? How do *our* cultural expectations influence the effects of the drug?

relaxed and less anxious. In a quiet setting she may feel somewhat sleepy, but in a social setting where sensory stimulation is increased, the relaxed state is demonstrated by reduced social inhibition, which may make the individual more gregarious, talkative, and friendly or inappropriately outspoken. Self-perception and judgment are somewhat impaired, and one may feel more confident than reality proves true. Reduced judgment and overconfidence may increase risk-taking behaviors and may make sexual encounters more likely. In a large representative sample of 12,069 young men and women, a significant relationship between alcohol use and sexual risk taking was found even after controls were applied for age, education, and family income (Parker et al., 1994). Because the relationship between alcohol use and unsafe sex is correlational, no clear cause-and-effect relationship can be assumed, and other factors such as rebellion against societal expectations may be responsible for both.

Of additional concern is the effect of alcohol-induced loss of judgment on the initiation of unsafe sex practices that may lead to increased risk for AIDS and other sexually transmitted diseases. Using a natural quasi-experimental design including cross-state comparisons, Staras and colleagues (2016) investigated the relationship between alcohol taxes, which have been shown to inversely impact consumption, and the incidence of sexually transmitted infections as quantified by the National Notifiable Disease Surveillance System. When compared with states with no change in the alcohol tax, they found that after the 50% increase in the tax in Maryland in 2011, gonorrhea rates decreased 24% over the 1.5-year study period. The researchers argue that the practical application of their results should provide incentive to change tax rates for the public health benefits.

Acute effects of alcohol on memory vary with dose and task difficulty (Jung, 2001). At low doses, memory deficits are based more on expectation than on the quantity of alcohol actually consumed. Further, under high-stress conditions, alcohol may enhance performance by minimizing the damaging effects of anxiety. However, high doses of alcohol rapidly consumed may produce total amnesia for events that occur during intoxication, despite the fact that the individual is behaving quite normally. This amnesia is called a **blackout**, and it is a common occurrence for individuals with alcohol use disorder but also occurs in about 25% of social drinkers (Campbell and Hodgins, 1993).

Reduced coordination leads to slurred speech, impaired fine motor skills, and delayed reaction time. Impaired reaction times for multiple stimuli, along with reductions in attention, increased sedation and drowsiness, and impaired judgment and emotional control, contribute to the increased probability of being involved in automobile accidents. In 2014, approximately one-third of all traffic-related fatalities in the

United States involved alcohol-impaired drivers (CDC, 2016). **FIGURE 10.8A** shows the distinct temporal pattern of high-risk alcohol-related deaths. In addition, a clear statistical relationship between BAC and the relative risk for an accident has been reported. At a BAC lower than 0.05%, the chances of having an accident are about the same as for nondrinking drivers, but between 0.05% and 0.10%, the curve rises steeply to seven times the nondrinking rate. It is this large increase that has prompted all states to change their blood level for legal intoxication from 0.10% to 0.08%, while most European and Asian countries consider driving with a BAC of 0.02% to 0.05% as driving under the influence. Beyond 0.10%, risk increases dramatically by 20 to 50 times. However, the relationship is complex, and BAC interacts with both age and driving experience (**FIGURE 10.8B**). Crash risk is higher for young people than for older people at all levels of BAC. In 2014, 3 out of every 10 individuals involved in fatal auto accidents were between the ages of 21 and 24 years (30%). Other significant age groups were those 25 to 34 (29%) and 35 to 44 (24%) (CDC, 2016). See Table 10.1 for estimates of the amounts of alcohol that must be consumed in 1 hour to reach the BAC that increases risk.

In addition to involvement in automobile fatalities, alcohol use is associated with homicide, rape, and other violent activities, although the direct pharmacological effect of alcohol is less clear. **Web Box 10.1** looks at this relationship. Aggression and many of the other effects of alcohol on behavior are highly dependent on the environment, the user's mental set, and one's expectations.

With increasing doses, mild sedation deepens and produces sleep. Alcohol suppresses rapid-eye-movement (REM) episodes (periods when the most dreaming occurs), and withdrawal after repeated use produces a rebound in REM sleep that may interfere with normal sleep patterns and produce nightmares. Higher doses produce unconsciousness and death. The blood alcohol level that is lethal in 50% of the population is in the range of 0.45%, which is only about five or six times the

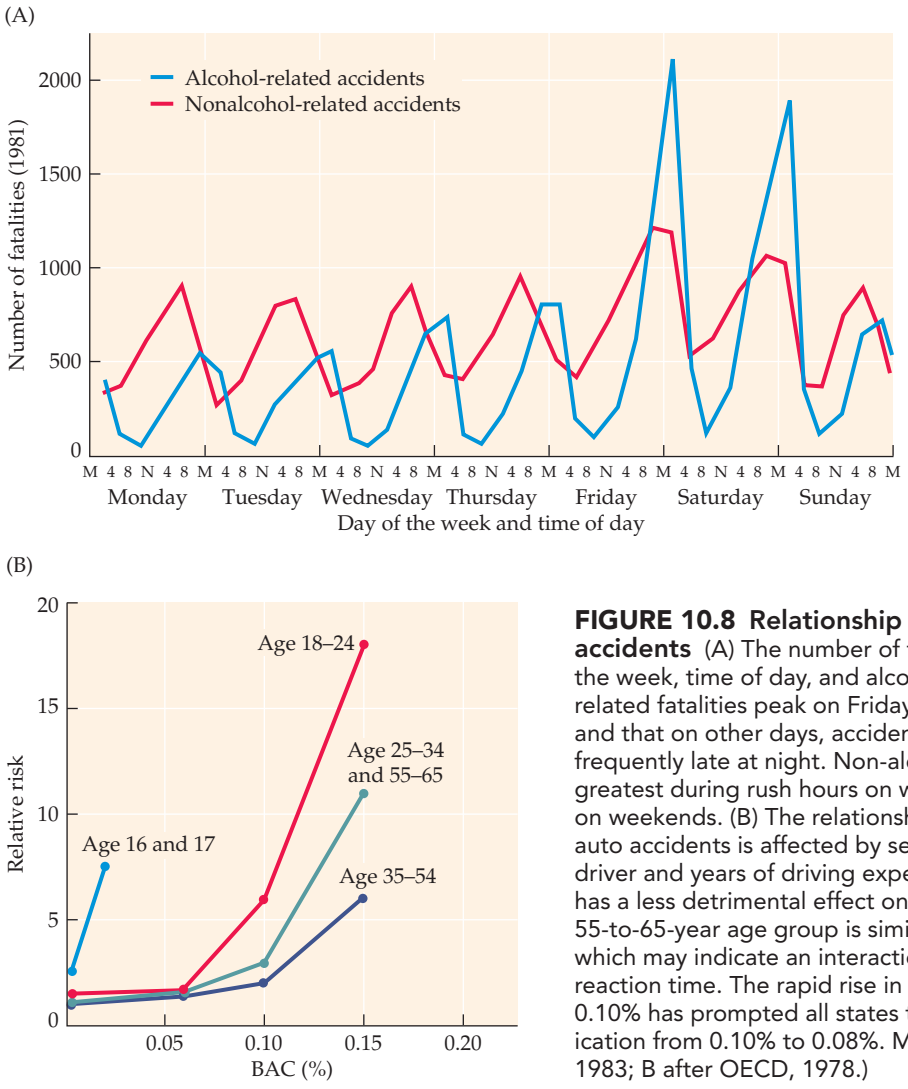


FIGURE 10.8 Relationship between alcohol use and traffic accidents (A) The number of fatal auto accidents varies by day of the week, time of day, and alcohol involvement. Note that alcohol-related fatalities peak on Friday and Saturday nights after midnight and that on other days, accidents involving alcohol also occur most frequently late at night. Non-alcohol-related fatalities appear to be greatest during rush hours on weekdays and just before midnight on weekends. (B) The relationship between BAC and relative risk for auto accidents is affected by several factors, including age of the driver and years of driving experience. Note that, in general, alcohol has a less detrimental effect on driving as drivers get older, but the 55-to-65-year age group is similar to the 25-to-34-year age group, which may indicate an interaction with age-related decreases in reaction time. The rapid rise in the number of accidents at BAC over 0.10% has prompted all states to reduce the definition of legal intoxication from 0.10% to 0.08%. M, midnight; N, noon. (A after NIAAA, 1983; B after OECD, 1978.)

blood level (0.08%) that produces intoxication. Fortunately, most people do not reach a lethal blood level because at about 0.15%, vomiting may occur, and a BAC of 0.35% usually causes unconsciousness, thereby preventing further drinking. However, if alcohol is consumed very rapidly, as might occur in binge drinking, lethal blood levels may be reached before the individual passes out.

The usual symptoms of **alcohol poisoning** include unconsciousness; vomiting; slow and irregular breathing; and skin that is cold, clammy, and pale bluish in color. Death from acute alcohol ingestion is caused by depression of the respiratory control center in the brainstem. Once the respiratory mechanism is depressed, the drinker can survive for about 5 minutes, although brain damage may result from oxygen deprivation. Kanny and colleagues (2015) at the CDC reported an average annual death rate of 2221 from alcohol poisoning between 2010 and 2012. The occurrence was the highest among men age 35 to 64 (76%). Although most deaths are attributed to binge drinking, the majority of binge drinkers are not individuals with alcohol use disorder. Nevertheless, alcohol dependence was a contributing factor to the deaths in 30% of cases, along with hypothermia (6%) and other drug use (6.7%). Some of the dose-dependent effects of alcohol are summarized in **TABLE 10.2**.

BRAIN DAMAGE Brain damage that occurs after many years of heavy alcohol consumption is caused by the interaction of several factors, including high levels of alcohol, elevated acetaldehyde, liver deficiency, and inadequate nutrition. In particular, heavy alcohol use produces a serious deficiency in vitamin B_1 (thiamine) as the result of both a poor diet and failure to absorb that vitamin, as well as other nutrients, during digestion. Because thiamine is critical for brain glucose metabolism, its deficit causes cell death. Lack of vitamin B_1 may lead to Wernicke's encephalopathy (WE). WE is characterized by confusion and disorientation, as well as poor coordination, tremors, weakness, and ataxia. Often there is some form of occulomotor dysfunction such as abnormal eye movements or double vision. WE results from lesions in the periaqueductal gray, medial thalamus, and mammillary bodies of the hypothalamus. When treated early, the symptoms are readily reversible with massive vitamin supplementation that reverses the biochemical damage. Without treatment the brain damage is permanent and leads to death in approximately 20% of cases. WE is associated with excessive alcohol use but can be caused by malnutrition, weight loss surgery, HIV/AIDS, anorexia nervosa, and other conditions that may cause vitamin B_1 deficiency. A high proportion of patients who survive WE develop Korsakoff syndrome (in combination, called Wernicke–Korsakoff syndrome), which is characterized by potentially irreversible memory loss, anterograde amnesia (inability to remember new information), decreased spontaneity, confabulation (creating false memories), hallucinations, and personality changes. Korsakoff syndrome is a result of permanent damage to thalamic nuclei and brain regions involved with memory subsequent to lack of thiamine. Although nutritional deficits are not the sole cause of the disorder, the importance of thiamine to the degenerative process is evident in animal studies. Feeding animals a thiamine-deficient diet or treating them with a thiamine antagonist produces lesions in the same brain areas and also impairs learning and memory (Langlais and Savage, 1995).

Although thiamine deficiency causes the selective damage described in the previous paragraph, other brain areas frequently show cell loss that seems to be unrelated to diet. The enlarged ventricles in the brains of individuals with alcohol use disorder attest to the extensive shrinkage of brain tissue (**FIGURE 10.9A**). Exterior views of brains from alcohol abusers compared with controls show smaller brain mass (**FIGURE 10.9B**). Frontal lobes are most affected, and this may be responsible for the personality changes, including apathy, disinhibition, and diminished executive functioning (ability to formulate strategies and make decisions) seen. Tissue shrinkage that occurs in medial

TABLE **10.2** Blood Alcohol Concentration and Effects on Behavior

BAC (%)	Effects on behavior
0.02–0.03	Minimal effects, slight relaxation, mild mood elevation
0.05–0.06	Decreased alertness, relaxed inhibitions, mildly impaired judgment
0.08–0.10	Loss of motor coordination, slower reaction times, less caution
0.14–0.16	Major impairment of mental and physical control, slurred speech, exaggerated emotions, blurred vision, serious loss of judgment, large increases in reaction time
0.20–0.25	Staggering, inability to walk or dress without help, tears or rage with little provocation, mental confusion, double vision
0.30	Being conscious but in a stupor, unawareness of surroundings
0.45	Coma, death for 50% of the population

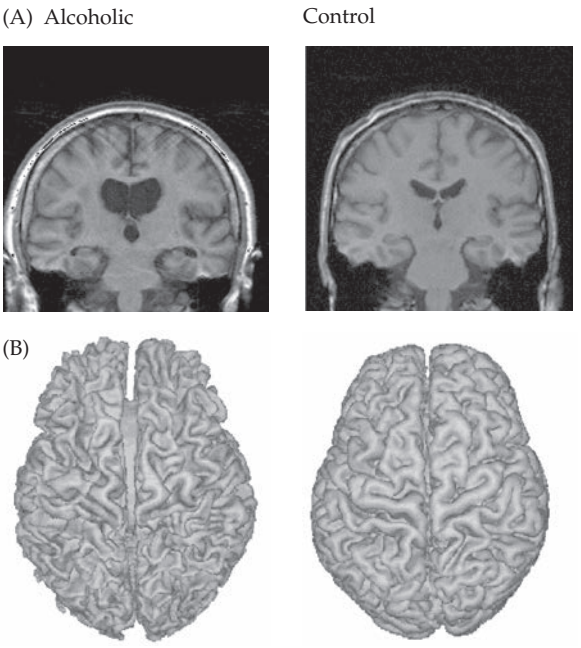


FIGURE 10.9 Alcohol-induced brain damage
(A) Brain images of a man with alcohol use disorder and a healthy male control. Note the extreme difference in ventricle size, indicating tissue shrinkage in the brain of the heavy user of alcohol. (B) Exterior views of the brains above. In the alcohol abuser, the gyri are more narrow, and the sulci and fissure between the hemispheres are very enlarged, showing significant loss of tissue volume. (A from Pfefferbaum and Sullivan, 2004; B from Sullivan, 2000.)

temporal lobe structures, including the hippocampus and cholinergic cells in the basal forebrain, contributes to memory disturbances. Symptoms that implicate the hippocampus and the basal forebrain include failure to remember recent events and failure to form new memories. Cerebellar cell loss is correlated with ataxia and incoordination, particularly of the lower limbs. These brain changes are probably caused by multiple mechanisms, but glutamate-induced hyperexcitability of neurons during abstinence (see the section on neurotransmitters later in the chapter) may play a central role (Fadda and Rossetti, 1998).

EFFECTS ON OTHER ORGAN SYSTEMS Alcohol has many effects on the body outside the CNS, including the following:

- Cardiovascular system
- Renal–urinary system
- Reproductive system
- Gastrointestinal system
- Liver

One well-known cardiovascular effect of alcohol is the dilation of peripheral blood vessels, which brings them closer to the surface of the skin and makes an individual look flushed and feel warm. Of course, vasodilation means that heat is actually being lost from the body rather than being retained. Although the myth of the Saint Bernard dog rescuing stranded skiers with a keg of brandy around his neck is widespread, in reality, drinking alcohol when you are truly cold produces an even more serious drop in body temperature. Heavy drinkers who fall asleep outside in cold climates risk death from hypothermia. Within the brain, vasodilation may improve cognitive function in older adults. At the end of a 6-year period, researchers found that people 55 years and older who consumed one to three drinks a day were less than half as likely to have developed dementia linked to poor oxygen supply to the brain as people who did not drink at all.

In addition to aiding circulation, a low to moderate daily dose of alcohol may reduce the risk of heart disease, because it increases the amount of "good" cholesterol in the blood while reducing the "bad" (Gaziano and Hennekens, 1995) and seems to reduce the incidence of blood clots and stroke. According to the Dietary Guidelines for Americans, moderate alcohol consumption is defined as having up to one drink per day for women and up to two drinks per day for men (USDHHS, 2015). However, these beneficial effects are counteracted when consumption is greater. Alcohol use disorder is associated with a higher-than-expected incidence of high blood pressure, stroke, and inflammation and enlargement of the heart muscle, which may be alcohol induced or due to malnutrition and vitamin deficiency.

The action of alcohol on the renal–urinary system produces larger volumes of urine that is far more dilute than normal. The loss of fluids is caused by reduced secretion of antidiuretic hormone. Although this is not normally a matter of concern, alcohol consumption should be avoided by individuals involved in strenuous athletic activities for which fluids need to be maintained. Further, athletes should not try to rehydrate with any beverage that contains alcohol.

The effect of alcohol on reproductive function is complex. Alcohol is widely believed to enhance sexual arousal and lower inhibitions. However, as Box 10.1 shows, expectation plays a large part in the effects of alcohol on sexual response. Furthermore, we need to distinguish between psychological arousal and physiological response. In one study, male college students consumed alcohol to achieve a BAC of 0%, 0.025%, 0.050%, or 0.075% while watching an erotic film (George and Norris, 1991). A plethysmograph was attached around the penis to measure degree of erection (both rate of tumescence and maximum achieved) during the film viewing. **FIGURE 10.10A** shows that low doses of alcohol enhanced arousal to a small extent, but higher blood levels reduced the male sexual response. Parallel studies with college women measured sexual arousal

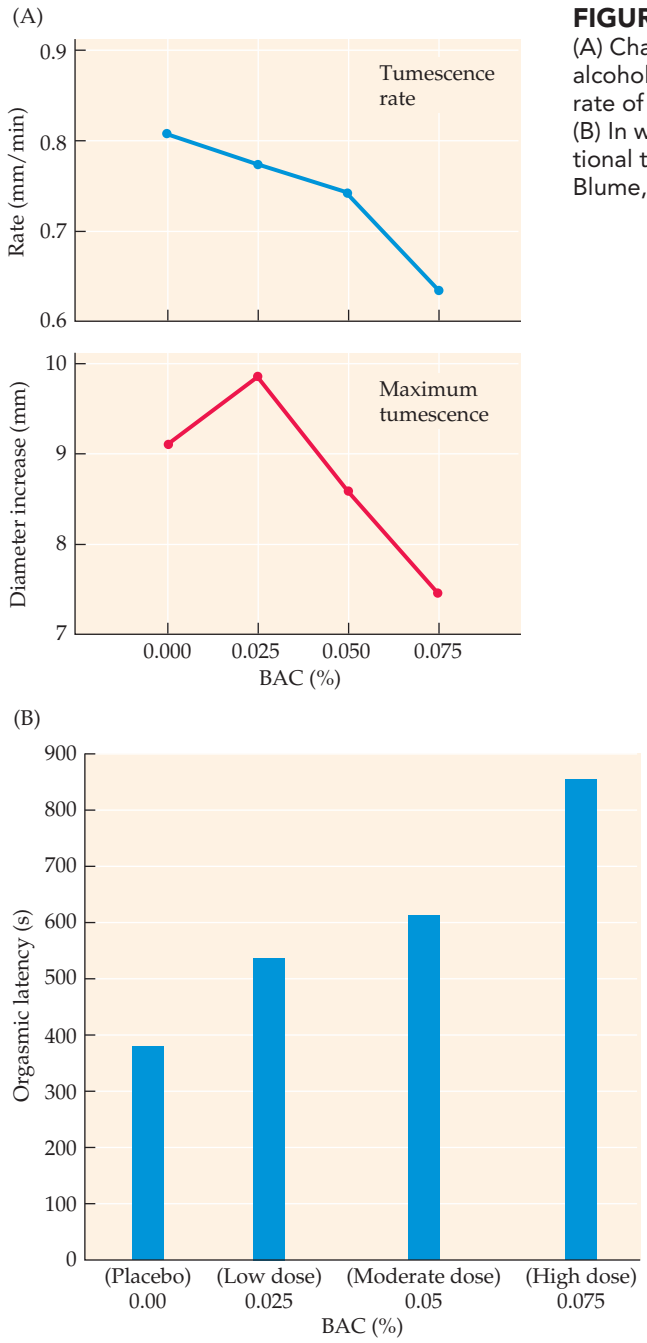


FIGURE 10.10 Effects of alcohol on sexual response
(A) Changes in male sexual response occur after varying amounts of alcohol. With increasing concentrations of blood alcohol, both the rate of penile tumescence and the maximum size achieved decreased. (B) In women, increasing blood alcohol levels were directly proportional to orgasmic latency. (A after Farkas and Rosen, 1976; B after Blume, 1991.)

by assessing vaginal blood pressure or orgasmic latency. Physiological measures of sexual arousal decreased with increasing alcohol levels (**FIGURE 10.10B**); however, reported subjective arousal was increased. Although laboratory evaluations of sexual response are necessarily artificial, and ethical restraint prohibits testing higher levels of alcohol, research in general supports the inverse nature of physiological and subjective arousal with low to moderate alcohol use.

When alcohol use is heavy and chronic, males may become impotent and may show atrophy of the testicles, reduced sperm production, and shrinkage of the prostate and seminal vesicles. Women abusing alcohol often experience disrupted ovarian function and show a higher-than-normal incidence of menstrual disorders.

Alcohol alters gastrointestinal tract function in several ways. It increases salivation and secretion of gastric juices, which may explain its ability to increase appetite and aid digestion, although higher concentrations irritate the stomach lining, and chronic use produces inflammation of the stomach (gastritis), as well as of the esophagus. Heavy alcohol use causes diarrhea, inhibits utilization of proteins, and reduces absorption and metabolism of vitamins and minerals.

Among the most damaging effects of heavy chronic alcohol consumption is liver dysfunction. Three distinct disorders may develop. The first is **fatty liver**, which involves the accumulation of triglycerides inside liver cells. The liver normally takes up and metabolizes fatty acids as part of the digestive process; however, when alcohol is present, it is metabolized first, leaving the fat for storage. Alcohol also affects adipose tissue. Adipose tissue stores energy in the form of fat. It also secretes a wide variety of hormones and inflammatory peptides called cytokines. For example, alcohol intake enhances cortisol secretion that causes changes in the way fat is distributed in the body, leading to greater abdominal and liver accumulation. Alcohol also causes inflammation of the tissue along with increased breakdown of fat into fatty acids. These changes in adipose tissue release fatty acids into the blood, which contributes to the increased deposit of triglycerides by liver cells (Kema et al., 2015). The condition produces no warning symptoms but is reversible, so if drinking stops, the liver begins to use the stored fat and returns to normal. However, some individuals who have abused alcohol for many years develop a serious and potentially lethal condition called **alcohol-induced hepatitis**, which leads to the death of liver cells.

Heavy drinkers may progress from fatty liver to alcohol-induced hepatitis and then to cirrhosis. Symptoms of alcohol-induced hepatitis include inflammation of the liver, fever, yellowing of the skin (jaundice), and pain. The death of liver cells stimulates the formation of scar tissue, which is characteristic of **alcohol-induced cirrhosis**. As scar tissue develops, blood vessels carrying oxygen are cut off, leading to further cell death. **FIGURE 10.11** compares pieces of normal liver, fatty liver, and cirrhotic liver from an individual with alcohol use disorder. Cirrhotic livers are usually firm or

FIGURE 10.11 The effects of chronic alcohol use on the liver Portions of a healthy liver (left), a fatty liver (center), and a cirrhotic liver (right). (© Arthur Glauberman/Science Source.)

hard to the touch and develop nodules of tissue that give them a pebbly appearance. As cirrhosis continues, liver function decreases proportionately. Consumption of large quantities of alcohol over a prolonged period is necessary for the development of cirrhosis, and even among heavy drinkers, only 10% to 15% are likely to develop the disease. Cirrhosis is most common in men between 40 and 60 years of age. However, although more men develop cirrhosis, probably because men typically drink more than women, at any given level of alcohol consumption, women have a greater chance of developing the disease than men. Cirrhosis mortality has been gradually declining since the peak following World War II, perhaps because of increased utilization of treatment programs that reduce drinking behavior. In addition, during that time alcohol consumption, particularly of spirits, has fallen. Although the liver damage is irreversible, cessation of drinking slows the rate of damage. People with cirrhosis who stop drinking have a 90% 5-year survival rate, while for those who continue to consume alcohol, the rate drops to 70%. However, for those in the late stages of the disease, survival rates drop significantly (Mann et al., 2004). For severe liver damage, the most effective treatment is liver transplant surgery. Although the damaging effects of alcohol are found in both men and women, there are significant gender differences, detailed in **Web Box 10.2**.

The precise cause of liver cell damage is not known, but there are promising leads. There is an abundance of endoplasmic reticulum (ER) in hepatocytes (liver cells), and abnormal ER function may be a major contributor to the liver cell damage following heavy and prolonged alcohol consumption. Alcohol metabolism is one of the many roles of the ER in the liver. The alcohol metabolizing enzyme CYP2E1 located on the smooth ER (see Chapter 1) is induced by chronic alcohol use. Subsequently the enhanced metabolism causes the release of free radicals (i.e., especially reactive atoms that can damage cells, proteins, and DNA by changing their chemical structure), producing oxidative stress (i.e., physiological stress on the body caused by free radical-induced damage) that induces cellular injury.

A second significant function of the ER involves the processing and maturation of newly synthesized proteins. Normally there is an equilibrium between the input of new proteins that need processing and the capacity of the ER to do the work. If changing conditions increase protein synthesis, a series of intracellular events is initiated to help the cells to adapt and survive. If the adaptive mechanism is insufficient to handle the increased processing demands (called ER stress), the cell undergoes a pathological response that includes increased fat formation, inflammation, and cell death, all signs of liver injury. Laboratory animals that have been fed chronic high doses of alcohol showed significant increases in multiple intracellular markers that indicate high levels of ER stress response, which may explain why they develop signs of liver disease. Alcohol ingestion for as short a time as 2 weeks has been shown to induce ER stress in healthy humans as well. In addition, abnormally high plasma levels of homocysteine were found in both individuals with alcohol-induced liver disease and the mouse intragastric alcohol feeding model. This amino acid interferes with ER processing of proteins and hence increases the ER stress response leading to cell toxicity. Homocysteine is just one of the possible alcohol-related factors that increase ER stress. Other possibilities include acetaldehyde, oxidative stress, epigenetic modifications, insulin resistance, and others (reviewed by Ji, 2014).

Of special concern is the circular nature of the interaction between the ER stress response and the events that promote it and the resulting outcome. Apparently, not only does the ER stress response initiate fat accumulation, inflammation, and cell injury, but those same outcomes further increase the ER stress response. Drugs targeting this vicious cycle may be beneficial in reducing further liver cell damage.

EFFECTS ON FETAL DEVELOPMENT Because alcohol readily passes through the placental barrier, the alcohol that a pregnant female consumes is delivered almost immediately to her fetus, who reaches the same BAC. The damaging developmental effects of prenatal alcohol exposure lead to **fetal alcohol spectrum disorders** (**FASD**) or the more severe **fetal alcohol syndrome** (**FAS**). One of the greatest tragedies of FAS is that it occurs at all. Fetal alcohol exposure is the most common preventable cause of intellectual disability in the United States. Although the damaging effects of alcohol on an adult generally take decades of heavy drinking, the developing embryo is far more susceptible. The major and

minor birth defects that constitute FASD and FAS present a challenge to families, social services, law enforcement, and the educational system. The cost for serving those individuals with only the most severe symptoms was estimated at more than $4 billion a year in the United States, and billions more might be spent on special care for the less impaired (CDC, 2015a).

Diagnostic signs and symptoms include the following:

- *Intellectual disability and other developmental delays*. The average IQ for an individual with FAS is 68. Such an individual generally attains an average reading level of a fourth grader and average second-grade math skills. The development of typical motor milestones is delayed, and evidence of poor coordination, slow response times, and language disabilities is common.
- *Low birthweight (below the 10th percentile)*. In addition, infants fail to thrive, exhibiting poor catch-up growth.
- *Neurological problems*. Some infants are born with high alcohol levels and experience withdrawal from the drug, which includes tremors and seizures starting within 6 to 12 hours of birth and lasting as long as a week. Abnormal electroencephalogram recordings persist, and the infant shows a high degree of irritability and hypersensitivity to sound. These infants show poor sucking reflexes, hyperactivity, attentional deficits, and poor sleep patterns.
- *Distinctive craniofacial malformations*. These include a small head, small wide-set eyes with drooping eyelids, a short upturned nose, a thin upper lip, and flattening of the vertical groove between the nose and upper lip (**FIGURE 10.12A**). The infants may also show low-set and nonparallel ears, malformations of the ear that produce hearing deficits, cleft palate, and reduced growth of the lower jaw.
- *Other physical abnormalities*. Cardiac defects such as a hole between the chambers or deformed blood vessels in the heart, failure of kidney development, undescended testes, and skeletal abnormalities in fingers and toes are common.

Although the estimates of the prevalence of FAS vary considerably depending on criteria used, the range is from 0.2 to 2.0 cases per 1000 live births. In addition, it has become clear that prenatal alcohol can have effects that are distinct from FAS. Fetal alcohol spectrum disorders are a cluster of disorders characterized by neurological abnormalities leading to attention deficits, hyperactivity, poor coordination, poor impulse control, delayed speech, deficits in memory, and learning disabilities. Other individuals show defects in skeletal and major organ systems. Incidence rate for FASD may be as high as 10 in 1000. Because the symptoms are so varied, diagnosis is difficult. However, although there is no cure for FASD, diagnosis before age 6 can improve the child's development by implementing such things as behavior therapy, medication for specific symptoms, and parent training in structuring the environment to optimize the child's functioning and coping with particular disabilities (CDC, 2015b).

How sure are we that alcohol itself is teratogenic (i.e., causes birth defects)? After all, women who use high doses of alcohol often have poor nutrition, smoke cigarettes or use other drugs, have poor health overall, and receive poor prenatal care. These issues have been well controlled in animal research that can regulate the amount of alcohol, the pattern of consumption, the timing of alcohol use during the pregnancy, and the diet of the mother. Early conclusions and subsequent research agree that prenatal alcohol does induce both physical defects and behavioral deficits in animals that closely resemble those seen in humans. Single large doses of alcohol given to pregnant mice produced abnormalities in the developing fetuses (**FIGURE 10.12B**), including eye damage, smaller brains, and facial deformities similar to those seen in human babies with FAS. The amount of alcohol responsible was equivalent to that consumed when a woman drinks a quart of whiskey over 24 hours.

Blood alcohol level is important in estimating the risk and severity of teratogenic effects, but the pattern of alcohol use that contributes to the peak maternal blood alcohol level is equally important. In one rodent study, 12 equally spaced doses of alcohol that produced maternal blood levels up to 0.12% did not affect fetal brain growth. In contrast, the same total amount of alcohol given in condensed fashion raised maternal blood levels to a range between 0.20% and 0.35% and caused a significant decrease in brain weight (Randall et al., 1990). Although this blood level is quite high, it is consistent with blood levels seen after binge drinking in humans.

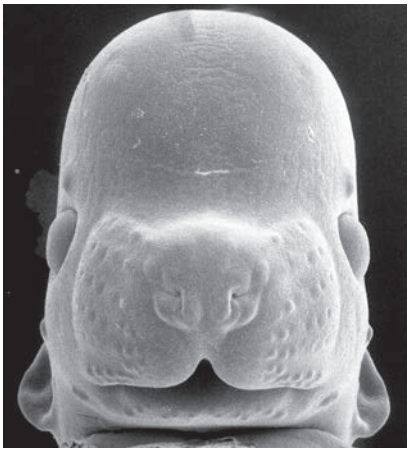
Animal research is well supported by many studies with humans showing that heavy maternal drinking, particularly of the binge type, is associated with significant behavioral and emotional problems, as well as cognitive deficits, in offspring. However, the effects of low to moderate alcohol consumption on fetal development are somewhat less clear. Although multiple studies have shown deficits in attention, aggressive behavior, and learning difficulties after moderate prenatal alcohol exposure, others have reported no measurable adverse outcomes following low to moderate amounts.

The discrepancies among studies are troubling because evidence suggesting that low levels of alcohol are relatively safe could encourage women to drink during pregnancy and if erroneous could lead to less optimal fetal outcomes. On the other hand, if small amounts of alcohol are safe to consume, women who drank lightly early in their pregnancy (when many are unaware of their status) would experience less guilt,

(A)

FIGURE 10.12 Fetal alcohol syndrome (A) Distinctive craniofacial malformations in a child with FAS. (B) Fetal abnormalities in mice exposed to alcohol in utero. (A, © Rick's Photography/Shutterstock; B from Sulik et al., 1981, courtesy of Kathleen K. Sulik.)

(B) Normal



Exposed to alcohol

anxiety, and stress over unintentionally harming their children. Unfortunately, correlational epidemiological studies such as these are plagued with methodological difficulties because in this retrospective research, women may inaccurately recall the quantity, timing, frequency, and pattern of alcohol use, all of which are critical factors determining fetal outcome. Some may underreport their consumption because of social pressure against drinking during pregnancy. In addition, many other variables such as the psychological health of the mother, maternal medical issues and other drugs being used, socioeconomic differences, level of stress experienced, adequacy of maternal nutrition, genetics, and parenting styles are likely to modulate the effects of prenatal alcohol and behavioral outcomes in the child. Others have suggested that the differences among studies may reflect the particular cognitive test used and its sensitivity or the manner in which behavioral outcomes were evaluated. Additionally, sociocultural differences, including the type of liquor consumed and whether it is part of a meal, may make results less generalizable across cultures (Todorow et al., 2010). Although controversy is likely to continue in the future, given that the threshold for adverse effects is unknown and how the threshold might vary from individual to individual is equally unclear, the *safest option at this time is abstinence* during pregnancy. In fact, both the Surgeon General and the American Academy of Pediatrics have stated that drinking *any* alcohol at *any* stage of pregnancy can cause some disability in the child (American Academy of Pediatrics, 2015). In addition, the CDC recommends that women who are considering becoming pregnant should stop using alcohol because they will be unaware of their status during the early weeks of pregnancy.

In addition to the amount and pattern of alcohol ingestion, the developmental stage of the fetus when exposed to alcohol is critical in determining the specific effects. Organ systems are most vulnerable to damage during the period of most rapid development. Alcohol ingestion at the time of conception significantly increases the risk of teratogenic effects, and within the first 3 weeks, the fetus may not survive. Alcohol use during the fourth to ninth weeks—a time when many women are unaware of their pregnancy—produces the most severe formative damage and severe mental retardation. Alcohol use later in pregnancy causes slowed growth. Since the brain is one of the first organ systems to begin to develop but is the last to be complete, alcohol use at any point in the pregnancy can have damaging effects on the CNS. Obviously, if drinking is constant throughout fetal development, the effects will be much greater than if drinking is stopped midpregnancy.

Although the damaging effects of fetal alcohol exposure are clear, its precise mechanism is less certain. It has been suggested that acetaldehyde may be the toxic agent; other possible mechanisms include decreased blood flow in the uterine artery, reduced oxygen availability, and placental dysfunction, which reduces the transport of vital amino acids, glucose, folate, and zinc. Hormone-like substances called prostaglandins are suspected of mediating teratogenic effects because inhibitors of prostaglandins, such as aspirin, reduce alcohol-induced birth defects in animals. Additional research showed that ethanol acting on both glutamate and γ-aminobutyric acid (GABA) neurons may trigger significant cell death (apoptosis) in the developing brain (Ikonomidou et al., 2000).

An additional potential mechanism responsible for alcohol-induced fetal brain damage is the occurrence of

epigenetic events. The transcription of multiple genes involved in cell maturation and neurodevelopment is epigenetically altered by exposure to alcohol. Laufer and colleagues (2013) showed that fetal alcohol exposure following voluntary maternal alcohol consumption in mice produced widespread disruption in DNA methylation (see Chapter 2) that persisted into adulthood. The altered gene transcription involved genes previously implicated in several other neurodevelopmental disorders. One of the many genes epigenetically modified (*Akt*) regulates multiple processes of neuronal development, including dendritic development, synapse formation, and synaptic plasticity, all processes found altered in FASD. A second gene (*Nmnat1*), whose function was down-regulated, normally protects against neurodegeneration by inhibiting cell death. Loss of function leaves many brain regions, such as the hippocampus, vulnerable to damaging events. An additional down-regulated gene (*Otx2*) is associated with mood disorders. Hence epigenetically altered gene expression may contribute to the neurobiological and behavioral characteristics associated with FASD.

Section Summary

- The small non-ionized alcohol molecule is absorbed from the GI tract by passive diffusion—a process slowed by food in the stomach. Absorption in women is faster because reduced gastric metabolism and smaller body size increase the concentration.
- About 95% of ingested alcohol is metabolized by the liver at a constant rate of 1 to 1.5 ounces per hour; about 5% is excreted by the lungs.
- Alcohol dehydrogenase converts alcohol to the toxic product acetaldehyde. Further metabolism produces carbon dioxide, water, and energy.
- The cytochrome P450 enzyme CYP2E1 metabolizes alcohol, as well as other drugs. Consuming them together may lead to dangerous blood levels because they compete for the limited amount of enzyme.
- Acute tolerance occurs within a single drinking episode and may lead to dangerous driving when binge drinkers perceive that they are less intoxicated on the descending limb of the blood alcohol curve.
- Chronic alcohol use increases cytochrome P450 enzymes (enzyme induction), so metabolism is more rapid, causing metabolic tolerance to the effects of alcohol and cross-tolerance to other drugs metabolized by the same enzyme.
- Continued presence of alcohol produces compensatory changes in neuron function (pharmacodynamic tolerance).
- Practicing an operant task under the influence of alcohol leads to improved performance (behavioral tolerance of the operant type). Repeated alcohol administration in the same environment leads to the development of a compensatory response that occurs only in that environment (behavioral tolerance of the classical conditioning type).
- Alcohol produces physical dependence and cross dependence with other sedative–hypnotic drugs.
- Withdrawal after chronic heavy use lasts for days and includes tremor, anxiety, high blood pressure and heart rate, sweating, rapid breathing, and nausea and vomiting. Severe withdrawal effects called delirium tremens include hallucinations, convulsions, disorientation, and intense anxiety.
- Behavioral effects of alcohol are directly related to BAC, but at low doses, the environment and expectations of effects also have significant effects.
- Dose-dependent effects on the CNS include relaxation, reduced anxiety, intoxication, impaired judgment, impaired memory, and sleep. Higher doses produce coma and death as the result of respiratory depression.
- Heavy long-term alcohol use causes a vitamin B_1 deficiency leading to cell death in the periaqueductal gray, medial thalamus, and mammillary bodies, causing Wernicke's encephalopathy: tremors, weakness, ataxia, confusion, and disorientation. Multiple brain regions may be damaged by glutamate-induced excitotoxicity. Korsakoff syndrome is caused by permanent damage to thalamic nuclei and brain regions involved in memory subsequent to vitamin B_1 deficiency. Symptoms of Korsakoff syndrome include potentially permanent memory loss, personality changes, and hallucinations.
- Beneficial alcohol-induced cardiovascular effects include vasodilation, elevation of good cholesterol, and lowering of bad cholesterol. Alcohol use disorder increases the risk of high blood pressure, stroke, and heart enlargement.
- Alcohol has a diuretic effect, increases sexual arousal while decreasing performance, increases appetite, and aids digestion by increasing gastric secretions.
- Liver damage associated with alcohol use disorder includes fatty liver, alcohol-induced hepatitis, and cirrhosis.
- Prenatal exposure to alcohol may produce FAS, which is characterized by intellectual disability and other developmental delays, low birth weight, neurological problems, head and facial malformations, and other physical abnormalities. A larger number of infants are affected by a cluster of FAS-related

disorders (fetal alcohol spectrum disorders) having highly varied symptom presentation, which makes them harder to diagnose and treat.

- Effects of prenatal exposure are dependent on blood alcohol level, pattern of alcohol use, fetal developmental stage at time of exposure, maternal nutrition, genetics, and comorbid drug use. Multiple possible mechanisms for alcohol-induced FASD have been identified including maternal alcohol-induced epigenetic effects that alter various stages of neuronal development.

Neurochemical Effects of Alcohol

The neurochemical effects of alcohol have proved more difficult to examine than those of some other drugs. One important reason is the chemical nature of the alcohol molecule, which not only provides the means for easy penetration into the brain but, more importantly, influences the phospholipid bilayer of neurons. The latter action has a widespread impact on many normal cell functions and also modifies the actions of many neurotransmitter systems. In addition, the initial effects of alcohol must be separated from the neuronal changes that occur after long-term drug use. For these and other reasons, research using animal experimentation is particularly important.

Animal models are vital for alcohol research

Animal models are particularly important in alcohol studies for several reasons. First, because research animals are maintained in controlled and healthful environments, some of the common human correlates of heavy alcohol use are eliminated: poor nutrition, liver damage, associated psychiatric disorders, and use of multiple drugs. Second, animal models allow us to use methods that are not appropriate for humans. For example, studies using controlled chronic alcohol consumption can tell us about the long-term damaging effects on body functions and behavior and can model the effects of alcohol withdrawal. The effects of prenatal alcohol can be evaluated independently of issues such as maternal nutrition and substance abuse. Also, invasive procedures, including genetic engineering, can be used to manipulate and measure the neurobiological correlates of intoxication, reinforcement, and behavioral effects of alcohol. Third, animal models serve as screening tools for evaluating treatment strategies.

When evaluating animal models, it is important to remember that alcohol addiction is not a unitary concept, but rather one that develops through several stages, beginning with the early pleasurable positive reinforcing effects or the elimination of a noxious state, for example, stress (early acquisition stage). These reinforcing events establish the repeated drug-taking behavior (maintenance stage), but it is not until neuroadaptive mechanisms develop in response to chronic use that the development of dependence and compulsive drug taking occurs. Animal models need to reflect these stages of drug abuse as well as the occurrence of binge-type drinking, withdrawal signs, and relapse behaviors (alcohol-seeking behavior) following prolonged abstinence. Since animals do not naturally develop alcohol use disorder, no direct animal model of the complex human condition with its drinking pattern and quantity of consumption, development of compulsion to drink, and escalation of drinking despite aversive events is possible. The best efforts can model only specific components of alcohol use disorder (i.e., biomarkers that contribute to the disorder).

The operant self-administration model used to evaluate the reinforcing effects of various drugs is described in Chapter 4. It differs from the two-bottle free choice technique in which animals are free to choose between a low concentration of alcohol or water, because in the operant technique the animals must make an effort (usually pressing a lever) to earn access to the alcohol. Although the paradigm is an important way to evaluate positive reinforcement and potential substance abuse, self-administration procedures are more difficult with alcohol than with other drugs of abuse because most animals will not spontaneously consume enough alcohol to produce intoxication. However, one of several initiation procedures can be used to get animals to self-administer alcohol. The first method is the sucrose substitution procedure (Samson, 1986). In this technique animals that have access to both food and water readily learn to bar press in an operant chamber for a 20% sucrose solution. Over a series of sessions, the sucrose concentration is gradually reduced while ethanol is gradually added. After about 25 of these sessions, the animals will bar press for the ethanol without sucrose and when given a choice between water or ethanol, will choose the ethanol solution. In a second procedure, the animals are given dry food once a day during the drinking session. Initially, water is available, but gradually increasing amounts of alcohol are added over a number of sessions. Finally, food presentation occurs at a different time, but the animals continue to consume the alcohol.

Manipulation of the access to alcohol has been used to model relapse behavior characterized by excessive and uncontrolled drinking following abstinence. In either operant self-administration or free choice drinking, animals that consume alcohol for at least 6 to 8 weeks and then are deprived access to the drug for several days, weeks, or months will show a two- to threefold increase in consumption over baseline when provided the opportunity. Since not all strains of rat will show this behavior, differences in genetic background play a significant role. When the drinking and deprivation stages are repeated multiple times, the animals show the compulsive drinking behavior that resembles the human

condition, including selecting high-concentration alcohol solutions to rapidly elevate blood levels, choosing alcohol even if adulterated with bitter-tasting quinine, and choosing the bitter drink over the normally preferable sucrose solution. These behaviors resemble those of alcohol use disorder: the classic loss of control over drinking despite negative consequences, as well as failure to choose a normally reinforcing alternative. This model, described by Vengeliene et al. (2014), provides the opportunity to test drugs that would potentially remediate the most important aspect of human alcohol use disorder—relapse. Additionally, repeated episodes of withdrawal lead to impaired fear conditioning and poor cognitive processing. Similar impairments were noted in individuals with alcohol use disorder who had experienced repeated episodes of detoxification. These cognitive impairments were further found to be associated with withdrawal-induced loss of gray matter in the ventromedial prefrontal cortex. These types of models represent efforts to improve translational research and to move discoveries from animal research more quickly into clinical applications (see Chapter 4). Several excellent reviews of animal models of various components of alcohol use disorder, including adolescent onset, motivation, abstinence, and relapse, are available for further study (Ripley and Stephens, 2011; Camarini et al., 2010).

Another approach to animal models involves genetic manipulations (reviewed by Crabbe et al., 2010). Although most laboratory animals drink very little of an ethanol solution, when large numbers of animals (usually mice or rats) are screened, a few will be found to voluntarily drink large quantities of alcohol rather than water. Two populations of animals can be developed by selectively breeding the heaviest drinkers and the abstainers over several generations (**FIGURE 10.13**). Numerous strains of alcohol-preferring (AP) and high-alcohol-drinking (HAD) rodents have been developed. These animals, given 24-hour access to alcohol, will freely choose a 10% ethanol solution over water that elevates their blood alcohol to pharmacologically relevant and intoxicating levels, even in the presence of food or highly palatable sweet solutions. When tested in an operant chamber, these rats have high breaking points (see Chapter 4) for alcohol, suggesting that the animals are willing to work for access to the drug and that it has high reinforcement value for them. AP rats also demonstrate other characteristics typical of individuals with alcohol use disorder. The animals show not only metabolic tolerance but also behavioral tolerance in tasks such as balancing on a swinging platform. They ingest enough alcohol during free access sessions to produce physical dependence and withdrawal signs at abstinence. Following prolonged abstinence, the rats exhibit alcohol-seeking behavior, that is, choosing alcohol over water, when returned to the

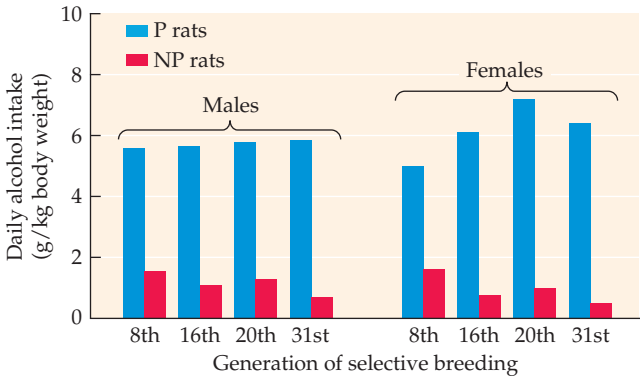


FIGURE 10.13 Average daily alcohol consumption for selected generations of rats These rats were bred for alcohol-preferring (P) and alcohol-nonpreferring (NP) behavior. Males and females show similar alcohol consumption. (After Stewart and Li, 1997.)

initial alcohol self-administration environment. These animals model human alcohol consumption and abuse and can be used to study behavioral, biochemical, and genetic differences (reviewed by McBride et al., 2014). Animals can also be bred for numerous alcohol-related characteristics such as sensitivity to alcohol, intensity of withdrawal, and so forth.

Using these inbred strains, Bell and colleagues (2014) have modeled human adolescent binge-like drinking, the typical form of early onset alcohol use. Their procedure uses scheduled access to alcohol, such as three or four 1-hour sessions a day during the dark cycle, which is the rodents' active period. Under these conditions the rats, in either the home cage or operant chamber, consume enough alcohol to reach binge-like blood levels (greater than 0.08%) on a daily basis, even when provided a highly palatable sweet alternative. Furthermore, they demonstrate intoxicated behaviors in tasks involving balance and coordination. It is of interest that the drinking pattern resembles human consumption. For instance, *adolescent* AP rats drink more during the limited-access sessions than when given continuous 24-hour access. In contrast, *adult* AP rats consume greater quantities when given continuous access than during scheduled binge-like sessions. Further, if the AP rats are provided with a selection of alcohol concentrations during the restricted access, they choose to drink the higher concentrations of alcohol, while control animals are apparently more sensitive to the aversive effects. Under those conditions blood levels two or three times higher are achieved, indicating loss-of-control drinking. The adolescent binge-type drinking in these rodents provides a means to study neural mechanisms contributing to early onset drinking, a frequent precursor to alcohol use disorder in adults.

In addition to selective breeding, a second type of study uses genetically altered animals that fail to

express a particular protein. These knockout animals are used to evaluate the role of one particular protein, for example, a specific receptor, in the effects of ethanol. The AP and HAD rat strains have been useful in identifying genes that influence alcohol consumption. Comparing the genomic sequencing of the AP and nonpreferring (NP) rats, researchers identified several regions linked to alcohol preference (Zhou et al., 2013). Further, they found a variant in the gene (*Grm2*) that encodes the metabotropic glutamate receptor 2 (mGluR2) in AP but not NP rats that impaired the expression of the receptor. Electrophysiological measures showed that application of an mGluR2 agonist in brain slices from the hippocampus and striatum produced a 40% to 60% reduction in excitation in NP rats, but less than 10% depression in AP rats, which reflects their loss of mGluR2 function. Using normal Wistar rats, chosen because they are the parental strain of both AP and NP rats, the investigators found that those animals that were administered an mGluR2 antagonist, compared with controls administered vehicle, showed a significant increase in self-administration of alcohol. Those results further indicate a role for the glutamate receptor in alcohol reinforcement. To further demonstrate the significance of mGluR2 in alcohol consumption, the researchers utilized *Grm2*-knockout mice. Homozygous knockout mice lacking mGluR2 escalated their alcohol consumption in a two-bottle free choice design in which alcohol concentration was gradually increased from 3% to 17% over 80 days. This increase was significantly greater than that found in wild-type mice (**FIGURE 10.14A**). Furthermore, in control mice the percent preference for alcohol was reduced at the highest concentrations of alcohol, which is a typical response to the aversive taste, while the knockout mice continued to prefer consistently high levels of alcohol (**FIGURE 10.14B**). Overall, mGluR2 knockout mice resemble AP inbred mice in their alcohol self-administration. Although similar gene variants have been identified in human populations, they are rare and usually contribute only a small amount to the risk factor for excessive alcohol use. Nevertheless, this type of research with animal models may help to identify potential drug targets for alcohol abuse treatment.

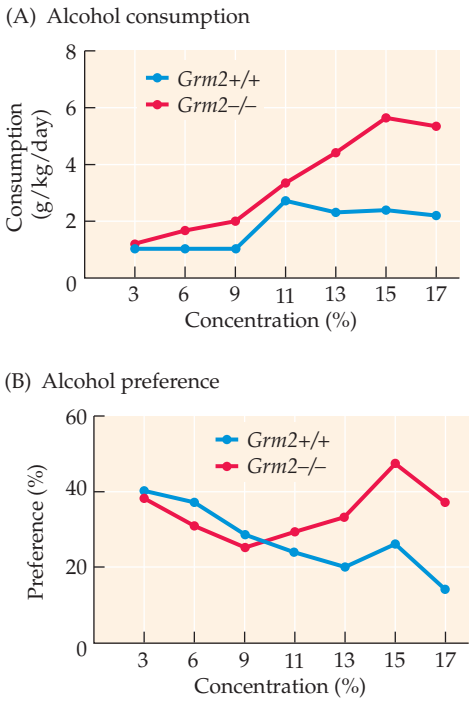


FIGURE 10.14 *Grm2*-knockout mice show increased alcohol consumption and preference
(A) Homozygous knockout mice (*Grm2*–/–) drink increasing amounts of alcohol as the alcohol concentration increases from 3% to 17%. Wild-type controls (*Grm2*+/+) consume significantly less than the knockout mice at the higher concentrations. (B) *Grm2*–/– mice showed a consistent percent preference for alcohol over water in a two-bottle free choice situation, even at high alcohol concentrations, while control mice reduced their preference for alcohol at high concentrations. (From Zhou et al., 2013.)

Alcohol acts on multiple neurotransmitters

Because alcohol is such a simple molecule, it readily crosses cell membranes, including the blood–brain barrier, and can be detected in the brain within minutes after consumption. Alcohol has both specific and nonspecific actions. *Nonspecific actions* depend on its ability to move into membranes, changing the fluid character of the lipids that make up membrane (**FIGURE 10.15**). As you might expect, the protein molecules that are embedded in that membrane are likely to function differently when their "environment" changes so dramatically and becomes less rigid. In contrast, at low to moderate doses, alcohol seems to interact with specific sites on particular proteins, and these *specific actions* are probably responsible for most of the acute effects of ethanol at intoxicating doses. Alcohol not only influences several ligand-gated channels but also directly alters second-messenger systems. For example, ethanol stimulates the G protein (G_s) that activates the cyclic adenosine monophosphate (cAMP) second-messenger system (see Figure 10.15, step 7). The ability to identify specific sites of ethanol action ultimately will lead to discovery of new drugs that will compete with ethanol to prevent particular undesirable effects.

Although alcohol affects virtually all neurotransmitter systems, this section describes the acute effects of alcohol on a limited number of neurotransmitters, suggesting possible connections between the transmitter action and specific effects of alcohol. In addition, it examines the neuroadaptations that occur with repeated alcohol use as they link to pharmacodynamic tolerance and dependence. Throughout this discussion, keep in mind that no neurotransmitter system works in

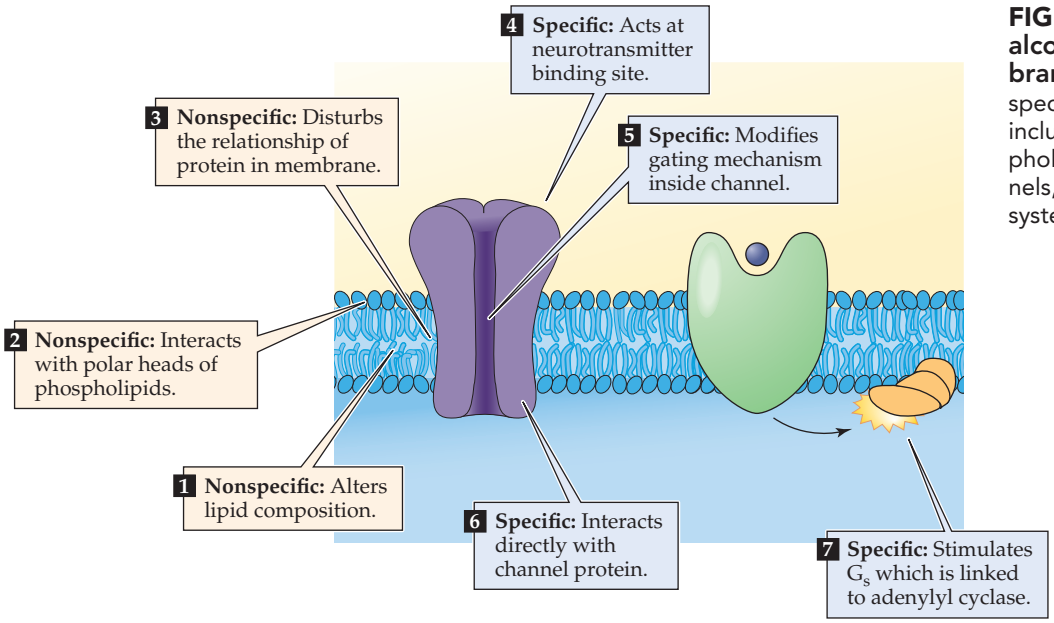


FIGURE 10.15 Effects of alcohol on neuronal membranes Alcohol acts at both specific and nonspecific sites, including membrane phospholipids, ligand-gated channels, and second-messenger systems.

isolation; changes in each one certainly modify other neurotransmitters in an interdependent fashion.

GLUTAMATE As you may recall from Chapter 8, glutamate is a major excitatory neurotransmitter in the nervous system and has receptors on many cells in the CNS. All glutamate receptors are inhibited by acute alcohol exposure, but some are only affected by high concentrations. Of the several subtypes of glutamate receptor, alcohol has its greatest effect on the NMDA (*N*-methyl-D-aspartate) receptor, which is a ligand-gated channel that allows positively charged ions (Ca^{2+} and Na^{+}) to enter and cause localized depolarization. Glutamate action at NMDA receptors mediates associative learning (see Chapter 8) and also has a role in the damaging effects of excessive glutamate activity (excitotoxicity), as in the case of prolonged seizures or after stroke. Let's look at the role of NMDA receptors in several effects of alcohol: (1) memory loss associated with intoxication, (2) rebound hyperexcitability associated with the abstinence syndrome after long-term use, and (3) NMDA-mediated excitotoxicity associated with alcohol-induced brain damage.

Alcohol acutely inhibits glutamate neurotransmission by reducing the effectiveness of glutamate at the NMDA receptor. These effects occur at concentrations as low as 0.03%, blood levels normally achieved by social drinkers. Alcohol, like other glutamate antagonists, impairs learning and memory, as has been shown in studies of long-term potentiation and conditioning (Fadda and Rossetti, 1998). In addition, alcohol significantly reduces glutamate release in many brain areas, including the hippocampus, as measured by microdialysis. Reduced glutamate release in the hippocampus is correlated with deficits in spatial memory. The combination of temporary inhibition of NMDA receptors by alcohol and reduced glutamate release may produce the amnesia that occurs for events that take place during intoxication (i.e., the blackouts so typical of heavy drinking; Diamond and Gordon, 1997).

In the adult brain, repeated use of alcohol leads to a neuroadaptive *increase* in the number of NMDA receptors (up-regulation) in response to reduced glutamate activity. The number of NMDA receptors in both the cerebral cortex and hippocampus is elevated in human alcohol abusers, as well as in animal models of chronic alcohol exposure. In addition, in dependent rats, glutamate release, normally inhibited by alcohol, is dramatically increased at about 10 hours after withdrawal of alcohol. The time course (**FIGURE 10.16**) of CNS hyperexcitability and the seizures that are typical of the alcohol abstinence syndrome matches the pattern of increased glutamate release during withdrawal. Further, there is a strong positive correlation between the magnitude of glutamate output during withdrawal and the intensity of abstinence signs. This means that the increased glutamate acting on up-regulated NMDA receptors may be one neurochemical correlate of alcohol withdrawal. Additionally, the elevated glutamate activity during withdrawal causes excessive calcium influx, which contributes to cell death. Frequently experienced withdrawal may be responsible for some of the irreversible brain damage described earlier.

Additionally, studies with rats show that maternal BACs as low as 0.04% during the last trimester of pregnancy can impair NMDA receptors and decrease glutamate release in the newborn. Unlike in the mature brain, inhibition of glutamate systems in the fetus may disrupt normal brain development, resulting in *reduced* NMDA receptors in the adult. It is reasonable to suspect

that a reduction in NMDA receptors is related to subtle impairments in learning and memory in children born to mothers with alcohol use disorder, but further investigation is required.

GABA GABA (γ-aminobutyric acid) is a major inhibitory amino acid neurotransmitter described in Chapter 8. It binds to the $GABA_A$ receptor complex and opens the chloride (Cl^-) channel, allowing Cl^- to enter the cell to hyperpolarize the membrane. Many classic sedative–hypnotic drugs (see Chapter 17) such as the benzodiazepines (e.g., Valium) and the barbiturates (e.g., phenobarbital) are known to enhance the effects of GABA at the $GABA_A$ receptor by binding to their modulatory sites on the receptor complex. Since the drugs in this class and alcohol produce many of the same actions and show both cross-tolerance and cross dependence, it is not surprising to find that alcohol also modulates GABA function, both directly via $GABA_A$ receptors and indirectly by stimulating GABA release.

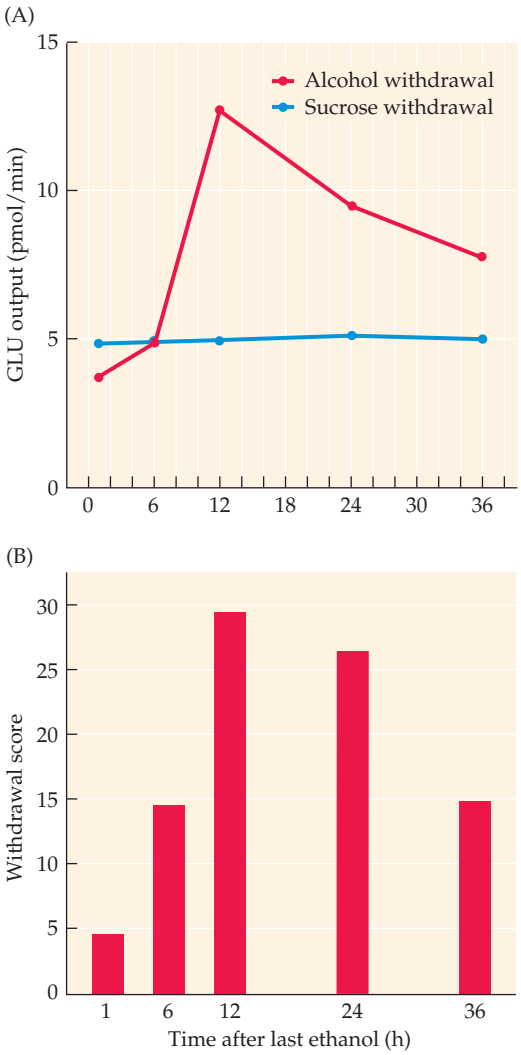


FIGURE 10.16 Relationship between alcohol withdrawal and glutamate release Withdrawal from chronic alcohol in dependent rats increases glutamate (GLU) release in the striatum (A) and behavioral rebound withdrawal hyperexcitability (B). The time course of the two withdrawal-related events is very similar. Experimental animals received intragastric delivery of ethanol at intoxicating concentrations (2–5 g/kg) every 6 hours for 6 consecutive days. Control animals received an equally caloric sucrose solution. On day 7, ethanol administration was terminated, and behavioral testing of abstinence signs and simultaneous microdialysis collection began. (After Rossetti and Carboni, 1995.)

What kind of biochemical and electrophysiological evidence suggests that alcohol increases GABA-induced Cl^- flux and hyperpolarization? First, picrotoxin (which blocks the Cl^- channel) and bicuculline (which competes with GABA for its receptor) antagonize both the hyperpolarization and some of the behavioral effects of alcohol. This suggests that both Cl^- conductance and GABA binding to the receptor are necessary for the effects of alcohol to occur. Second, manipulations that increase GABA (e.g., inhibiting its degradation) also increase alcohol-induced behavioral effects. Likewise, reducing GABA function with antagonists reduces signs of ethanol intoxication and its anti-anxiety effects (Grobin et al., 1998). Third, lines of mice bred for their sensitivity to some of the behavioral effects of alcohol show a relationship between the ability of ethanol to increase GABA-induced Cl^- entry and the intensity of their response to alcohol. Greater incoordination and loss of righting reflex in vivo corresponded to greater alcohol-induced Cl^- influx into the animals' brain preparations in vitro (Mihic and Harris, 1997).

Just as benzodiazepines modulate some $GABA_A$ receptors and not others, depending on the isoform of subunits of the receptor that are present (see Chapter 8), so too alcohol acts on some $GABA_A$ receptors and not others. For instance, male knockout mice lacking $GABA_A$ receptors that have an α1 or β2 subunit show less loss of the righting reflex after alcohol administration, indicating that those receptors mediate the sedative–hypnotic effects of alcohol. Furthermore, some receptors respond to the low doses of alcohol achieved by social drinking, and others modulate GABA function only at the high concentrations associated with greater intoxication. $GABA_A$ receptors that are highly sensitive to alcohol contain a δ subunit (instead of the more usual γ), along with α4 or α6 subunits and are located *extrasynaptically*. Although most $GABA_A$ receptors located synaptically respond transiently to GABA released into the synapse, extrasynaptic receptors respond in a more persistent fashion to GABA that remains in the extracellular space to produce tonic inhibition. Because they are more sensitive to the effects of alcohol, these extrasynaptic receptors are more likely to have a role in the behavioral and subjective effects produced by low or moderate amounts of drinking.

Additionally, evidence from several studies suggests that extrasynaptic GABA receptors have a role in the reinforcing effects of oral ethanol and, in that way, may contribute to the development of alcohol abuse (Nie et al., 2011; Rewal et al., 2012). Using a gene-silencing technique in adult rodents, researchers produced rats that had fewer δ- and α4-subunit-containing $GABA_A$ receptors. These animals showed a lower preference for alcohol, consumed less alcohol, and bar pressed less for access to oral alcohol. Furthermore, this effect was specific for alcohol and did not alter the animals' response to other reinforcers (sucrose solution), nor did it interfere with bar-pressing motor performance. It is significant that reducing receptors specifically in the shell of the nucleus accumbens but not in the core region altered alcohol intake, because the shell has long been associated with self-administration not only of alcohol but of other drugs of abuse (see the section below on dopamine). Additional evidence from knockout, knockin, and pharmacological studies is needed to clarify which receptor subtypes in which cellular and anatomical locations respond to high and low concentrations of alcohol to produce various ethanol-related responses.

In contrast to the acute GABA-enhancing effects of alcohol, repeated exposure to ethanol *reduces* $GABA_A$-mediated Cl^- flux. Also, chronic alcohol makes animals more sensitive to seizure-inducing doses of the GABA antagonist bicuculline. We might expect this result, since it should take less of the antagonist to reduce GABA function, because it has already been down-regulated by chronic alcohol. This neuroadaptive mechanism apparently compensates for the initial GABA-enhancing effect of alcohol and may contribute to the appearance of tolerance and some of the signs of withdrawal, such as hyperexcitability, seizures, and tremors because of reduced GABA-mediated inhibition. Benzodiazepines also lose their GABA-enhancing effects at the receptor in mice treated chronically with ethanol, and this may serve as a mechanism for cross-tolerance with other sedative–hypnotic drugs.

$GABA_B$ RECEPTORS In addition to altering $GABA_A$ receptor function, ethanol also impacts both pre- and postsynaptic metabotropic $GABA_B$ receptors. As you learned in Chapter 8, postsynaptic $GABA_B$ receptors inhibit cell firing by opening K^+ channels and additionally inhibit synthesis of the second messenger cAMP. Also, $GABA_B$ receptors may be presynaptic autoreceptors or axoaxonic heteroreceptors and reduce neurotransmitter release by reducing voltage-gated Ca^{2+} channel opening. $GABA_B$ receptors have a role in a variety of behaviors including modulation of alcohol consumption (reviewed by Agabio and Colombo, 2014). The classic $GABA_B$ agonist baclofen prevented the selection of alcohol over water in a two-bottle choice design in alcohol-preferring rats until baclofen administration was terminated, at which time alcohol consumption increased. Not only did the drug prevent the initial acquisition of alcohol drinking in the rats, but it also reduced alcohol consumption once acquisition was established, that is, in the "maintenance" phase of alcohol use, as well as in mouse models of binge drinking. Relapse alcohol consumption in response to an alcohol-associated cue after withdrawal was also reduced by acute administration, raising the possibility that baclofen may be an effective treatment for loss of control and relapse in human alcohol abusers. However, despite the strong preclinical results, there have been an insufficient number of large-scale, randomized, placebo-controlled clinical trials to draw a conclusion. Those studies completed thus far have produced conflicting results regarding the efficacy of baclofen in reducing consumption and craving or achieving abstinence. The multiple actions of baclofen on alcohol use may be due to its inhibitory effects on ventral tegmental area (VTA) cell firing and subsequent reduction of dopamine (DA) release in the nucleus accumbens. Unfortunately, the reduced reinforcing value found for alcohol in animal studies was also demonstrated for other substances, including sucrose solutions and regular food pellets.

Just as benzodiazepines enhance $GABA_A$ receptor function by binding to specific modulatory sites on the receptor complex, modulatory sites for $GABA_B$ function also exist. Agonists at those sites, called $GABA_B$ PAMS (positive allosteric modulators), have no agonist effect alone but enhance the effectiveness of GABA binding at $GABA_B$ receptors. Since these agents have effects similar to baclofen on alcohol consumption with fewer adverse effects such as sedation and motor incoordination, they represent an alternative therapeutic approach to treating alcohol use disorder.

It seems appropriate to once again mention that neuronal excitability is caused by a balance between excitation and inhibition. Since GABAergic neurons frequently have glutamate receptors and GABA frequently modulates glutamate release, the compensatory changes in each of the neurotransmitter systems may reflect an interaction of the two (Fadda and Rossetti, 1998).

DOPAMINE Evidence from biochemical, electrophysiological, and behavioral studies suggests that the dopaminergic mesolimbic system plays a significant role in the reinforcement and motivational mechanisms underlying behaviors that are vital to survival (see Chapter 5). The pathway begins in the VTA of the midbrain and courses rostrally to innervate various limbic structures, including the nucleus accumbens (NAcc) and the central nucleus of the amygdala (**FIGURE 10.17**). The NAcc is particularly important because part of it (the shell) belongs to a network of structures called the extended amygdala, which is involved in integrating

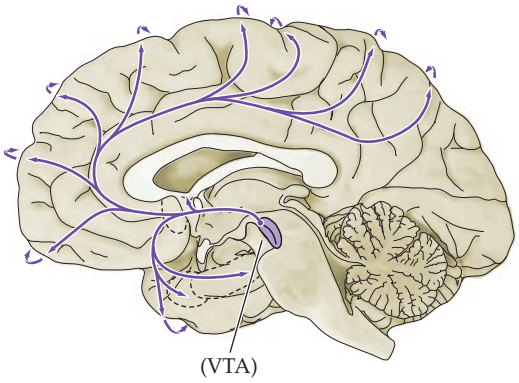
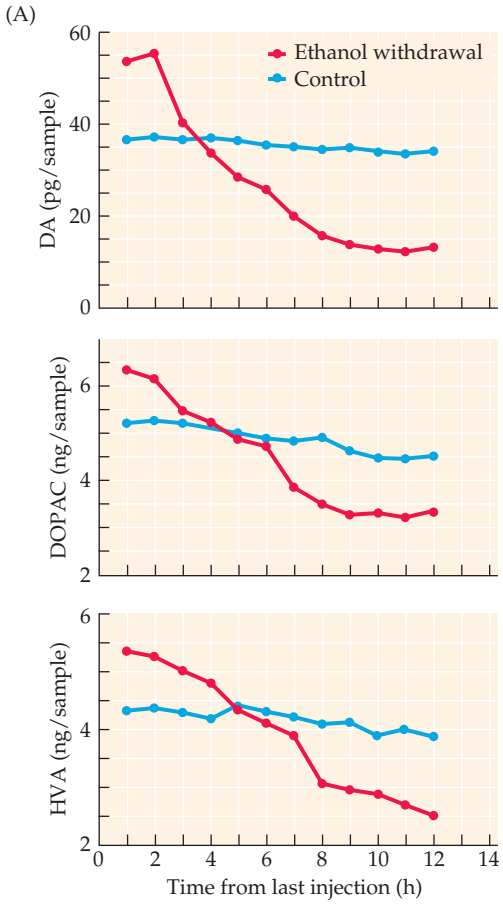


FIGURE 10.17 Dopaminergic mesolimbic and mesocortical pathways Midsagittal view of dopaminergic neurons involved in alcohol reinforcement and withdrawal-induced dysphoria. The ventral tegmental area (VTA) is the origin of neurons that innervate the nucleus accumbens, amygdala, hippocampus (mesolimbic), and the cortex (mesocortical).

emotion with hormonal responses and sympathetic nervous system activity. The core of the NAcc is associated with the striatum, which modulates movement and establishes habitual behavior. Afferents to and efferents from the NAcc provide a means for combining motivational status, emotional content, and motor responses (DiChiara, 1997). The role of the NAcc in substance abuse seems to consist of supplying the primary reinforcing qualities that lead to repeated drug use and the incentive or motivation for the drug, which helps to explain why alcohol is so addictive for some people.

Increased dopaminergic transmission in the mesolimbic pathway occurs in response to administration of most drugs of abuse, including alcohol. In rats, alcohol consumption at intoxicating concentrations increases the firing rate of dopaminergic neurons in the VTA and subsequently elevates the amount of DA released in the NAcc, as measured by microdialysis, although the release is greater in genetically selected alcohol-preferring rats than in nonpreferring animals. Rats in operant chambers self-administer ethanol directly into the VTA, which presumably activates those neurons (probably by inhibiting inhibitory cells). Microinjection of dopamine receptor antagonists into the NAcc reduces (but does not abolish) the self-administration of ethanol in rats, as you would expect because alcohol-induced DA release is ineffective when postsynaptic receptors are blocked.

Because alcohol consumption clearly increases mesolimbic cell firing, we might ask how alcohol withdrawal affects the same reward pathway. In contrast to the acute effects, when animals have been allowed to consume alcohol in a chronic fashion, withdrawal of the drug dramatically reduces the firing rate of mesolimbic neurons and decreases DA release in the NAcc (Diana et al., 1993). **FIGURE 10.18** shows the decrease in synaptic DA in the NAcc, as well as the reduction



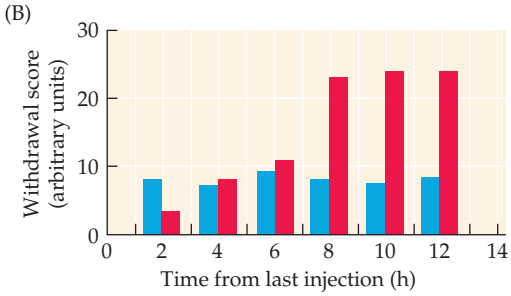


FIGURE 10.18 Dopamine turnover and alcohol withdrawal (A) Microdialysis collection of DA and its major metabolites, DOPAC and HVA, in the NAcc of rats at varying times after ethanol withdrawal shows significant reduction in DA turnover compared with control. (B) Development of abstinence signs in the same animals. Note the similarity in time course of biochemical measures and the occurrence of behavioral signs. The alcohol group received intoxicating doses of alcohol every 6 hours for 6 days before withdrawal. Control animals received sucrose of caloric value equal to the alcohol. The microdialysis collection of extracellular fluid and the behavioral measures of abstinence occurred at various times over the 12-hour period following ethanol withdrawal. Withdrawal scores were a composite of separate measures of tremor, vocalization on handling, bracing posture, rigidity, and others. (After Diana et al., 1993.)

in the DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), as measured by microdialysis at various times after the last injection of ethanol. This decrease began at approximately 5 hours after the last alcohol administration and reached a maximum at around 10 to 12 hours. The same animals showed behavioral signs of withdrawal that began at 8 to 10 hours and continued throughout the testing period. One might conclude that the behavioral signs, the reduced DA outflow, and the reduced mesolimbic cell firing, all of which were reversed by a single administration of alcohol, may represent the neurobiological correlate of the dysphoria associated with the alcohol withdrawal syndrome.

The reduction in mesolimbic DA is also reflected in a rebound depression of reinforcement mechanisms, as is shown by elevation of the threshold for intracranial stimulation. Elevated thresholds mean that more electrical current is needed to activate the reinforcing pathway (i.e., reinforcement is less rewarding than it was during the initial alcohol use). **FIGURE 10.19** shows that elevation of thresholds reached a maximum 6 to 8 hours after abrupt withdrawal and disappeared by 72 hours. This down-regulation of the neuronal system that mediates reward may be the neuroadaptive mechanism that is responsible for the negative emotional signs (dysphoria and depression) characteristic of withdrawal from many abused substances, including psychostimulants and opioids, as well as alcohol (Schulteis et al., 1995).

Although release of DA within the NAcc is clearly instrumental in ethanol-induced reinforcement, VTA cell firing during previously established, compulsive alcohol self-administration may have very different behavioral effects depending on the frequency of cell firing. This was elegantly demonstrated by Bass and colleagues (2013) using optogenetic technology. Using viral-mediated gene delivery to selectively express channelrhodopsin-2 (ChR2) on dopaminergic cells in the VTA (see Chapter 4 for a description of optogenetics), they stimulated the VTA neurons with pulses of blue light during binge-type alcohol drinking periods. Optical stimulation of the VTA produced corresponding time-locked release of DA in the NAcc as measured with voltammetry. When stimulation was of high frequency (50 Hz) and delivered in a phasic fashion, there was a large spike of DA released at the terminal regions that was short-lived. This stimulation had no effect on the rats' compulsive drinking. In contrast, more prolonged tonic stimulation at lower frequency (5 Hz) produced less DA efflux that was more prolonged. This tonic VTA activation produced a significant decrease in the number of licks on the alcohol drinking tube and amount of alcohol consumed, without having an effect on water consumption. It is well known that different populations of VTA neurons have low-frequency tonic and high-frequency phasic firing properties that serve different functions and modulate behavior in distinct ways. While phasic firing is associated with motivating goal-directed behaviors, the tonic firing that produces more prolonged synaptic DA may reduce phasic DA release by acting on presynaptic autoreceptors. The researchers conclude that such a negative feedback mechanism would reduce the salience of the contextual cues associated with the drinking chamber and reduce the binge-drinking behavior.

Dopamine is clearly important in the reinforcing effects of alcohol, but alcohol may increase mesolimbic firing indirectly secondary to its modulation of other neurotransmitter actions in the VTA, such as those produced by GABA, acetylcholine, serotonin, or endorphins. Despite the apparent importance of the dopaminergic neurons, almost total destruction of the mesolimbic terminals with 6-hydroxydopamine (6-OHDA) does not abolish self-administration of alcohol, suggesting that other dopamine-independent mechanisms contribute to ethanol reinforcement. One such mechanism may be the opioid peptide transmitters.

OPIOID SYSTEMS A family of neuropeptides (called endorphins) that have opiate-like effects modulate pain, mood, feeding, reinforcement, and response to stress, among other things (see Chapter 11). Opioids also contribute to the reinforcing effects of alcohol. Support for this statement comes from three types of studies (Froehlich,

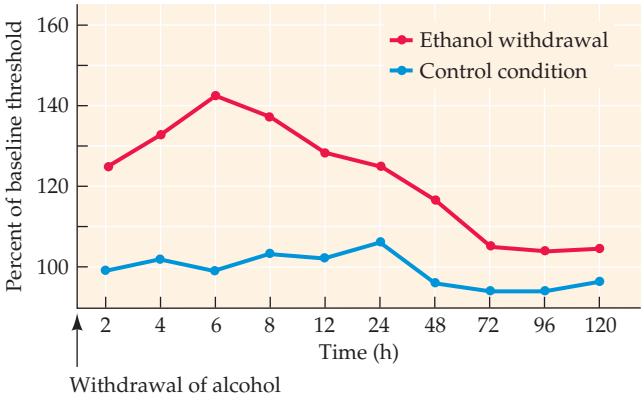


FIGURE 10.19 Rebound depression of reinforcement during withdrawal After alcohol withdrawal from dependent rats, a time-dependent increase in the current thresholds for electrical brain stimulation occurs, with maximum effect at 6 to 8 hours. The increase in threshold indicates that the animals need more stimulation to achieve reinforcement. Note that the *x*-axis is not a linear scale. (After Schulteis et al., 1995.)

1997). First, alcohol enhances endogenous opioid activity in both rodents and humans. Acute administration of alcohol increases endogenous opioid (endorphin and enkephalin) release from brain slices and the pituitary gland in vitro and also increases blood levels of opioids in humans in vivo. Opioids are released into the blood from the pituitary gland. Acute alcohol administration also increases gene expression of both endorphin and enkephalin in selected brain areas of rats, which would increase the amount of peptides available. In contrast, chronic alcohol administration reduces gene expression, making less of the peptides available for release. In humans, chronic alcohol use leads to reduced brain levels of endorphin. Since the release of DA in mesolimbic neurons is regulated by opioid cells in both the VTA and NAcc, alcohol-induced opioid release may produce reinforcement by modulating dopamine (Herz, 1997). Reduced opioid levels may contribute to the dysphoria that accompanies chronic alcohol use and withdrawal.

Second, if alcohol-induced enhancement of opioid systems is at least partially responsible for reinforcement, then blocking opioid receptors should reduce alcohol consumption. Opioid receptor antagonists like naloxone and naltrexone, which compete for the endogenous opioid receptors, do significantly reduce alcohol self-administration in animals. Antagonists that act on specific subtypes (μ and δ) of opioid receptors (see Chapter 11) also reduce operant self-administration. Several clinical trials of opioid antagonists in patients with alcohol use disorder found reduced alcohol consumption, relapse, and craving, as well as a reported decrease in the subjective "high." (Further discussion can be found in the section on treatment of alcohol use disorder later in this chapter.) Finally, μ-opioid receptor knockout mice fail to self-administer ethanol and in some experimental conditions show an aversion to the drug (Roberts et al., 2000).

Third, if opioids have a role in reinforcement, then perhaps we should expect to see a difference in the opioid systems of genetic strains of animals that show greater or lesser preference for alcohol. In rat strains that have been genetically bred for alcohol preference, endogenous opioid systems are generally more responsive to the effects of alcohol. For example, alcohol-preferring rats, when compared with alcohol-nonpreferring rats, released significantly more β-endorphin from the hypothalamus when infused with alcohol in vitro and showed enhanced β-endorphin gene expression in the pituitary. Additionally, alcohol-preferring rats have higher baseline levels of μ-opioid receptors in selected limbic areas, including the shell of the NAcc and the amygdala.

In addition to having a role in reinforcement, evidence suggests that opioids play a part in alcohol craving. In individuals with alcohol use disorder, positron emission tomography (PET) imaging was used to determine the number of μ-opioid receptors in the NAcc and in other brain areas associated with reinforcement and craving following several weeks of abstinence. Abstinence increased the μ-opioid receptors found in these individuals, and the number of receptors was positively correlated with scores on the Obsessive-Compulsive Drinking Scale developed by Raymond F. Anton, indicating that as μ-opioid receptor numbers increased, craving scores also increased (Heinz et al., 2005). A similar correlation of receptor number in the frontal cortex with craving scores suggested to researchers that activation of opioid receptors may impair executive control, leading to an increased probability of relapse. These results may explain individual differences among alcohol abusers in their ability to abstain from alcohol use.

OTHER NEUROTRANSMITTERS **TABLE 10.3** summarizes some of the cellular effects of alcohol and their contributions to behavior. Both laboratory animal and human studies implicate many more interacting neurotransmitter systems in the action of alcohol than we have had the space to discuss. The effects of acute and chronic alcohol administration on these receptors are responsible for the changes in cell signaling, both rapid ionotropic and slower metabotropic actions such as those regulated by second messengers. Second messengers such as cAMP and their cascade of effects within the cell that ultimately lead to phosphorylation of the transcription factor CREB (pCREB) may be responsible for more long-term changes in cell function, including altered gene expression. Those changes may represent the transition from casual drinking to dependence to compulsive alcohol use. Among the target genes for CREB are those for neuropeptide Y and brain-derived neurotrophic factor (BDNF), both implicated in long-term memory, neuroplasticity associated with alcohol-drinking, and anxiety-like behaviors. The effects of alcohol on adenylyl cyclase–cAMP–PKA activity depend upon the nature of alcohol status (acute, chronic, withdrawal) as well as the brain region (PFC, hippocampus, amygdala) investigated. In the cortex and hippocampus, acute alcohol enhances adenylyl cyclase–cAMP–PKA activity, while more prolonged exposure to alcohol reduces it, possibly by inducing the expression of a protein kinase inhibitory factor. A weakened control of the amygdala by PFC has been suggested as a preliminary step in the neuroadaptation underlying alcohol dependence and may be responsible in part for the enhanced anxiety at acute alcohol withdrawal. In the central and medial nuclei of the amygdala, acute alcohol withdrawal reduces pCREB and subsequent gene expression. In alcohol-preferring rats this reduction is associated with heightened anxiety and excessive alcohol consumption. These types of studies suggest that drugs that enhance the cAMP cascade, such as rolipram, a drug that inhibits the breakdown of cAMP, may be therapeutically useful in treating alcohol use disorders.

TABLE **10.3** Role of Selected Neurotransmitters in Cellular and Behavioral Effects of Alcohol

Neurotransmitter	Acute cellular effects	Chronic cellular effects	Behavioral effects
Glutamate	Receptor antagonism and reduced glutamate release	——	Memory loss
	——	Up-regulation of receptors and rebound increase in glutamate release	Rebound hyperexcitability of the abstinence syndrome
	——	Extreme hyperexcitability and massive Ca^{2+} influx (rebound)	Brain damage
GABA	Increase in GABA-induced Cl^- influx to hyperpolarize	——	Sedative effects: anxiety reduction, sedation, incoordination, memory impairment
	——	Neuroadaptive decrease in GABA function without change in receptor number	Tolerance and signs of hyperexcitability during withdrawal (seizures, tremors)
Dopamine	Increase in dopamine transmission in mesolimbic tract	——	Reinforcement
	——	Reduced firing rate, release, metabolism	Negative affect as a sign of withdrawal
Opioids	Increase in endogenous opioid synthesis and release	——	Reinforcement
	——	Neuroadaptive decrease in endorphin levels	Dysphoria

An excellent discussion of the role of this intracellular signaling pathway in alcohol drinking can be found in Mons and Beracochea (2016). Further discussion of the neurochemical effects of alcohol is beyond the scope of this chapter, but details can be found in several reviews (Sprow and Thiele, 2012; Most et al., 2014).

Section Summary

- Ethanol has specific actions on multiple neurotransmitter systems but also has nonspecific actions that change the fluid nature of membrane phospholipids.
- Animal models in alcohol research do the following: (1) control for poor nutrition, liver damage, comorbid drug use, and psychiatric disorders associated with human alcohol abusers; (2) permit the use of techniques inappropriate in humans; (3) allow genetic manipulations; and (4) provide means for investigators to evaluate treatment strategies.
- Rodent models exist for acquisition and maintenance of drinking, physical dependence, relapse (alcohol seeking), compulsive drinking, and binge-type drinking.
- Inbred strains of alcohol-preferring and high-alcohol-drinking rats provide the means to evaluate behavior, neural mechanisms, and genetics of alcohol use disorders.
- Alcohol acutely inhibits glutamate neurotransmission by reducing the effects of glutamate at the NMDA receptor and reducing glutamate release.
- Modulation of glutamate by alcohol has a role in ethanol-induced memory impairment, rebound hyperexcitability during withdrawal, NMDA-mediated excitotoxicity causing brain damage, and the intellectual disability associated with FAS.
- Chronic ethanol up-regulates NMDA receptors in humans and animal models and increases glutamate release, providing an explanation for the hyperexcitability and seizures seen at abrupt withdrawal.
- Alcohol enhances GABA-induced chloride entry and hyperpolarization by modulating $GABA_A$ receptors and stimulating GABA release.
- The effect of alcohol on synaptic $GABA_A$ receptors requires high concentrations that are associated with high levels of intoxication. Extrasynaptic $GABA_A$ receptors having δ and $\alpha 4$ or $\alpha 6$ subunits are extremely sensitive to the low concentrations of GABA that remain in the extracellular space, making them more likely to be mediators of low to moderate amounts of drinking.

- Extrasynaptic $GABA_A$ receptors in the shell region of the nucleus accumbens seem to mediate some of the reinforcing effects of alcohol.
- Chronic ethanol leads to down-regulation of $GABA_A$ receptors, making the organism more sensitive to seizure-inducing agents.
- Alcohol acts on both pre- and postsynaptic metabotropic $GABA_B$ receptors and modulates alcohol consumption.
- Ethanol activates dopaminergic cells in the VTA, causing the release of DA in the NAcc to provide the positive reinforcement that leads to repeated drug taking.
- In physically dependent rodents, withdrawal of alcohol reduces the firing rate of mesolimbic neurons, decreases DA release in the NAcc, and causes rebound depression of reinforcement mechanisms as shown by an elevation in the threshold for intracranial self-stimulation.
- Low-frequency tonic stimulation of VTA cells, in contrast to high-frequency phasic stimulation, has produced prolonged low DA efflux and has inhibited compulsive drinking in rats.
- Acute alcohol increases opioid release and increases gene expression of opioid peptides. Chronic alcohol reduces gene expression and lowers levels of peptides.
- Blocking opioid receptors with naloxone reduces alcohol self-administration. Mu opioid receptor knockout mice fail to self-administer ethanol. High levels of μ-opioid receptors correlate with scores on craving.
- Alcohol-preferring rats release more opioids in response to ethanol and show enhanced opioid peptide gene expression.
- Ethanol modulates the function of many additional neurotransmitters to alter ionotropic and metabotropic signaling.

Alcohol Use Disorder (AUD)

AUD is a serious and complex phenomenon that consists of psychological, neurobiological, genetic, and sociocultural factors, making it both difficult to define and treat. According to the latest *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-5*) the term *alcoholism* has been replaced with *alcohol use disorder*. You can find the specific criteria for alcohol use disorder according to the *DSM-5* in Chapter 9. Regardless of the specific definition, AUD damages the health and well-being of the individual and those around him. Financial costs of the disorder are huge and include medical treatment of alcohol-induced disease, loss associated with accidents on the road and at work, lost productivity, criminal justice costs, and financial disruption that accompanies the breakdown of families (**TABLE 10.4**). Many volumes have been written about alcohol use and AUD from various points of view—some emotionally evocative, others based on theoretical models or empirical research. The final section of this chapter presents a brief synopsis of some of the research findings.

Defining alcohol use disorder and estimating its incidence have proved difficult

Chapter 9 describes substance use disorder and identifies some of the criteria used by professionals to diagnose the condition. In addition, the chapter provides multiple theoretical models of substance abuse and provides a critique of each. In this section, we look specifically at **alcohol use disorder**, a form of substance abuse that has been historically difficult to define because the drug is legal and is used by most individuals in a way that does not harm themselves or others. For the layperson, someone with an "alcohol problem" may be anyone who drinks more than he or she does. It is important to realize that not everyone who drinks alcohol consumes excessive amounts, and not all heavy drinkers develop dependence. However, it is difficult to objectively define inappropriate amounts of alcohol because the frequency and pattern of use are as significant as the total amount consumed. For example, consuming five alcoholic drinks over 1 week's time does not have the same physical and social consequences as consuming five drinks in a row for men, or four for

TABLE **10.4** Estimated Costs of Excessive Drinking by Type of Cost[a]

Cost category	Total cost
Specialty care for alcohol abuse treatment	$10,668
Hospitalization attributed to alcohol-related illness	$5,156
Medical care for fetal alcohol syndrome	$2,538
Special education for fetal alcohol syndrome	$369
Decreased productivity at work	$74,102
Lost productivity due to absenteeism	$4,237
Lost productivity due to incarceration	$6,329
Lost productivity due to premature death	$65,062
Motor vehicle crashes	$13,718

Source: After Bouchery et al., 2011.
[a]Costs are in millions of dollars for the year 2006 in the United States.

women, which is the usual definition of **binge drinking**. An individual with AUD does not necessarily have to start each day with a drink, nor does he necessarily drink all day long, but he may drink very heavily periodically. Instead of emphasizing quantity, the diagnosis of AUD depends on identifying a cluster of behavioral, cognitive, and physical characteristics. For the clinician, the essential features of AUD are compulsive alcohol seeking and use despite damaging health and social consequences. Unfortunately, because other groups and government agencies use different criteria, there is still a great deal of variability in how professionals use the terms *addiction*, *misuse*, *abuse*, *dependency*, and *problem drinking*.

Part of the difficulty in defining AUD is the tremendous heterogeneity among those individuals abusing alcohol. A clear classification scheme of alcohol-abusing individuals is needed for studying neurobiological etiology and genetic analysis, predicting the course of the disorder and optimal treatment approach, and developing prevention strategies. The interest in identifying subtypes of alcohol abusers was initiated by Cloninger (1987), who proposed a popular categorization of alcohol abusers into type I and type II alcoholics. These categories were based on differences in alcohol-induced defining characteristics, such as inability to control drinking and age of onset; personality characteristics such as behavioral inhibition and novelty seeking; and etiology including family history of AUD and environmental influence. Type I alcoholics generally begin drinking later in life and experience guilt and fear about their AUD. These individuals rarely have trouble with the law or display antisocial activities. Many drink to escape stress or unpleasant situations in their environment. Most females with AUD are type I, although many men also fit this description. Type II alcoholics are almost always male and display thrill-seeking, antisocial, and perhaps criminal activities. They have lower cerebrospinal fluid levels of the serotonin (5-HT) metabolite 5-hydroxyindoleacetic acid (5-HIAA)—a result that matches the human and animal literature regarding impulsivity, aggression, and suicide. Type II drinkers have greater genetic vulnerability and begin drinking at an early age.

Since Cloninger's early attempt at categorization, a variety of multidimensional typologies have been developed that include larger sample sizes and improved sample selection methods, though they have many similarities to the original. Babor and coworkers (1992) used a biopsychosocial approach because the variability among those with AUD is likely due to an interaction of those multiple factors. Their clustering technique looked for a pattern of naturally occurring common features (derived from 17 severity-related dimensions) to increase homogeneity within the types. Using that technique, they identified two types, A and B. The characteristics for types A and B are shown in **TABLE 10.5**. There have been a number of subsequent efforts at typology, with some researchers identifying as many as five types with a large number of defining characteristics. These more complex and specific categorizations are important for neurobiological and genetic research but may prove unwieldy in the clinic because of the increased time needed for assessment of patients. Also, the individuals within the types are rarely followed up longitudinally, so clinicians do not have data regarding the expected course of illness or response to treatment. Finally, most previous typologies used individuals from treatment-seeking samples, and they are likely to be quite different from alcohol abusers in the general population. To rectify some of these problems, Tam et al. (2014) used in-person interviews of over 43,000 nationally representative alcohol-dependent individuals (both men and women) over the age of 18. Over 34,000 of the individuals were reinterviewed 3 years later to evaluate their status at that time. Tam and colleagues found that the type A/B typology was validated in the general population and the distribution of type A and type B was similar to that found in earlier studies. Approximately one-third of the individuals resembled Babor's more severe type B drinkers, including 31% of dependent men and 27% of dependent women. Because the sample size was so large, it could be determined that the incidence of type B drinkers was especially high among Native Americans and high but slightly less so in U.S.-born Hispanics. The 3-year follow-up data showed that without treatment, type B drinkers were likely to remain alcohol-dependent and had more severe alcohol and psychiatric problems than those who sought treatment. Although they were heavy drinkers who were dependent on drugs other than alcohol, those individuals who sought treatment showed

TABLE **10.5** Babor's Type A/B AUD

Characteristics	Type A	Type B
Age of onset	Late	Early
Childhood risk factors	Fewer	More
Severity of dependence	Less	Greater
Alcohol-related physical consequences	Fewer	More
Alcohol-related social consequences	Fewer	More
Previous treatment history for alcohol problems	Fewer	More
Psychopathological dysfunction	Less	Greater
Life stress	Less	More
Polydrug use	Less	More
Distress in work and family	Less	More

lower incidence of alcohol dependence, suggesting that treatment can effectively modify alcohol dependence in type B drinkers. These results have practical application because clinicians can potentially screen patients for vulnerability to type B dependence and initiate appropriate intervention.

Disparities in the definition of AUD have led to equally large differences in estimated incidence. Although you may think of an individual with alcohol use disorder as a poor, aging, homeless individual living on the street, in reality there is no such thing as typical (**FIGURE 10.20**). Although many homeless individuals do suffer from a variety of psychiatric disorders and abuse both illicit drugs and alcohol at high rates, only 5% of alcohol-dependent individuals fit that category. Based on the U.S. Government's annual survey of drug use, the rates of alcohol use in the United States have remained relatively steady for the last 10 years although other major public health surveys suggest a steady increase in both alcohol use and abuse. The most commonly accepted statistic is that approximately 10% of Americans have some problem with alcohol use, and as many as 15 million adults are dependent on alcohol (Substance Abuse and Mental Health Services Administration, 2016). Significant gender differences are seen in alcohol use and abuse for all age groups. The 2011 National Survey on Drug Use and Health found that more men (91.2%) than women (83.7%) consumed alcohol to some extent over their lifetimes. Among drinkers age 18 and older, heavy drinking, defined as five or more drinks on the same occasion on each of 5 days in the past month, was reported in 10.2% of males and 3.4% of females. Binge alcohol use during the past month was also higher in men (32.5%) than women (16.7%). Although the percentage of men declined on most drinking measures during their 30s, women had higher rates on some drinking indices between the ages of 30 and 40. However, by age 60, indicators of problem drinking fell into the single digits for both genders (Substance Abuse and Mental Health Services Administration, 2011). Although problem drinking drops significantly in the elderly, problems associated with drinking can be more serious because of drug interactions and medical complications.

A closer focus on the drinking patterns of college students might be appropriate, since there has been a great deal of public concern regarding student accidents and fatalities where alcohol was involved. Several large-scale studies are available to guide you in further research (Hingson and Zha, 2009; Hingson et al., 2009; White et al., 2013). A variety of U.S. Government surveys have estimated that 44.7% of 18-to-24-year-old college students engaged in binge drinking at least once in the last month. That behavior has led to a variety of serious outcomes for many. An estimated 15% of full-time 4-year college students suffered alcohol-related injury, and 12% were assaulted by other drinking students. Furthermore, alcohol overdose deaths remain a significant problem. Each year more than 5000 deaths in that age group have been attributed to alcohol use. Although this group reported a significant number of alcohol-related problems (**TABLE 10.6**), only 1% believed that they had a drinking problem.

Significant variation in estimates of alcohol-induced problems occurred across campuses, and these differences were reflected in the impact of binge drinking on the welfare of other students. Students who were not heavy drinkers on campuses that had the highest rates of heavy drinkers reported significantly more negative experiences associated with other students' drinking, including vandalism, violence, theft, and unwanted sexual advances.

Underage drinking has become the focus of a major initiative by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) and several other governmental agencies

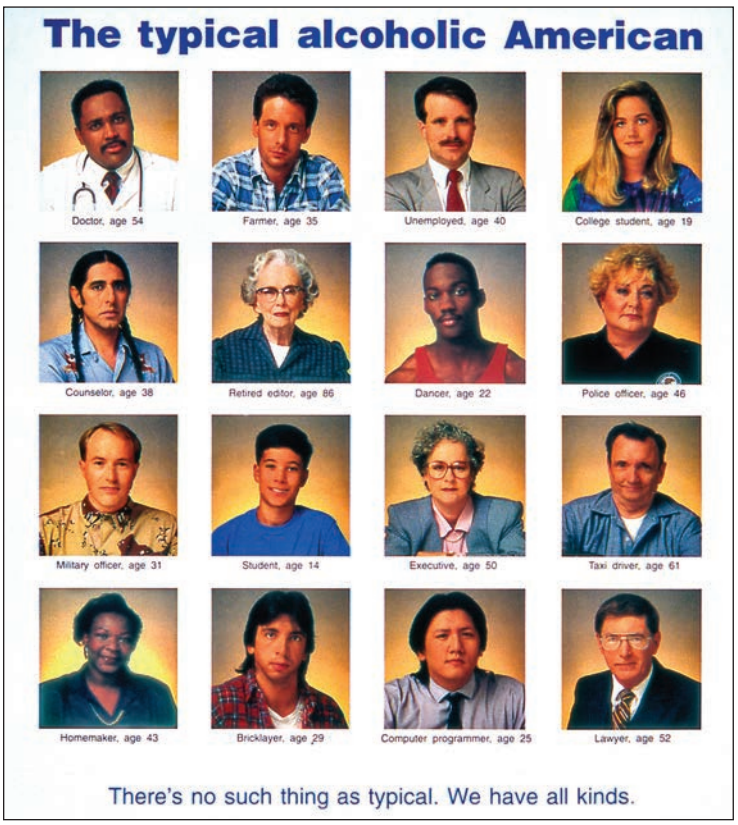


FIGURE 10.20 A wide variety of individuals suffer from alcohol use disorder About 15.7 million Americans have AUD, making it our largest drug problem. (Courtesy of the National Library of Medicine.)

TABLE 10.6 Percentage of Binge Drinkers Reporting Alcohol-Related Problems since the Beginning of the School Year by Gender[a]

	Percentage[b]	
Alcohol-related problem	**Women**	**Men**
General disorientation		
Have a hangover	81%	82%
Do something you later regret	48%	50%
Forget what you did	38%	41%
Sexual activity		
Engage in unplanned sex	26%	33%
Not use protection before sex	15%	16%
Violence		
Argue with friends	29%	32%
Damage property	6%	24%
Disciplinary action		
Have trouble with campus/local police	4%	10%
Personal injury		
Get injured	14%	17%
Get medical treatment for overdose	<1%	1%
School performance		
Miss a class	42%	45%
Get behind in schoolwork	31%	34%

Source: Wechsler et al., 1995a.
[a]Women binge drinkers report having four or more drinks in a row at least once during the past 2 weeks. Men binge drinkers report having five or more drinks in a row.
[b]Percentage of binge drinkers who report that, since the beginning of the school year, their drinking has caused them to experience each problem one or more times.

aimed at developing effective prevention and intervention programs. The focus is on this age group because according to the CDC, 16% of all the alcohol consumed in the United States is consumed by individuals between 12 and 20 years of age, much of it (90%) during binge drinking. Numerous analyses of national surveys show that the younger the individuals are when they first begin to drink (less than 13 years old), the greater is the probability that they will develop AUD later in life. This association occurs even for low levels of alcohol consumption but is exaggerated by binge drinking. Additionally, early drinking predicts other risky behaviors, including driving after drinking, riding with drinking drivers, becoming involved in physical fights, carrying weapons, and unintentionally injuring another person (Research Society on Alcoholism, 2015). Impulse control problems such as compulsive gambling and antisocial behaviors are also predicted by early adolescent drinking. Additionally, adolescents are more vulnerable to alcohol-induced neurological deficits because neurodevelopmental events occurring at that time of life include synaptic pruning, changes in receptor number and sensitivity, and the refinement of neural circuits. A disruption of neurodevelopment is suggested by animal research showing that immature neuronal function and behavior *persist* in the adult animals that were exposed to alcohol as adolescents. Among the altered behaviors is an increase in social anxiety in the adults, as demonstrated by a reduction in social investigation and play with a novel partner. Fear conditioning and spatial learning that are dependent on the hippocampus are also impaired in adulthood by adolescent, but not adult, administration of alcohol. This deficit has been associated with impairments in neurogenesis in the hippocampus (Spear, 2014) as well as changes in hippocampal dendritic morphology. These morphological changes include an increase in immature dendritic spines and corresponding decrease in mature spines, reduction in postsynaptic proteins that are associated with neurodevelopmental disorders, and alterations in memory-related synaptic plasticity (Risher et al., 2015). Such neurodevelopmental pathology may be responsible for promoting the retention of adolescent-like phenotypes such as impulsivity, cognitive deficits, and reduced sensitivity to alcohol leading to increased drinking.

The causes of alcohol use disorder are multimodal

No specific cause of AUD has been identified, but a variety of factors contribute to the vulnerability of any given individual. It is quite clear that multiple factors stem from three essential areas: (1) psychological, (2) neurobiological, and (3) sociocultural (**FIGURE 10.21**). Although we are forced to discuss them in a linear fashion, keep in mind that complex interactions exist among the areas. Because the literature is extensive, this section will provide an overview and suggest methods for future research.

PSYCHOLOGICAL FACTORS Although no alcoholic personality has ever been defined, one vulnerability factor for AUD is the response to stress. Both animal and human studies show an interaction between both acute and chronic stress and initiation of alcohol use,

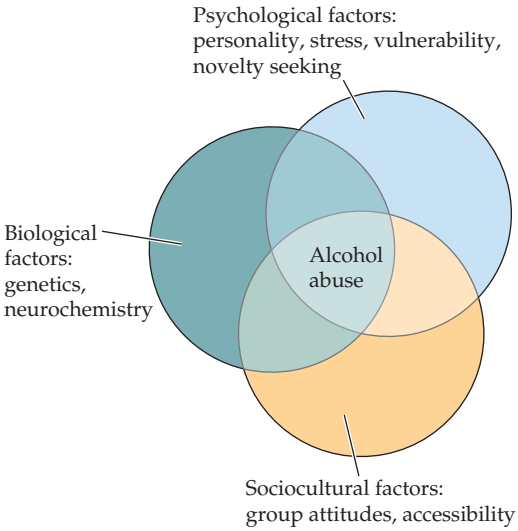


FIGURE 10.21 Three-factor vulnerability model
Biological, psychological, and sociocultural influences contribute to the development of alcohol abuse.

maintenance of drug consumption, and relapse after withdrawal (Brady and Sonne, 1999). Some have used the term *symptomatic* drinking to describe the reinforcing effects of alcohol when stress and tension are relieved. The individual who no longer drinks on a social occasion but drinks each day after work to relieve tension represents an example of symptomatic drinking. However, although alcohol has been found to reduce anxiety in some cases, the relationship between stress and alcohol use is complex and depends on multiple variables, including the nature and timing of the stressor. Animal studies show that the effects of stress on voluntary alcohol consumption depend on the nature of the stressor applied (e.g., restraint, forced swimming, social isolation), whether it is acute or chronic, and whether the animal can predict the onset of the stress and/or control the stressful event. Furthermore, a circular association is seen because although alcohol can be shown to relieve stress under some conditions, alcohol also increases the function of brain and endocrine stress systems, making alcohol itself an additional stressor that may lead to further alcohol use (see Box 2.4 for an introduction to the neuroendocrine stress response of the hypothalamic–pituitary–adrenal [HPA] axis). Numerous rodent studies have shown that acute alcohol administration elevates levels of the stress hormones corticotropin-releasing factor (CRF), adrenocorticotropic hormone (ACTH), and glucocorticoids (e.g., cortisol). Furthermore, withdrawal of alcohol after several weeks of chronic administration produces more anxious behavior in rodents in the elevated plus-maze following restraint stress compared with controls. The fact that the anxious behavior could be reduced by blocking CRF receptors demonstrates that CRF mediates the anxiety. Additionally, during withdrawal, CRF expression was greater in brain areas regulating anxiety, including the bed nucleus of the stria terminalis and the central nucleus of the amygdala (see Chapter 17 for a discussion of the neural substrates of anxiety), and the behavioral stress response was enhanced. Likewise, in humans, alcohol withdrawal increases anxiety as well as alcohol consumption. More important, it appears that although postwithdrawal anxiety subsides within a few weeks, sensitization to stressors and increased reactivity persists for long periods. This increased sensitivity is responsible for stress-induced craving and relapse. The fact that stressors frequently lead to further alcohol consumption suggests the potential usefulness of CRF receptor antagonists in preventing relapse.

The significance of stress to alcohol abuse is also demonstrated by the epidemiological evidence of high comorbidity of anxiety disorders and AUD. The central nucleus of the amygdala is the brain region responsible for orchestrating the various components of an emotional response to fearful or anxiety-provoking stimuli. As such, it activates the sympathetic nervous system and HPA axis and increases vigilance, among other things. Hence it should come as no surprise that the amygdala is overactive in many anxiety disorders, including PTSD, social phobia, panic disorder, and generalized anxiety disorder (see Chapter 17). Animal models of alcohol dependence and withdrawal have also shown neuroadaptations in the central nucleus of the amygdala that have been considered the basis for a stress "kindling" process, that is, an enhanced sensitivity to environmental stressors. This increased sensitivity intensifies the magnitude of the stress response to aversive environmental stimuli, as is well documented in humans attempting to abstain from alcohol use (see Gilpin et al., 2015).

Another significant risk factor for AUD is family history, which is associated with greater cortisol response following psychosocial stress compared with individuals without a family history of AUD. Furthermore, alcohol has a greater suppressing effect on the stress responses in those with a family history. Since HPA reactivity to stressors shows significant heritability, researchers are working to identify gene polymorphisms that are involved in the differences, with the hope of developing targeted drug treatment plans for selected individuals. Alcohol abuse has long been known to be associated with high levels of lifetime anxiety. Consistent with the findings of rodent studies, stress early in life is a significant environmental risk factor for alcohol abuse in the adult, just as it predicts adult depression (see Chapter 18) and anxiety disorders (see Chapter 17). However, in agreement with several other studies, Schmid and colleagues (2010) found that the number of early stressful life events could predict early onset of drinking and higher levels of alcohol consumption at age 19, but only for those individuals with a particular polymorphism of the *corticotropin-releasing factor receptor 1* (CRF_1) gene, clearly indicating

a gene × stress × alcohol interaction. The fact that gene polymorphisms of CRF_1 are associated with risky drinking behavior suggests a potential screening method for identifying future problem behaviors and perhaps indicates the optimal treatment approach for these individuals should such behaviors develop. Differences in individuals' stress response systems and in their perception and appraisal of stress may help to explain some of the differential vulnerability to alcohol abuse. These and other pivotal studies are summarized in two excellent reviews (Clarke and Schumann, 2009; Spanagel et al., 2014).

In an earlier section, we mentioned that alcohol increases DA transmission in the mesolimbic tract, thereby mediating reinforcement. It is significant that cortisol seems to sensitize the reward pathway, which means that alcohol-induced increases in the HPA axis function and subsequent elevations in cortisol make drug reinforcement more rewarding. It is intriguing to find that corticosterone (the rodent version of cortisol) itself is self-administered by laboratory animals, apparently because its binding to glucocorticoid receptors in NAcc activates a feedback loop to VTA that enhances mesolimbic cell firing. This idea is supported by rodent studies showing that glucocorticoids enhance mesolimbic DA and increase drug-seeking behaviors. A striking relationship exists: even among individuals without substance abuse problems, those who secreted more cortisol in response to stress also released more mesolimbic DA and experienced greater subjective effects of psychostimulants. It is suspected that this relationship may cause some individuals to increase consumption beyond casual use. In contrast, as was mentioned in the section on dopamine, withdrawal reduces the mesolimbic neuron firing rate, which dampens the reward function, leading to a negative affective state that acts as a chronic internal stressor. Hence, although mild stress enhances DA-mediated reward, chronic stress further reduces the reward pathway function and increases craving—a state that would make relapse to alcohol consumption more likely (Spangel et al., 2014; Uhart and Wand, 2009).

Novelty seeking by individuals may also increase the risk for using alcohol and other drugs of abuse because exposure to novel events activates the dopaminergic mesolimbic pathway in a manner similar to that seen with most abused substances (Bardo, 1996). Individual differences in the need for novelty and drug-seeking behavior are under genetic control and may explain why some individuals experiment with drugs initially and why some more readily become compulsive users. Differences in the need for stimulation have been applied to drug and alcohol prevention programs that use fast-paced, physiologically arousing, and unconventional message delivery to appeal to the target audience.

In replicating and expanding on earlier studies, Nees and colleagues (2012) found that behavior (risk taking and aversion for delayed reward), neural response in the brain reward circuitry in response to large versus small rewards, and personality (extraversion, impulsivity, sensation seeking, and novelty seeking) interacted and were predictive of early alcohol use and subsequent alcohol addiction. Personality traits represented the strongest predictor. Since genetics contributes to these phenotypic characteristics, future research may be directed toward evaluating genetic factors in early adolescent drinking models.

NEUROBIOLOGICAL FACTORS One significant neurobiological factor in AUD is genetic vulnerability. Close relatives of alcohol-dependent individuals have a three to seven times greater risk for AUD than the general population. Both twin and adoption studies show that genetics is correlated with vulnerability, particularly for the more severe type of AUD. In adoption studies, the risk of AUD is higher in children of alcohol-dependent parents even when they are adopted into nonalcoholic families. AUD concordance rates are higher in monozygotic (54%) than in dizygotic (28%) twins, demonstrating the influence of genes but leaving significant variability to be explained by other factors. Overall, genetics explains 50% to 60% of the variance of risk for dependence in both men and women, although the percentage may be higher for some types of AUD than others. The heritability of alcohol abuse is less, at about 38%.

Although the importance of heritability is clear, identifying specific genes is much more difficult because of the complexity of the disorder. To help with genetic analysis, researchers utilize subgroups of people with AUD on the basis of various characteristics such as severity, craving, occurrence of withdrawal, gender, and so forth. Many genes have been identified as potential vulnerability factors, but each of them contribute only a small amount to the risk of developing AUD.

To find genetic patterns in humans with AUD, several methods may be used. Rietschel and Treutlein (2013) summarize recent findings using each of the methods. One method, called **linkage studies**, examines the inheritance pattern of genes that increase disease susceptibility using DNA analysis and the occurrence of AUD in many members of a large number of families. Researchers look for easily identified genetic markers and determine which are most closely related to alcohol-associated behaviors. Affected family members would be expected to have more of those markers than their unaffected relatives. The most consistently identified region that is related to several traits of AUD and that has been found in multiple ethnic groups is on chromosome 4. Within a broad region of the chromosome (4q22–4q32) is a group of 7 genes for the enzyme alcohol dehydrogenase that metabolizes alcohol

(discussed further below). A region of chromosome 2 (2q35) has been linked to the alcohol-dependence phenotype and to low sensitivity to alcohol, which is a risk factor for AUD (see Rietschel and Treutlein, 2013). Individuals with AUD frequently report that early in their drinking, they experienced very little effect of alcohol unless they consumed large quantities. Schuckit (2000) measured subjective intoxication, "sway score" when balanced on a straight line, and hormonal response to alcohol in young sons of alcohol-dependent parents compared with controls. The men who later developed AUD showed a reduced response to moderate levels of alcohol for each of the measures (**FIGURE 10.22**). More important, Schuckit found that the low response rate increased the risk for AUD fourfold *regardless of family history* when the men were evaluated 8 years later.

The **case–control method**, a type of **association analysis**, compares the genes of unrelated affected and unaffected individuals to see whether more members of the affected population have a particular form (allele) of a gene. The gene does not necessarily have to be directly associated with the disorder but may be a marker associated with a characteristic that increases the risk of developing AUD. For example, meta-analyses of case–control studies show the strongest association for genes responsible for the manufacture of alcohol dehydrogenase (in agreement with linkage analysis) and aldehyde dehydrogenase. As you know, both enzymes are involved in alcohol metabolism (see Figure 10.5). Alcohol dehydrogenase converts alcohol to the toxic metabolite acetaldehyde that is subsequently metabolized to acetic acid by aldehyde dehydrogenase. Increased levels of acetaldehyde cause flushing, nausea, headache, and increased heart rate. Since individuals with the gene for the inactive form of the aldehyde dehydrogenase experience these unpleasant effects when drinking alcohol, they have essentially no risk for AUD. Alternatively, acetaldehyde concentration may be elevated by greater alcohol dehydrogenase activity, producing the same unpleasant effects when alcohol is consumed. Hence, individuals with the gene for the more active form of alcohol dehydrogenase have low probability of AUD. Rodents carrying a particular human allele have a 90% increase in enzyme activity, which produces a 3.5- to 5.0-fold elevation in blood acetaldehyde and reduces alcohol consumption by 50% (Rietschel and Treutlein, 2013), making that allele a protective factor against AUD.

Other case–control studies have looked for variants in genes involved in neurotransmitter function, such as receptor subtypes, synthesizing enzymes, and reuptake transporters. For example, a genetic variant of the 5-HT transporter is associated with anxiety and with low sensitivity to alcohol, and this makes affected individuals more vulnerable to developing AUD.

Genome-wide association studies (**GWAS**) represents the newest approach to genomic research; hundreds of thousands of genomic markers are analyzed to detect differences in the frequency of alleles across the entire genome. Describing the many research projects conducted that compare cases of AUD phenotype and controls is beyond the scope of this chapter, but Rietschel and Treutlein (2013) provide in-depth details for those interested. However, suffice it to say that specific markers located in the alcohol dehydrogenase gene cluster achieved genome-wide significance in multiple reports, which is in agreement with the most consistent linkage analyses and association studies.

Rodent models, as described in an earlier section, are extremely important in evaluation of the genetic contribution to isolated drinking behaviors. Researchers use selectively bred lines of animals that differ with

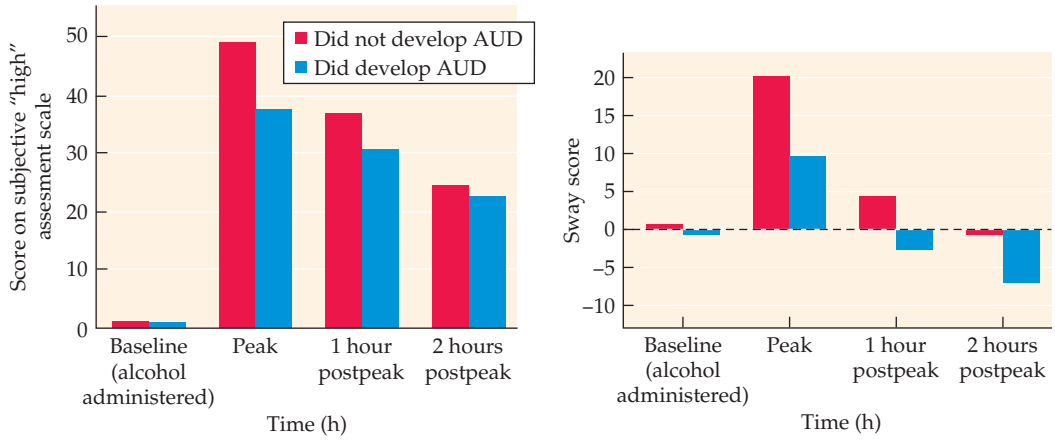


FIGURE 10.22 Low sensitivity to alcohol is a risk factor for AUD Subjective assessment of "high" and sway scores following a moderate dose (0.75 ml/kg) of alcohol in men who later developed alcohol dependence and in those who did not. Those vulnerable to alcohol dependence showed significantly less response to the same dose at peak blood level (1 hour) and 1 and 2 hours after the peak. (After Schuckit, 1994.)

respect to alcohol-related traits, such as withdrawal sensitivity, level of alcohol consumption, or alcohol-induced hypothermia, to search for clusters of genes linked to the particular trait. A second technique uses gene knockout animals, in which a particular gene is selectively deleted, before evaluating animal alcohol-associated behaviors.

SOCIOCULTURAL FACTORS Social and cultural factors mold changing attitudes about drinking, as well as the definition of problem drinking. Group attitudes also determine how much alcohol is available in a particular environment, for example, by controlling hours of sale, public consumption, and advertising. The examination of cultural influences on drinking was pioneered by Bales (1946), who suggested that cultures of those who abstain from alcohol use or who restrict it for religious purposes have the lowest rates of alcohol abuse. In contrast, societies that engage in social drinking in public settings and in which drinking is condoned for personal reasons, such as tension reduction, have higher rates of alcohol misuse. The importance of cultural influence is well demonstrated by the low rate of AUD among Muslims and Mormons—two groups with religious prohibitions on alcohol use. A low rate of AUD is also seen in Jewish populations, who use alcohol in a religious ceremonial way among family and do not condone intoxication. Further discussion of cultural influences on vulnerability to alcohol dependence can be found in several sources, including McNeece and DiNitto (1998). An excellent pictorial article by Boyd Gibbons (1992) in *National Geographic* describes many cultural differences in alcohol use and abuse.

The most obvious conclusion that can be drawn from our discussion of etiology is that multiple factors contribute to AUD. The neurochemical effects of alcohol and genetic predisposition to heavy drinking; the personality, cognitive structure, and expectations of the individual; and social, cultural, and economic variables all affect the use of alcohol and vulnerability to alcohol dependence.

Multiple treatment options provide hope for rehabilitation

An increasing number of treatment programs for AUD have developed over the past few years, with the goal of abstinence or reduced drinking, reducing the number and severity of relapses, and improving function in daily living. Multiple approaches give alcohol abusers increased hope for recovery.

The first step in treatment is **detoxification** because withdrawal symptoms (if present) are strong motivators and can be physiologically dangerous. Under medical care, detoxification involves substituting a benzodiazepine such as chlordiazepoxide (Librium) or diazepam (Valium). These drugs prevent alcohol withdrawal, including seizures and DTs. The long-acting nature of the drugs stabilizes the individual, and as the dose is gradually reduced, withdrawal symptoms are minimized. It should be mentioned that some symptoms of withdrawal increase in magnitude with successive withdrawals. This is true for seizure activity, and repeated episodes of withdrawal-induced seizures may be responsible for some of the long-term deficits in cognitive processing and emotional regulation that in turn may lead to less control over drinking.

PSYCHOSOCIAL REHABILITATION After detoxification, **psychosocial rehabilitation** programs help the alcohol abuser to prevent relapse through abstinence or to reduce the amount of alcohol consumed if relapse occurs (Fuller and Hiller-Sturmhofel, 1999). Many programs are available, but we provide here only a cursory survey of a few of them. For a more thorough discussion, see Jhanjee (2014). The three basic types include (1) individual and group therapy to provide emotional support and address psychological and social problems associated with dependence, (2) residential alcohol-free treatment settings, and (3) self-help groups such as Alcoholics Anonymous (AA). All of these methods reduce alcohol use among patients, although as is true for other chronic diseases, the relapse rate is high. Approximately 40% resume drinking within 3 years, and estimates of long-term relapse rates vary between 20% and 70% (Moos and Moos, 2006). However, rather than being considered a failure of treatment, relapse should be the signal for initiating a new treatment plan. Following multiple episodes of treatment and self-help, approximately 60% eventually reach a state of sustained abstinence.

AA is perhaps the best known of the self-help treatment programs and is the most frequently recommended. It is an organization in which all members suffer from AUD because an underlying assumption is that only a peer group is able to understand alcohol-dependent individuals and help them accept their disorder and admit their powerlessness over it. The emphasis of the group is a spiritual one in which the individual relies on a "higher power" to help him remain sober. The self-help group provides peer support, role modeling, practical problem-solving advice, and a social support network. **Web Box 10.3** provides the 12 steps in the program and describes some of the benefits and drawbacks of AA.

The community reinforcement approach (CRA) is one of several counseling approaches available that is more effective than standard care in reducing the number of drinking days (Roozen et al., 2004). CRA assumes that environmental contingencies (rewards and punishers) are powerful in encouraging drinking behavior but that they can be modified to become powerful reinforcers of nondrinking as well. If a nondrinking lifestyle is more appealing than a drinking one, the alcohol

abuser will no longer turn to the drug. CRA focuses on problems (e.g., job loss, marital issues, family stress) as perceived *by individuals with AUD* and helps them set their own goals, enhances their motivation to achieve these goals, and teaches the skills needed to create the positive lifestyle they desire.

In a similar manner, cognitive behavior therapy is based on the belief that alcohol abuse is a maladaptive behavior pattern that has been learned. In therapy, the individual learns to identify problematic behaviors that have led to alcohol use and develop effective coping strategies. For example, by recognizing the cues for craving and at-risk situations, the alcohol abuser can learn a variety of methods to control the craving and avoid high-risk situations. Cognitive behavior therapy is frequently combined with pharmacotherapeutic treatment for maximum effectiveness.

PHARMACOTHERAPEUTIC APPROACHES There is no wonder drug available that will magically eliminate the overwhelming urges to drink in an individual with AUD, but combined with psychosocial rehabilitation, pharmacotherapy can significantly reduce craving and heavy-drinking days. Presently there are three drugs approved by the FDA to treat AUD: disulfiram (Antabuse), naltrexone (Revia), and acamprosate (Campral). **Pharmacotherapeutic treatment** for AUD includes two basic strategies: making alcohol ingestion unpleasant and reducing its reinforcing qualities (Garbutt et al., 1999; Swift, 1999). The drug **disulfiram** (**Antabuse**) inhibits ALDH, the enzyme that converts acetaldehyde to acetic acid in the normal metabolism of alcohol. An individual who drinks as little as a quarter of an ounce of alcohol within a week of taking disulfiram experiences a sharp rise in blood acetaldehyde accompanied by facial flushing, tachycardia, pounding in the chest, drop in blood pressure, nausea, vomiting, and other symptoms. This method is clearly aimed at making ingestion of alcohol unpleasant. Patients must be cautious about unknowingly consuming alcohol in beverages, foods, over-the-counter medications like cough syrup, or mouthwash. Because disulfiram can cause hepatitis, frequent liver function tests must be done. For obvious reasons, compliance with the drug-taking regimen is quite low, with sometimes as many as half of the participants dropping out of the study. Disulfiram clearly does not treat AUD or reduce craving but may act as a motivational aid for those who are very determined to avoid alcohol. Unfortunately, rigorous double-blind studies show little difference in rates of abstinence between men on disulfiram and controls, although some studies show an increase in the duration of abstinence before relapse (see Jonas et al., 2014).

Preclinical animal studies described earlier (in the section on opioid systems) showed that μ-opioid agonists increase alcohol consumption and μ-antagonists decrease self-administration, presumably because they compete for the endogenous opioid receptors. In individuals with AUD, **naltrexone**, which is an opioid receptor antagonist, reduces alcohol consumption and craving and improves abstinence rates, according to several double-blind studies (**FIGURE 10.23**), although it seems to be less promising in longer studies. It is assumed that naltrexone reduces the positive feelings and the subjective "high" of alcohol by blocking the effects of alcohol-induced endorphin release. Social drinkers report more negative effects and greater sedation from a moderate amount of alcohol when taking naltrexone. Increased abstinence rates in humans during naltrexone treatment show parallels with rodent studies in

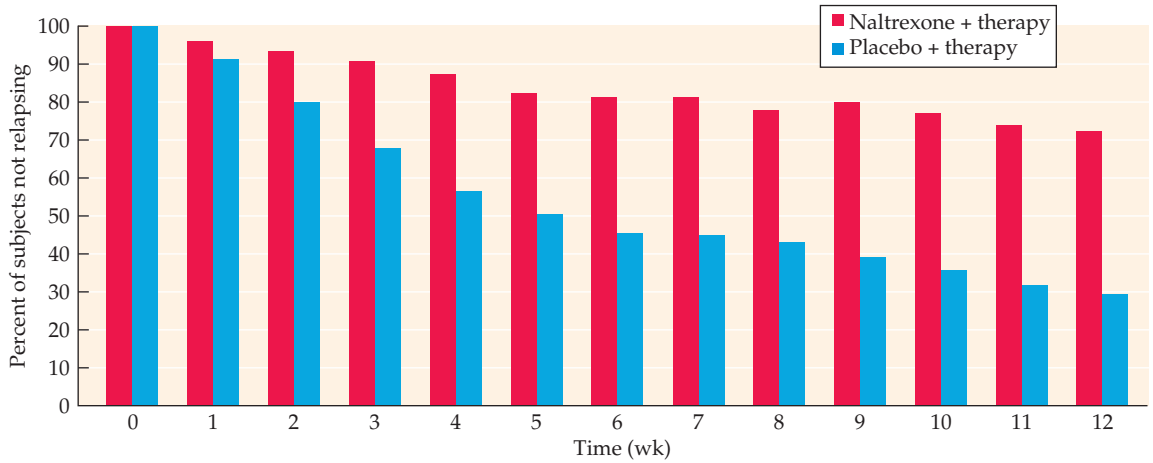


FIGURE 10.23 Effectiveness of naltrexone in treatment of AUD Naltrexone along with coping skills therapy is more effective in preventing relapse than placebo and therapy over a 12-week period. At 12 weeks, relapse rates were approximately 70% for the placebo group compared with 30% for the naltrexone treatment group. (After Volpicelli et al, 1992.)

which naltrexone reduced the reinstatement of alcohol consumption triggered by priming injections of ethanol or ethanol-associated cues. Although clinical studies show significant effects, the effect size is small because only a fraction of individuals show meaningful benefit. Some of the variability in response may be due to genetics, since evidence demonstrates that people with a family history of AUD respond better to naltrexone treatment than people with no family history. Furthermore a μ-receptor polymorphism that leads to reduced receptor expression predicts greater success for alcohol relapse rates with naltrexone therapy (see the meta-analysis by Chamorro et al., 2012). It is possible that naltrexone is more effective for those with a gene variant because they have a different type of AUD and may, for example, differ in their reinforcement mechanisms (see Ripley and Stephens, 2011). Hence targeting the opioid receptor seems to produce substantial benefit for some individuals and limited benefit for others, which indicates the potential value of pharmacogenetic screening for individualizing treatment approaches (see Heilig et al., 2010). As is often the case in AUD rehabilitation, the effectiveness of naltrexone is most pronounced when accompanied by counseling aimed at enhancing individual coping skills and relapse prevention. Naltrexone is now available as a once-a-month injectable (Vivitrol), which increases the ease of compliance.

Several laboratory studies demonstrated the potential use of κ-opioid receptor antagonists in AUD treatment by demonstrating effects of selective opioid receptor subtype antagonism. In one such study, following a month of alcohol exposure, dependent animals and their nondependent controls were allowed to self-administer alcohol 6 hours after their last alcohol exposure (considered acute withdrawal). As expected, alcohol-dependent rodents self-administered more alcohol than did nondependent animals. Both the μ-receptor antagonist naltrexone and the dual κ- and μ-opioid receptor modulator **nalmefene** were effective in reducing lever pressing for alcohol in a dose-dependent manner in both dependent and nondependent animals. However, nalmefene, because of its action at κ-opioid receptors as well as μ-receptors, was more effective than naltrexone in reducing consumption among dependent animals. The third drug tested, nor-binaltorphimine (nor-BNI), which is a selective κ-opioid antagonist, reduced self-administration only in dependent animals. These results suggest that the μ-opioid receptor mediates acute reinforcing effects, as shown by the ability to reduce alcohol self-administration with μ-antagonists. The fact that antagonists at κ-receptors reduced the withdrawal-induced increase in consumption of only *dependent* animals suggests that κ-receptors have a role in the development of alcohol dependence and withdrawal-induced alcohol consumption. This conclusion is supported by evidence that shows κ-receptor up-regulation during chronic alcohol administration. It is suggested that this up-regulation may be responsible for an anhedonic state (i.e., the inability to experience pleasure) that develops during the formation of physical dependence and leads to dysphoria when drug cessation occurs. This dysphoria produces a withdrawal-induced increase in alcohol consumption (Walker and Koob, 2008; Nealey et al, 2011). Studies such as these imply that blocking both μ-receptor-induced reinforcement and κ-receptor-mediated anhedonia may improve abstinence rates in those with AUD.

Nalmefene has been approved for use in treatment of heavy alcohol use in Europe but has not yet been FDA approved. Clinical evidence suggests that compared with placebo, nalmefene along with psychosocial support reduces the number of heavy-drinking days and total amount of alcohol consumed. It has been suggested that it can be used on an as-needed basis, that is, patients can choose to take the drug on days when they feel heavy drinking is likely, rather than taking it chronically. The clinical benefit seen is that patients could maintain moderate drinking over time and reduce the most damaging effects of heavy alcohol consumption. When abstinence is not required, more heavy-drinking individuals enter treatment, which leads to better health outcomes and may be an interim step toward abstinence (Mann et al., 2016). As you might expect, this as-needed approach has met with some criticism, and active debate is likely to continue.

Acamprosate (**Campral**) is a somewhat newer agent available for the treatment of AUD. Several large, well-controlled studies have shown that acamprosate increases nondrinking days by 30% to 50% and approximately doubles the rate of abstinence, even though most patients ultimately return to drinking. The drug seems to reduce the insomnia, anxiety, restlessness, and unpleasant mood changes experienced by heavy drinkers when they abstain. Eliminating those symptoms may help to prevent relapse. In head-to-head comparisons, acamprosate and naltrexone are essentially equivalent in effectiveness and their side effect profiles are similar, including dizziness, nausea and vomiting, diarrhea, and anxiety. Acamprosate acts as a partial antagonist at the glutamate NMDA receptor and significantly blocks the glutamate increase that occurs during alcohol withdrawal in rats (**FIGURE 10.24**), which may explain its therapeutic effects. Acamprosate has a chemical structure similar to that of GABA and returns basal GABA levels to normal in alcohol-dependent rats. Its ability to modify the functions of both GABA and glutamate in the nucleus accumbens may serve as the ultimate basis for its efficacy in preventing relapse.

Other available drugs have been tested in fewer well-controlled trials, but they have generally shown disappointing results despite encouraging findings with

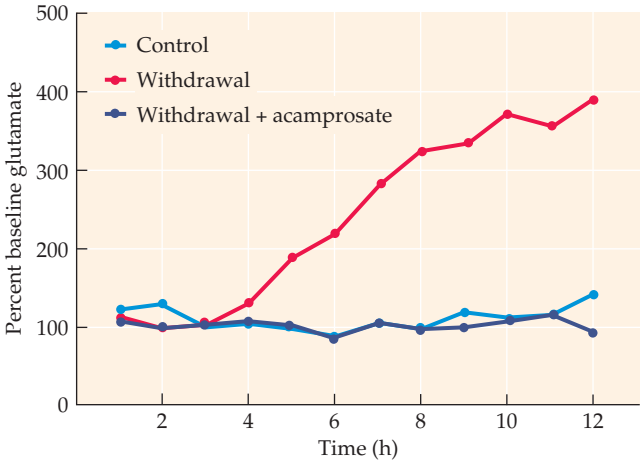


FIGURE 10.24 Acamprosate prevents withdrawal-induced glutamate release The red line shows the rebound release of glutamate (measured by microdialysis) that occurs in the nucleus accumbens when alcohol is withdrawn from dependent rats. Alcohol-dependent animals no longer receiving alcohol but receiving acamprosate (purple line) are no different in glutamate release from non-alcohol-treated controls. (After Dahchour and DeWitte, 2000.)

animal testing. Serotonergic agents such as the antidepressant fluoxetine, whose effectiveness is predicted by animal studies, have not consistently been effective in humans, except in cases when dependence is accompanied by depression or anxiety disorders. The dopamine antagonist tiapride, sold only in Europe, reduces the symptoms of alcohol withdrawal and increases abstinence. However, in at least one clinical trial, compliance was an issue, and only about half of the participants completed 1 month of treatment (Shaw et al., 1994). Likewise, although cannabinoid receptor antagonists reduce alcohol self-administration in numerous animal models, double-blind, placebo-controlled clinical trials report little efficacy in reducing alcohol consumption or relapse rates among alcohol-dependent individuals. Such failures of basic research to translate to effective clinical treatment may be due to several factors, including poor clinical research designs, inability to identify subpopulations of individuals who may benefit from treatment, and animal models that poorly predict efficacy in humans (see Ripley and Stephens, 2011). It is hoped that collaborative efforts in translational research, as described in Chapter 4, will produce more effective therapies in the future.

PROMISING NEW DIRECTIONS IN TREATMENT In an earlier section, we described the complex reciprocal interaction between stress and alcohol consumption and the importance of stress in leading to relapse in the abstinent individual with AUD. Since both animal and human studies show that repeated episodes of intoxication and withdrawal lead to increased CRF_1 receptors in the amygdala, sensitization of the reactivity to stressors, and significantly elevated rates of alcohol consumption, it is reasonable to consider pharmacological interventions to minimize this stress response. One approach is to block the CRF_1 receptors that mediate both the hypothalamic-regulated neuroendocrine response and the extrahypothalamic CRF_1 receptors that modulate the behavioral response to stress. Several animal studies have shown that either systemic or intracerebral injections of **CRF_1 antagonists** into the central nucleus of the amygdala reduced the postwithdrawal escalation of alcohol self-administration to baseline consumption levels. Additionally, although the antagonist had no effect on basal alcohol self-administration in animals that had no history of dependence, it did reduce excessive binge-like consumption in nondependent animals. Importantly, the CRF_1 antagonists prevented stress-induced relapse-type behavior. Unfortunately, there have been few efforts to test CRF_1 antagonists on the alcohol-abusing population, because medicinal chemists have had difficulty in creating agents that penetrate the blood–brain barrier and achieve the high receptor occupancy needed for efficacy as determined by animal research.

Taking a different approach to understanding the importance of the stress response in AUD, Vendruscolo and colleagues (2015) tested the hypothesis that glucocorticoid receptor (GR) signaling has a role in compulsive alcohol consumption. Glucocorticoids released by the adrenal cortex in response to stress bind to GRs located in many brain regions, but they are especially dense in the PFC, hippocampus, and amygdala, which explains the sensitivity of those regions to stress. The initial findings demonstrated that increased phosphorylation of GR occurred during acute withdrawal in alcohol-dependent compared with nondependent rodents. Administration of a systemically administered GR antagonist reduced alcohol self-administration in dependent rats without altering consumption of water or saccharin, demonstrating that response to nondrug reinforcers is not altered. The importance of the central nucleus of the amygdala was demonstrated by bilateral intracerebral injection that reduced self-administration of alcohol in dependent rats. In a proof-of-concept study to translate these results to humans, the GR antagonist was tested on the craving response to alcohol-related cues and self-reported drinking in a small number (n = 56) of human alcohol-dependent participants. After 1 week of treatment, craving was significantly reduced compared with placebo, as was the number of drinks per drinking day during treatment week and one week after treatment (**FIGURE 10.25**). Because there were no adverse effects reported for the participants, further investigation of GR antagonists as treatment for AUD is warranted.

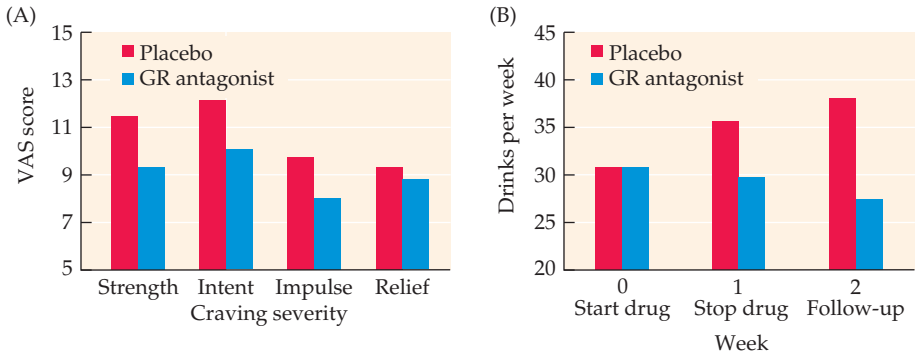


FIGURE 10.25 Glucocorticoid receptor (GR) antagonism reduced alcohol-cued craving and drinking in dependent individuals (A) Visual analog scale (VAS) scores on four VAS measures of craving severity following presentation of alcohol-related cues. Strength: how strong is your craving to drink alcohol? Intent: if I could drink alcohol now, I would drink it. Impulse: it would be hard to turn down a drink right now. Relief: having a drink would make things just perfect. Higher scores indicate greater craving. Significant differences were reported for strength, intent, and impulse. (B) Total number of alcoholic drinks consumed per week for individuals treated with the GR antagonist or placebo at baseline, following treatment, and at 2-week follow-up. (After Vendruscolo et al., 2015.)

An alternative target for modulating stress is the neuropeptide substance P and its neurokinin 1 receptor (NK1R). These receptors are densely located in the hypothalamus and the amygdala, and intracerebral injection of substance P into those areas produces anxiety in animal tests. Blocking the receptor or genetically deleting it reduces anxious behaviors. More to the point, NK1R knockout mice showed reduced alcohol consumption in a free choice situation—a result that also occurred following administration of the NK1R antagonist L-703,606. In an animal model of relapse in which several cycles of alcohol exposure followed by deprivation caused increased alcohol intake in control animals, the increase in alcohol consumption was absent in NK1R knockout mice. An initial clinical study using 50 recently detoxified anxious alcohol-dependent patients showed that the NK1R antagonist LY686017 significantly reduced craving and improved scores on a measure of general well-being. When study participants were exposed to both stress- and alcohol-related cues, LY686017 reduced craving as well as the rise in the stress hormone cortisol. With the same participants, researchers used functional magnetic resonance imaging (fMRI) to image regional brain activity in response to pictures that had either a negative or positive affect. Placebo-treated participants with AUD showed enhanced neural activity to negative images in brain regions associated with emotional processing and reduced activity to positive stimuli, compared with control individuals. The NK1R antagonist attenuated both of those neural responses. Hence participants treated with the antagonist had less activation in the insula, a brain region associated with craving and addictive behaviors, in response to negative stimuli than was seen in the placebo-treated controls with AUD. In response to positive visual images, NK1R-treated alcohol abusers showed elevated response in the nucleus accumbens and the anterior cingulate cortex compared with the placebo-treated controls with AUD. These changes represent a normalization of the brain response to emotional stimuli and may be a neural correlate of the enhanced clinical rating of well-being (reviewed by Heilig et al., 2010).

COMBINED PHARMACOTHERAPY AND TALK THERAPY

The National Institute of Alcohol Abuse and Alcoholism (NIAAA) initiated a multisite, controlled clinical trial of treatments for alcohol dependence utilizing almost 1400 alcohol-dependent study participants (Anton et al., 2006). The focus of this research was to evaluate the efficacy of pharmacotherapy, behavioral treatment, and a combination on a number of alcohol use parameters. The research, called Combining Medications and Behavioral Interventions (COMBINE), aimed to enhance treatment outcomes (www.cscc.unc.edu/COMBINE/ accesses the study's 1999–2007 website). Investigators used four treatments singly and in combination: naltrexone, acamprosate, medical management, and combined behavioral intervention (CBI). Medical management was designed to be used in a normal medical setting by medical doctors and nurses to increase medication adherence and provide support for abstinence, while CBI was used in specialized alcohol treatment programs and provided more intense individual psychotherapy. A wide variety of physiological and behavioral outcomes were evaluated at multiple time points during the 16-week active phase of treatment, as well as during the post-treatment phase, but the principal measures included percent days of abstinence and time to the first heavy drinking. Results showed that the most effective treatments were medical management with either naltrexone or CBI, which led to 79% days of abstinence. The interaction of naltrexone and the behavioral intervention was particularly important because naltrexone with medical management also

prolonged the time to the first heavy drinking. Oddly, in contrast to the findings of earlier studies, acamprosate had no effect either alone or in combination with either naltrexone or behavioral intervention. The authors suggest that the difference may be due to the fact that participants in earlier successful acamprosate trials were inpatients who had a significantly longer period of abstinence before the trial began. The importance of these findings for treatment options is that pharmacotherapy with naltrexone and medical management can be provided by family doctors in readily available health care settings, and treatment does not depend on specialized treatment programs that are not always accessible to the alcohol-dependent population. It was interesting to see that during longer follow-up, CBI began to show significant benefit over medical management that was not evident in the initial clinical trial, although all treatments showed a diminishing outcome over time. It is likely that alcohol dependence should be treated much like other chronic conditions such as diabetes and hypertension, with regular monitoring over time and rapid intervention with follow-up treatments as needed. Such monitoring and follow-up treatment should reduce the amount of health care needed to treat alcohol-related disease and associated costs in the long run.

Section Summary

- AUD involves compulsive alcohol seeking and use despite damaging health and social consequences. Frequency and pattern of drinking are as important as the quantity consumed.
- Approximately 10% of Americans have an alcohol use problem. There are significant gender differences in alcohol use, binge drinking, and heavy drinking.
- Defining subtypes of individuals with AUD is important for neurobiological and genetic research, predicting the course of the disorder, and identifying the most effective treatment approach. Babor's type A and type B have been validated in the general population and are defined by such characteristics as age of onset, childhood risk factors, presence of psychopathology, social impact of alcohol, and so forth.
- Underage drinking disrupts neurodevelopmental events, causing long-lasting change in neurological function and behavior.
- Neurobiological, psychological, and sociocultural factors contribute to the vulnerability of a given individual to AUD.
- Stress reduces or increases alcohol consumption under different conditions. Alcohol increases the activity of the brain stress systems and neuroendocrine stress systems that may lead to further alcohol use. Sensitization to stressors persists long after withdrawal.
- Early life stress is a risk factor for adult alcohol abuse. Family history is a risk factor and is associated with a greater stress response and greater alcohol-induced suppression of the response.
- Cortisol sensitizes the DA mesolimbic pathway, which makes drug reinforcement more rewarding.
- Early (before age 13) drinking, impulse control problems, high novelty seeking, low harm avoidance, and aversion to delayed gratification predict future substance abuse.
- Genetics explains 50% to 60% of the variance of risk for alcohol dependence. Twin and adoption studies, linkage analysis, association studies, and GWAS provide evidence for a genetic risk for AUD.
- Genes for the inactive form of aldehyde dehydrogenase, the enzyme that converts the toxic metabolite acetaldehyde, predict low risk for AUD because alcohol has unpleasant effects.
- Gene polymorphisms for the 5-HT reuptake transporter, associated with anxiety and low sensitivity to alcohol, increase vulnerability to alcohol abuse.
- Social and cultural factors determine attitudes about drinking and how much alcohol is available. Cultures that restrict use of alcohol have lower rates of AUD.
- Detoxification under medical supervision is the first step in treatment and is followed by benzodiazepine substitution to prevent withdrawal, and gradual dose reduction.
- Psychosocial rehabilitation includes individual and group therapies, residential treatment settings, and self-help groups.
- FDA-approved pharmacotherapies for AUD include disulfiram, naltrexone, and acamprosate. Disufiram inhibits the enzyme that converts acetaldehyde to acetic acid so that alcohol consumption causes very unpleasant effects such as nausea and vomiting, which discourages drinking.
- Naltrexone is an opioid receptor antagonist that reduces consumption and craving in some individuals with AUD, perhaps by reducing the positive feeling caused by alcohol. Those with a family history of AUD and those with a μ-receptor polymorphism associated with reduced receptor expression respond better to this treatment.
- Targeting the opioid κ-receptor with an antagonist reduced self-administration only in dependent animals, suggesting that the κ-receptor may have a role in the anhedonic states associated with physical dependence. Blocking μ- and κ-receptors with

a dual antagonist like nalmefene may improve abstinence rates in humans.

- Acamprosate reduces the relapse rate. It reduces the glutamate increase that occurs at withdrawal and returns basal GABA levels to normal in the nucleus accumbens.
- Promising rodent studies targeting the stress response with CRF_1 antagonists suggest that they may be effective in reducing withdrawal-induced increase in consumption, as well as stress-induced relapse behavior.
- A glucocorticoid receptor antagonist reduced self-administration of alcohol in rodents without altering water or saccharin consumption. The same antagonist reduced craving in response to alcohol cues in humans and reduced drinking.
- Animal studies suggest that blocking the NK1R for substance P reduces anxiety, alcohol consumption, and relapse behavior after withdrawal. In alcohol-abusing patients, the antagonist reduced craving when patients were exposed to stress- and alcohol-related cues. The drug also normalized the brain response to emotional stimuli.
- The COMBINE study showed the most effect treatment for alcohol dependence is medical management along with either naltrexone or combined behavioral intervention. Since medical management with naltrexone can be provided by family doctors without special training, it provides accessible health care.

STUDY QUESTIONS

1. How is alcohol produced?
2. Describe the pharmacokinetics of alcohol absorption and distribution. What are the variables that determine absorption? Why are blood levels higher for women than men given the same amount of alcohol?
3. Summarize the two ways alcohol is metabolized.
4. Name the four types of tolerance, and briefly describe their mechanisms. What roles do operant and classical conditioning have in behavioral tolerance?
5. List several signs of withdrawal from chronic alcohol use. Why is medically supervised detoxification recommended for heavy alcohol abusers?
6. Describe the CNS effects of alcohol, including those on judgment, memory, motor skills, and CNS depression.
7. What are the signs of alcohol poisoning?
8. Discuss the nature of brain damage caused by heavy alcohol use and its functional outcome.
9. Describe the effects of alcohol on the cardiovascular, renal–urinary, reproductive, and gastrointestinal systems.
10. Describe the progression in liver damage from fatty liver to alcohol-induced hepatitis to alcohol-induced cirrhosis. Where possible, describe one potential cause.
11. What are fetal alcohol syndrome and the less severe fetal alcohol spectrum disorders? Provide evidence from animal studies for a role of alcohol use in the disorders. Why are correlational epidemiological studies with humans much more difficult to conduct?
12. Why are animal models especially important in alcohol research? Compare the self-administration model and the two-bottle free choice procedure. How does the manipulation of access to alcohol model relapse behavior? Compulsive drinking?
13. Provide evidence for the validity of inbred alcohol-preferring (AP) and high-alcohol-drinking (HAD) rat strains in modeling characteristics of AUD.
14. Describe the most important effects of alcohol on the glutamate NMDA receptor and associated biobehavioral effects. Do the same for the $GABA_A$ receptor, mesolimbic dopamine pathway, opioid systems, and second-messenger signaling leading to phosphorylation of CREB.
15. What are some of the significant characteristics of Babor's type A and type B alcoholics?
16. Summarize the hazardous effects of college student and underage drinking. Explain why adolescents are more likely to develop long-lasting neurological and behavioral deficits.
17. Discuss the role of stress in the vulnerability to AUD.

STUDY QUESTIONS *(continued)*

18. Tell how stress interacts with mesolimbic DA to increase vulnerability to AUD.
19. What are the personality factors that predict early alcohol use and addiction?
20. How important is genetic vulnerability to AUD? Provide one piece of evidence each from linkage, case control, and genome-wide association studies. Why are gene polymorphisms for alcohol dehydrogenase and aldehyde dehydrogenase of particular significance?
21. How do social and cultural factors influence alcohol use?
22. Distinguish among the psychosocial rehabilitation approaches: Alcoholics Anonymous (AA), the community reinforcement approach (CRA), and cognitive behavior therapy.
23. What are the pros and cons of the three FDA-approved pharmacotherapies: disulfiram, naltrexone, and acamprosate?
24. Provide evidence for the effectiveness of nalmefene, a κ-opioid receptor antagonist, in AUD treatment programs.
25. Why are CRF_1 antagonists a focus of pharmacotherapeutic research? Glucocorticoid receptor antagonists? Substance P and its neurokinin receptor?
26. Why was the NIAAA-initiated trial of combined pharmacotherapy and talk therapy important?

Go to the *Psychopharmacology* Companion Website at oup-arc.com/access/meyer-3e for animations, web boxes, flashcards, and other study aids.

CHAPTER 11

Infections, festering sores, and tissue damage caused by toxic contaminants in the street drug krokodil. (© Emanuele Satolli.)

The Opioids

KROKODIL, A HOME-BREWED DESOMORPHINE, is a killer. Desomorphine is a codeine derivative with a simple chemical modification. It is an old opioid drug first synthesized in 1932 to produce rapid analgesia and sedation. It is about 8 to 10 times more potent than morphine, but its medical use was terminated in the early 1980s except for extraordinary circumstances because of its high addiction potential. Although medical use declined, clandestine manufacture particularly in Russian cookhouses increased dramatically around 2010, when heroin was more difficult to secure. It is easily prepared from over-the-counter codeine and iodine, plus phosphorus taken from matchbook strike pads, but it may also contain gasoline, paint thinner, hydrochloric acid, or lighter fluid. As is true for many homemade drugs, the product frequently has contaminants from the synthesis process that are not removed. These toxic products are the cause of commonly occurring dangerous outcomes, including infections, tissue damage, and festering sores, which led to its nickname the "flesh-eating" drug. Blood vessels at the injection site burst, and the surrounding flesh subsequently rots away. Frequently portions of tissue fall off the bone, which gives the drug another nickname: the "zombie" drug. These wounds were first recognized by Russian physicians while treating drug addicts, and the appearance of the skin lesions gave the drug its name, krokodil (crocodile). The attraction of this drug for addicts is its rapid "high" and low cost, although with regular use, addicts often do not survive for more than 2 to 3 years because of gangrene that may lead to limb amputation, weakened immune systems, blood clots, bone and blood infections, and kidney and liver damage (Shuster, 2013, provides further description and a photo essay dramatizing the problem). ■

Narcotic Analgesics

The opioid drugs all belong to the class known as **narcotic analgesics**. These drugs reduce pain without producing unconsciousness but do produce a sense of relaxation and sleep, and at high doses, coma and death. As a class, they are the very best painkillers known. In addition to inducing analgesia, opioids have a variety of side effects and also produce a sense of well-being and euphoria that may lead to increased use of the drugs. Continued use leads to tolerance and sometimes physical dependence. In contrast to analgesics, **anesthetics** reduce all sensations by depressing the central nervous system (CNS) and produce unconsciousness.

The opium poppy has a long history of use

Opium is an extract of the poppy plant and is the source of a family of drugs known as the *opiates* or sometimes *opioids*.[1] Opium is prepared by drying and powdering the milky juice taken from the seed capsules of the opium poppy, *Papaver somniferum* (meaning "the poppy that causes sleep"), just before ripening (**FIGURE 11.1**). When the capsules are sliced open, the juice leaks out and thickens into a reddish brown syrupy material. In its crude state, it is very dark in color and forms small balls, called "black tar." Cultivation of the opium poppy has been successful in the temperate zones as far north as England and Denmark, but the majority of the world's supply comes from Southeast Asia, India, China, Iran, Turkey, and southeastern Europe. The plant grows to about 3 or 4 feet in height and has large flowers in white, pink, red, or purple. This variety is the only poppy that has significant psychoactive effects.

[1] *Opiate* refers specifically to substances derived directly from the opium poppy. *Opioid* is a broader term that includes opiates, synthetic substances, and endogenous peptides that bind to opioid receptors.

FIGURE 11.1 Preparing opium The unripe opium poppy capsule has been sliced and the crude opium is dripping from the incision. (© Heather Angel/Alamy.)

The opiates have been used both as medicine and for recreational purposes for several thousand years. As early as 1500 BCE, the Egyptians described opiates' preparation and medicinal value. Archeological evidence from Cyprus dated as early as 1200 BCE includes ceramic opium pipes and vases with poppy capsules for decoration. By the second century CE, the famous Greek physician Galen prescribed opium for a wide variety of medical problems, including headache, deafness, asthma, coughs, shortness of breath, colic, and leprosy, among others. But the Greek author Homer, in *The Odyssey*, refers to the drug's recreational properties when he describes the plant as eliciting a feeling of warmth and well-being followed by sleep. More modern use began in Europe, when news of the "miracle cure" was brought back by the religious crusaders from the Near East. Eating or smoking opium was accepted in Islamic countries such as Arabia, Turkey, and Iran, where it replaced the consumption of alcohol, which was prohibited. By 1680, an opium-based medicinal drink was introduced by the father of clinical medicine, the English physician Thomas Sydenham. His recipe for the drink called *laudanum* (meaning "something to be praised") included "2 ounces of strained opium, 1 ounce of saffron, and a dram of cinnamon and cloves dissolved in 1 pint of Canary wine." Drinking laudanum-laced wine was the accepted form of opium use in both Victorian England and America, especially among women, who considered it far more respectable than "common" alcohol use. A number of notable literary figures of that period were addicted to laudanum and opium, including William Taylor Coleridge, Charles Dickens, and Elizabeth Barrett Browning. Laudanum was also a common ingredient of many popular remedies for a wide variety of problems, including teething pain and restlessness in infants, muscle aches and pains, and alcoholism.

Right up to the turn of the twentieth century, opium-containing products with names such as "A Pennyworth of Peace," "Mrs. Winslow's Soothing Syrup," and "White Star Secret Liquor Cure" could be ordered through the Sears, Roebuck and Co. catalog for about $4 a pint (**FIGURE 11.2**). In nineteenth-century America, neither the federal government nor individual states chose to control the availability and advertising of drugs such as opium and cocaine. There was clearly no significant concern about safety, long-term health

FIGURE 11.2 Mail-order source for opium preparations
Laudanum was used to treat pain and cough, and paregoric was used to treat diarrhea. The advertisement is from the 1897 Sears, Roebuck and Co. catalog.

issues, or dependence. Finally, in 1914, the Harrison Narcotics Tax Act was passed, which required physicians to report their prescriptions for opioids. Only in the 1920s did the Supreme Court broadly interpret the law to mean that prescriptions should be limited to *medical* use, making it illegal to provide opioids for addicted individuals or recreational use.

Minor differences in molecular structure determine behavioral effects

The principal active ingredient in opium was called *morphine* after the Roman god of dreams, Morpheus, and it was first isolated in the early 1800s by a German chemist, Friedrich Wilhelm Sertürner. His extraction of morphine crystals from the milky juice of the poppy seed capsules is considered a milestone in the history of pharmacology because it was the first time the active ingredient of any medicinal plant was isolated. Having the extract enabled physicians to prescribe the painkiller in known dosages. In addition to morphine, opium contains other active ingredients, including codeine, thebaine, narcotine, and others. Although morphine was isolated from opium in the early 1800s, the structure of morphine was not identified until 1925 (**FIGURE 11.3**). The naturally occurring opiate codeine is identical in structure to morphine except for the substitution of a methoxy ($-OCH_3$) for a hydroxyl (–OH) group. This small molecular difference produces a drug that has less analgesic effect and fewer side effects than morphine but is still a potent cough suppressant. It was exciting for pharmacologists to discover that simple modifications of the morphine molecule produce great variations in potency, duration of action, and oral effectiveness. In many cases, these differences are due to differences in pharmacokinetics rather than to intrinsic activity. For example, heroin was manufactured by adding two acetyl groups onto the morphine molecule. This drug was developed by the Bayer Company to be more effective in relieving pain without the danger of developing opioid use disorder. Today we know that the pharmacological effects of morphine and heroin are essentially identical because heroin is converted to morphine in the brain. Heroin is, however, two to four times more potent when injected and is faster acting because the change in the molecule makes the drug more lipid soluble and allows it to get into the brain much more quickly to act on receptors there. When taken orally, morphine and heroin are approximately equal in potency. The very rapid action of heroin is apparently also responsible for the dramatic euphoric effects achieved with that drug. Like heroin, methadone is a full agonist at opioid receptors; however, its slow onset after oral administration and its long half-life make it useful in treating opioid dependence (see the last section of this chapter).

Some of the modifications to morphine's molecular structure produce **partial agonists**, which are drugs that bind readily to (i.e., have a high affinity for) the receptors but produce less biological effect (i.e., low

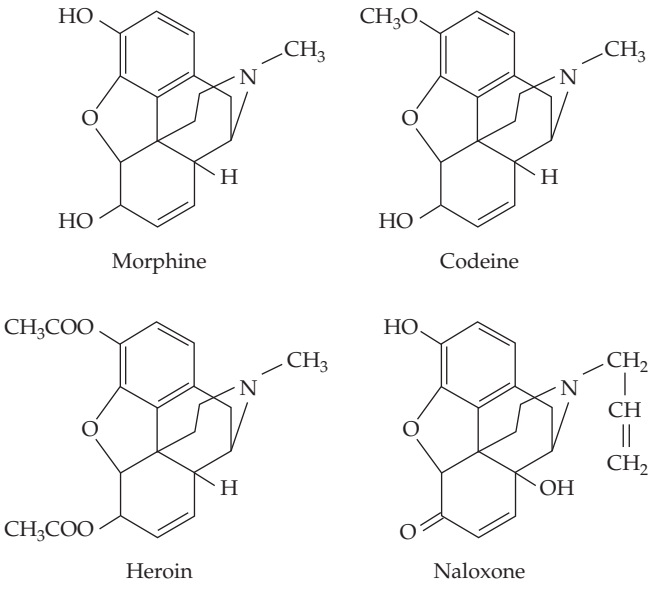


FIGURE 11.3 Molecular structure of morphine, codeine, heroin, and naloxone It is easy to see the similarities in structure. The minor differences contribute to effectiveness and side effects.

efficacy). Therefore, when administered alone, they produce partial opioid effects, but when given along with an opioid that has higher effectiveness, they compete for the receptor and subsequently reduce the action of the more effective drug. The prototypic partial agonist is pentazocine (Talwin), but others in this category are nalbuphine (Nubain) and buprenorphine (Buprenex). Although as analgesics they are much less potent than morphine, they do not cause significant respiratory depression or constipation, and they have a reduced risk for dependence.

Other chemical modifications of the morphine molecule produce **pure antagonists** such as naloxone and nalorphine. These are drugs that have structures similar to those of the opiates but produce no pharmacological activity of their own (i.e., no efficacy). The receptor antagonists can prevent or reverse the effect of administered opioids because of their ability to occupy opioid receptor sites. **BOX 11.1** describes how intravenously administered naloxone can revive an unconscious individual in a matter of seconds and reverse all of the opioid effects and how it can be used to save the lives of those brought to the emergency room after opioid overdose. Specific receptor antagonists are also important for understanding the mechanism of action of opioid analgesics. As you will learn a bit later, the four principal types of opioid receptors (μ, δ, κ and NOP-R) mediate different opioid actions.

While some narcotic drugs are natural derivatives, others are considered semisynthetic because they require chemical modifications of the natural opiates. For instance, hydromorphone (Dilaudid) and heroin are modifications of the morphine molecule; others are entirely synthetic and may have quite distinct structures (e.g., propoxyphene [Darvon], meperidine [Demerol]). Thebain, another constituent of opium, is chemically converted into several opioid compounds, including oxycodone, oxymorphone, buprenorphine, and etorphine. The relationship of the major opiates and some of their derivatives, as well as some of the synthetic opioids, is shown in **FIGURE 11.4**.

Bioavailability predicts both physiological and behavioral effects

When morphine is administered for medical purposes, it is usually injected intramuscularly or given orally. Oral administration is convenient but produces more variable blood levels for the reasons discussed in Chapter 1. Recreational users often smoke opium for its rapid absorption from the lungs, although "snorting" heroin also leads to rapid absorption through the nasal mucosa. In addition, subcutaneous administration ("skin popping") may precede the more dangerous "mainlining" (intravenous injection).

Although morphine has pronounced psychoactive effects, only a small fraction of the drug is capable of crossing the blood–brain barrier to act on opioid receptors in the brain. Opioid distribution is fairly uniform in the rest of the body, and the drugs easily pass the placental barrier, exposing the unborn child to high levels. The newborn of an opioid-addicted mother suffers withdrawal symptoms within several hours after birth, which may have severe consequences for the infant, especially if the child is weak from inadequate prenatal nutrition. However, infants are readily stabilized with low doses of opioids to prevent abstinence signs, and the dose is gradually reduced. Following metabolism in the liver, most of the opioid metabolites are excreted in the urine within 24 hours.

Opioids have their most important effects on the CNS and on the gastrointestinal tract

The multiple effects of morphine and other opioids on the CNS are dose related and are also related to the rate of absorption. At low to moderate doses (5 to 10 mg), pain is relieved, respiration is somewhat depressed, and pupils are constricted. The principal subjective effects are drowsiness, decreased sensitivity to the environment, and impaired ability to concentrate, followed by a dreamy sleep. Because opioids have actions in the limbic system, some researchers suggest that the drugs relieve "psychological pain," including anxiety, feelings of inadequacy, and hostility, which may lead

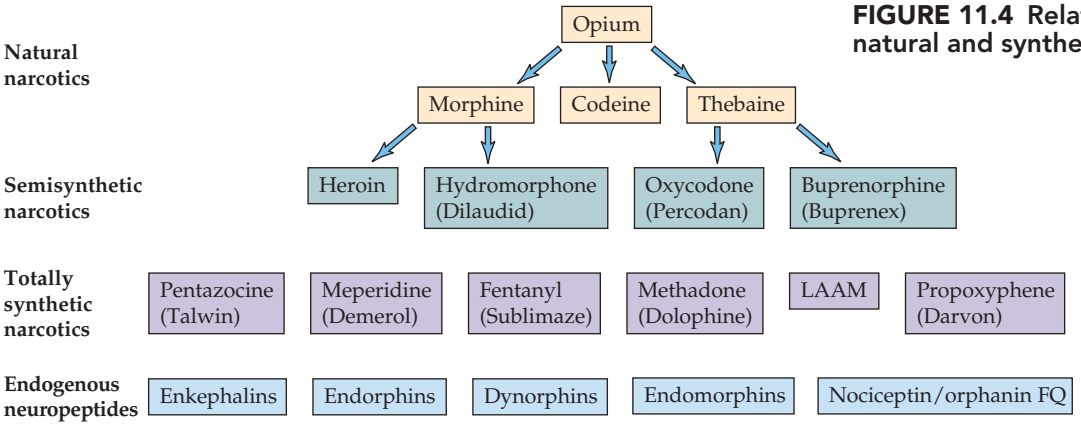


FIGURE 11.4 Relationship of selected natural and synthetic opioid drugs

BOX 11.1 Clinical Applications

Saving a Life: Naloxone for Opioid Overdoses

Moments after the arrival of the ambulance, a patient was rolled in on a stretcher to receive almost instant care by the emergency room staff. The patient was about 24 years old, Caucasian, and unconscious. Cardiac monitor leads were placed on his chest, and oxygen was immediately provided. His pulse was weak and his blood pressure extremely low. Heart rate and respiration rate were also depressed. The neurological exam revealed extreme pinpoint pupils, failure to respond to pain, and no response to verbal instructions. Although he was rather thin, his appearance was otherwise unremarkable except that one rolled-up shirt sleeve exposed needle track marks indicating intravenous (IV) drug use. The triad of coma, pinpoint pupils, and depressed respiration is a strong indicator of opioid poisoning, and physical evidence of IV drug use further confirms the diagnosis. To any bystander, death seemed imminent. Nevertheless, 0.8 mg of IV naloxone (Narcan) was ordered immediately. Within a minute or two, respiratory rate had returned to normal, and soon after, the young man was alert enough to respond to a few questions. Although capable of walking out of the emergency room, the patient was convinced to remain overnight for further observation since the half-life of naloxone is somewhat shorter than that of heroin, and he might need a second naloxone infusion.

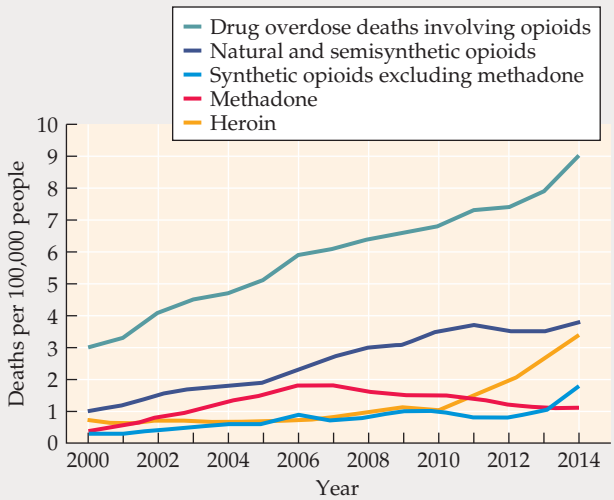
Naloxone administration in response to suspected opioid overdose has been the standard care of emergency medical professionals for many years. Based on this well-documented success, the distribution of naloxone to first responders, police, and firefighters has increased dramatically in much of the country. In addition, in many places family and friends of opioid users have been encouraged to have a source of naloxone available at all times to treat any sudden overdoses. It has been so effective in helping opioid abusers that take-home naloxone programs have been expanded in some regions to provide naloxone to all primary care patients who receive chronic opioid treatment for pain.

The number of opiate overdose deaths has jumped dramatically, to over 33,000 people in the United States in 2015 (CDC, 2016a). Opioid overdoses have tripled since 2000 because of the increased prescription of opioids for chronic pain and the upsurge in the use of illicit synthetic fentanyl along with heroin (see **Figure**; Rudd et al., 2016). Drug deaths are rising faster than ever, so the problem is extremely acute. The figure shows the number of opioid overdose deaths per 100,000 population according to the type of opioid involved.

Take-home naloxone is one of the few approaches with proven effectiveness in reducing opioid mortality. The rationale is that the use of naloxone in combination with calling 911 can provide enough time for the arrival of medical help to save lives. With a minimum of instructions, nonmedical personnel can easily administer the life-saving drug. The drug can be delivered by intramuscular injection, using a premeasured handheld auto-injector, or intranasally with an atomizer. A training video can be accessed at www.kelley-ross.com/naloxone-program/. Naloxone has no psychoactive effects except to reverse opioid-induced effects, hence there is no abuse potential. Naloxone distribution is not intended to be a treatment program but merely a temporary means of harm reduction in response to the epidemic of opiate-related deaths.

Despite its recognized effectiveness, there are multiple barriers to its widespread use beyond distribution by local agencies (Bazazi et al., 2010). There is low social priority to deal with this issue because of the stigma surrounding drug users and drug overdose. Unlike syringe exchange programs that reduce infection by HIV/AIDS and save health care costs, preventing overdose deaths does not have a clear economic benefit, which further reduces the political will. Despite expressed fears, there is no evidence

(*Continued*)



Opioid overdose deaths from 2000 to 2014 by type of opioid Opioids include drugs such as morphine, oxycodone, hydrocodone, heroin, methadone, fentanyl, and tramadol. Natural and semisynthetic opioids include morphine, codeine, oxycodone, and buprenorphine. Synthetic opioids include fentanyl, meperidine (Demerol), and propoxyphene (Darvon). (After Rudd et al., 2016.)

BOX 11.1 Clinical Applications (*continued*)

that providing a harm reduction safety net encourages increased drug consumption and riskier behavior. Individuals providing the naloxone may have concerns about administering it incorrectly or causing an adverse outcome, although adverse reactions are rare and those that occur are usually due to a secondary drug in the system. However, naloxone can precipitate an immediate and severe withdrawal, so the victim may be angry and agitated when resuscitated. Some bystanders administering naloxone may also be concerned about criminal liability if they were using illegal drugs with the overdose victim. Police response varies widely, and some states have laws providing some amount of legal protection.

One final obstacle to increased use of naloxone is cost. Although naloxone is a drug old enough to be available as a generic, prices for generic drugs have been rising. Naloxone in 2005 cost about $1 per milliliter vial, while in 2014 the price was $15. The cost of naloxone in more convenient new delivery systems can vary from $125 (for a nasal spray two-pack) to as much as $3,750 for two prefilled injectors that provide oral instructions for its use. High prices may limit accessibility to the drug-using community as well as to local governments providing naloxone to their first responders. U.S. Congress has made several attempts to address the problem and the U.S. House Judiciary Subcommittee on Regulatory Reform, Commercial and Antitrust Law has held a special hearing on the issue (Tirrell, 2017, provides more details).

to increased drug use. In a longitudinal survey with over 37,000 participants, Martins and colleagues (2012) found that presence of any mood disorder at time 1 predicted nonmedical opioid use at time 2 (3 years later), supporting the self-medication model of drug abuse, that is, drug use is initiated to reduce the individual's symptoms (see Chapter 9). However, there was also evidence that nonmedical opioid use or dependence at time 1 was associated with psychopathology at time 2, indicating that the relationship between opioid use and mood disorders is complex and bidirectional. Morphine also suppresses the cough reflex in a dose-dependent manner and has actions on the hypothalamus that lead to decreased appetite, drop in body temperature, reduced sex drive, and a variety of hormonal changes. Each of these effects can be associated with opioid receptors in particular areas of the CNS.

At slightly higher doses, particularly if the drug is administered intravenously or inhaled, the individual experiences an abnormal state of elation or euphoria, which is referred to as the "kick," "bang," or "rush" and is compared to a "whole-body orgasm." Nonaddicts describe it as a sudden flush of warmth located in the pit of the stomach. To achieve the maximum euphoria, very rapid penetration into the brain is needed. Although it is experienced as intense pleasure, the "rush" is not the principal basis for abuse but acts as a powerful reinforcer that encourages repeated drug use.

It is also important to know that the euphoric effect does not always accompany intravenous (IV) administration. For many individuals being medically treated, the drug may produce dysphoria, consisting of restlessness and anxiety. In addition, the nausea and vomiting that may accompany low doses of morphine are increased with higher doses. They are directly related to morphine's effect on the chemical trigger zone (the area postrema) in the brainstem that elicits vomiting. Although clearly unpleasant for most individuals, for the addict, the nausea may become a "good sick" because it is closely associated with the drug-induced euphoria by classical conditioning.

At the highest doses, the opioids' sedative effects become stronger and may lead to unconsciousness. Body temperature and blood pressure fall. Pupils are now quite constricted and represent a clinical sign of opioid overdose in a comatose patient. Respiration is dangerously impaired because of morphine's action on the brainstem's respiratory center, which normally responds to high blood CO_2 levels by triggering increased respiration. Respiratory failure is the ultimate cause of death in overdose.

Apart from the CNS, the effects of morphine are greatest on the gastrointestinal tract. Opium was used for relief of diarrhea and dysentery even before it was used for analgesia. It remains one of the most important lifesaving drugs because of its ability to cause constipation and stop the life-threatening fluid loss associated with diarrhea that accompanies many bacterial and parasitic illnesses especially prevalent in developing countries. Unfortunately, when opioids are used for pain management, constipation is a common and disturbing side effect that does not diminish even after prolonged use.

More modern treatment for diarrhea utilizes modified opioid molecules such as loperamide, which was designed so that it cannot cross the blood–brain barrier. The major advantage is that it effectively slows gastrointestinal function but does not have any effect

on the CNS at recommended doses. Unfortunately, at extraordinarily high doses it can flood the blood–brain barrier to enter the CNS and produce a mild euphoria. Opioid addicts have discovered that by taking 200 to 600 mg of loperamide rather than the maximum recommended dose of 8 mg, they can achieve a euphoric effect. Others use the drug to reduce withdrawal symptoms such as vomiting and muscle pains when morphine or heroin are not available. Such high doses are associated with extreme constipation as well as cardiovascular toxicity including irregular heartbeats and abnormal electrical conduction through the heart, leading to fatalities (Saint Louis, 2016). At present these over-the-counter antidiarrhea drugs are inexpensive and can be purchased in drug stores and supermarkets in large quantities. However, emergency medicine physicians and toxicologists are encouraging the FDA to limit the amount of the medication that can be purchased at one time, just as the decongestant pseudoephedrine purchase was limited to reduce the manufacturing of crystal meth.

Opioid Receptors and Endogenous Neuropeptides

The opioid drugs produce biobehavioral effects by binding to specific neuronal receptors. Since minor modifications of the morphine molecule produce significant changes in effect, analyses of the molecular structure of the drugs provide sufficient information to hypothesize definite structural features of the opioid receptor. Further, naloxone's blocking effects can be overcome by increasing concentrations of morphine, which demonstrates competition for the receptor. Not long after opioid receptors were identified, the natural neuropeptide ligands that act at the receptors were characterized.

Receptor binding studies identified and localized opioid receptors

Although the existence of opioid receptors was evident, the initial attempts to label and locate these receptors in brain tissue using standard radioligand binding methods (see Chapter 4) proved to be a difficult task. Ultimately, the opioid receptor was labeled (Pert and Snyder, 1973) by making several technical refinements in the assay and separation procedure, as well as by having access to newer radioactive ligands that had a greater amount of radioactivity per drug molecule. The receptors that they identified met the criteria described in Chapter 4. First, **FIGURE 11.5A** shows the classic binding curve, demonstrating that as the amount of radioactive opioid (in this case, the antagonist naloxone) is increased, binding also increases and gradually tapers off until the receptors are fully occupied. The leveling off of the binding curve at B_{max} shows that a finite number of receptors exist in a given amount of tissue. This saturation would not occur if the radioligand happened to be "sticky" and attached randomly to many cellular materials. Second, looking at the concentrations used in the assay makes it clear that the

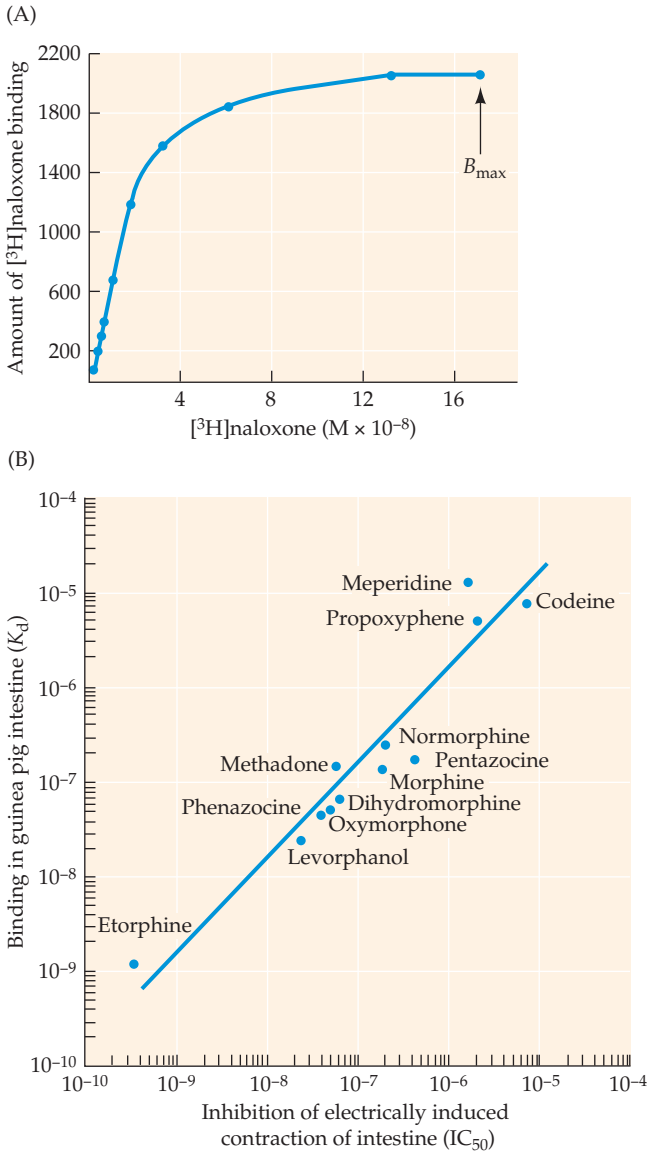


FIGURE 11.5 Opioid receptor binding (A) Binding of [^{3}H]naloxone to rat brain shows the saturation of opioid receptors. As the concentration of the opioid ligand (naloxone) increases, binding to the receptors increases steadily until the receptors are filled at B_{max}. (B) There is a strong positive correlation between the concentration of opioid drugs needed to inhibit electrically induced contraction of the intestine (IC_{50}) and the concentration needed to bind to opioid receptors in the same tissue. The results show clearly that drugs that bind readily at low concentrations of ligand (e.g., etorphine) also are effective in inhibiting the intestinal contraction at low doses. Drugs that bind less well (e.g., codeine) also require higher concentrations to inhibit the contraction. (A after Pert and Snyder, 1973; B after Snyder, 1977.)

binding sites have a high affinity for the opioids. Third, the binding was shown to be reversible, with a time course that matches the loss of physiological effectiveness. Fourth, the concentrations needed in the binding assay are meaningfully related to the concentration of agonist needed to elicit a biological response.

But how do we know that these sites are responsible for the opioids' pharmacological activity? Snyder (1977) calculated binding affinity by measuring the ability of a number of nonradioactive opioids to compete with radioactive naloxone for the receptors. They found that the relative potency of various opioids in the competition experiments closely paralleled their relative potencies in pharmacological effects on the intestine (**FIGURE 11.5B**). In this case, the pharmacological effect measured is the ability of opioids to inhibit electrically induced contraction of the ileum (the lowest portion of the small intestine). This inhibition occurs because opioids inhibit the release of neurotransmitter from stimulated nerves. Although many more-sophisticated methods are possible, opioid action on the ileum is considered a classic bioassay and is described in **Web Box 11.1**.

Once the receptors were labeled and characterized, autoradiography could be used to locate the receptors in the brain. **FIGURE 11.6** shows a color-enhanced distribution of opioid receptors in rat brain.

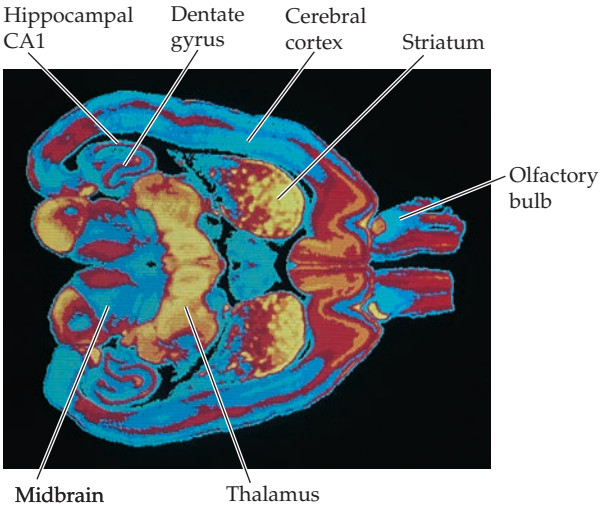


FIGURE 11.6 The distribution of opioid receptors in rat brain In this autoradiogram higher densities, seen as warmer colors, occur in the striatum, medial thalamus, locus coeruleus, periaqueductal gray, and raphe nuclei. (Courtesy of Miles Herkenham, NIMH.)

Four opioid receptor subtypes exist

Although the classic dose–response curves used by Martin and colleagues (1976) suggested that several subtypes of opioid receptors exist, researchers needed to develop highly *selective* radioactive ligands to directly label the subtypes. Selectivity means that a given molecule readily binds to one receptor subtype and has relatively low binding affinity for the others (Simon, 1991). The three classical subtypes have been called μ (mu), δ (delta), and κ (kappa). A more recent addition to the opioid receptor family is the nociceptin/orphanin FQ receptor (NOP-R). Based on genetic criteria, the NOP-R and classical opioid receptors belong to the same family. The same is true for the opioid peptides and the ligand for the NOP-R, nociceptin/orphanin FQ (see the following section). However, despite their familial relationship, opioids do not bind to NOP-R, nor does nociceptin/orphanin FQ bind to classical opioid receptors. The four opioid receptor subtypes have distinct distributions in the brain and spinal cord, which suggests that they mediate a wide variety of effects.

The **μ-receptor** is the receptor that has a high affinity for morphine and related opioid drugs. The location of the μ-receptors has been mapped by autoradiography in several species (Mansour and Watson, 1993), including human brain postmortem (Quirion and Pilapil, 1991). Other researchers have performed in vivo mapping using positron emission tomography (PET) imaging (Mayberg and Frost, 1990). The results consistently show a wide distribution of μ-receptors in both the brain and the spinal cord. The brain areas rich in μ-receptors (e.g., the medial thalamus, periaqueductal gray, median raphe, and clusters within the spinal cord) support their role in morphine-induced analgesia. Other high-density areas suggest a role in feeding and positive reinforcement (nucleus accumbens), cardiovascular and respiratory depression, cough control, nausea and vomiting (brainstem), and sensorimotor integration (thalamus, striatum) (Mansour and Watson, 1993; Carvey, 1998). **FIGURE 11.7A** is an autoradiogram of μ-receptor binding in the rat CNS.

The **δ-receptors** have a distribution similar to that of μ-receptors (**FIGURE 11.7B**) but are more restricted. They are predominantly found in forebrain structures such as the neocortex, striatum, olfactory areas, substantia nigra, and nucleus accumbens. Many of these sites are consistent with a possible role for δ-receptors in modulating olfaction, motor integration, reinforcement, and cognitive function. Delta receptors in areas overlapping μ-receptors suggest modulation of both spinal and supraspinal analgesia. Delta-receptor knockout mice and animals treated with δ-receptor antagonists show increased anxiety and depressive-like behavior in rodent tests while agonists reduce the emotional behaviors. However, human clinical trials with δ-receptor agonists showed minimal effects and suggest significant species differences (see Chu Sin Chung and Kieffer [2013] for a review of δ-opioid receptor function).

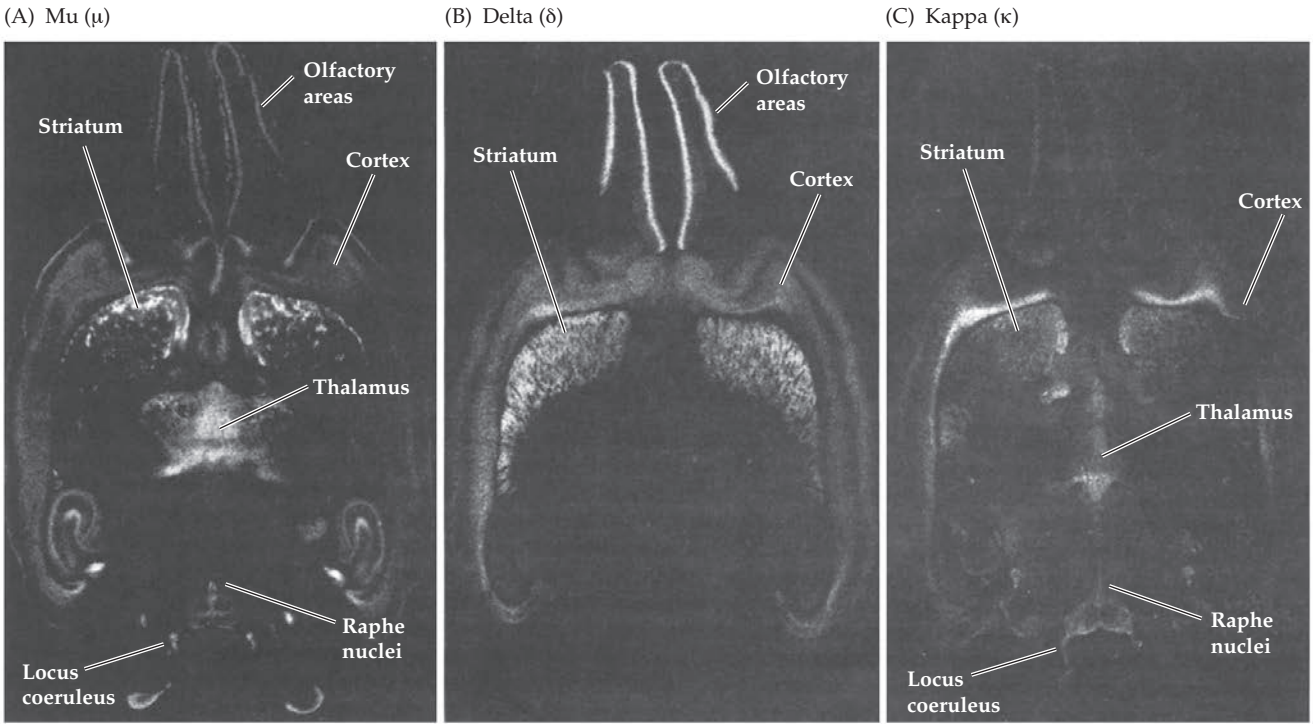


FIGURE 11.7 Autoradiograms of opioid receptor subtype binding in rat brain Notice the distinct locations of (A) μ-, (B) δ-, and (C) κ-receptors. (From Mansour et al., 1988.)

The **κ-receptors** (**FIGURE 11.7C**) have a very distinct distribution compared with the μ- and δ-receptors. The κ-receptor was initially identified by high-affinity binding to ketocyclazocine, which is an opioid analog that produces hallucinations and dysphoria. This receptor is also found in the striatum and amygdala but additionally has a unique distribution in the hypothalamus and pituitary. These receptors may participate in the regulation of pain perception, gut motility, and dysphoria but also modulate water balance, feeding, temperature control, and neuroendocrine function.

The **NOP-R** are widely distributed in the CNS and the peripheral nervous system. They are found in high concentration in the cerebral cortex and the limbic areas, including the amygdala, hippocampus, and hypothalamus, as well as the periaqueductal gray, thalamus, brainstem nuclei including the raphe nuclei, and spinal cord. The receptor localization suggests a role in analgesia, feeding, learning, motor function, and neuroendocrine regulation.

Almost 20 years after the initial successful receptor binding studies, the genetic material for each of the four receptor types was isolated. Then it was inserted into cells (a process called **transfection**) maintained in culture to produce large numbers of identical cells (cloning). The **receptor cloning** and molecular sequencing of the opioid receptor subtypes provided several key pieces of information:

1. For each of the receptors, we now know the specific nucleic acid sequence making up the DNA that directs the synthesis of each receptor protein.
2. Using the nucleic acid sequence, the amino acids of the protein can be identified and compared with other families of receptor proteins.
3. The transfected cells can be used to study the intracellular changes induced by receptor agonists.
4. By radioactively labeling the genetic material, in situ hybridization makes it possible to visualize those cells in the brain that synthesize the receptor protein and more precisely localize the receptors themselves. (See Chapter 4 for a review of the techniques of molecular biology.)

Although the first opioid receptor to be successfully cloned was the δ-receptor (Evans et al., 1992; Kieffer et al., 1992), cloning of the others was soon to follow. Each of the four receptors has between 370 and 400 amino acids, and they bind with the ligands specific to each. All four of the protein receptors have a structure similar to the family of receptors that are linked to G proteins, which suggests that they mediate metabotropic (rather than ionotropic) responses. The structure of the δ-receptor, with the classic seven transmembrane portions, is shown in **FIGURE 11.8**.

A great diversity in opioid signaling exists because interactions occur among the receptor subtypes

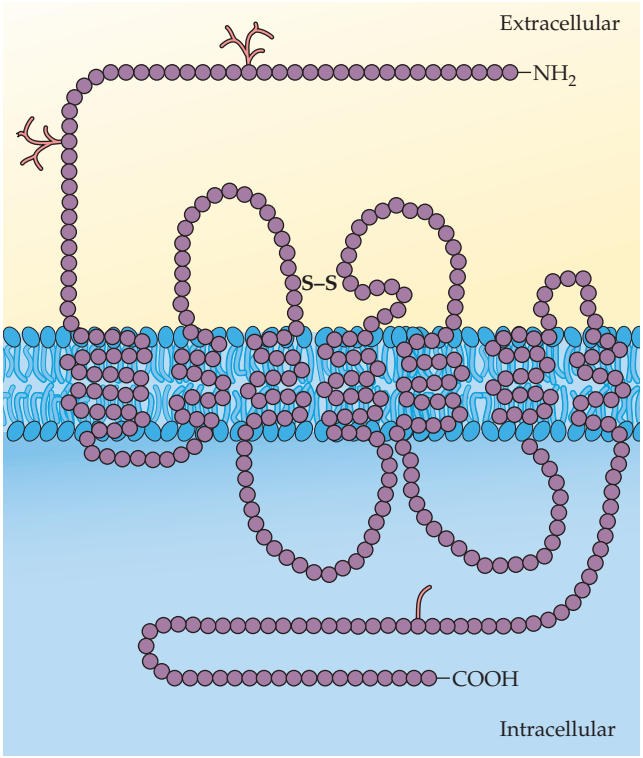


FIGURE 11.8 Proposed structure of the δ-opioid receptor Each circle represents an identified amino acid. The seven regions spanning the cell membrane are typical for receptors that are coupled to G proteins.

forming heteromeric complexes. Such heteromeric associations exist for κ–δ, μ–nociceptin, μ–κ, and μ–δ interactions (recently reviewed by Costantino and colleagues [2012]). These associations of two different receptor subtypes alter opioid binding and intracellular events. What this means is that the interaction of the two receptors could enhance or diminish opioid drug effects. For example, low subanalgesic doses of a δ-receptor ligand enhanced morphine-induced analgesia (acting on the μ-receptor) in the tail-flick test. Of further significance is the finding that both in vivo and in vitro exposure to chronic (but not acute) morphine increases the co-localization of μ–δ-receptor heteromers in the cell membrane of neurons in brain areas important to pain signaling. This enhanced receptor interaction is apparently a homeostatic process related to the development of tolerance. In fact, interfering with the μ–δ heteromer-mediated functions prevented the development of tolerance to chronic morphine. Understanding the function and pharmacology of this heteromer has the potential to improve opioid therapies by producing analgesia with a minimum of tolerance. **BOX 11.2** will tell you more about further developments in the fascinating story of opioid pharmacology.

Several families of naturally occurring opioid peptides bind to these receptors

It was not long after the receptors were initially identified that researchers were quick to ask why the nervous system would have receptors for the derivatives of the opium poppy. It seemed more reasonable to hypothesize that an endogenous neurochemical must exist to act on opioid receptors.

DISCOVERY In 1974 two different laboratories identified a peptide in brain extracts and other tissues that mimicked opioid activity (electrophysiologically and in a mouse vas deferens bioassay) and could also bind to opioid receptors (Terenius and Wahlstrom, 1974; Hughes, 1975). They named the first peptide *enkephalin*, meaning "in the brain." Soon a number of peptides were found to have these properties and were called endogenous opioids, or **endorphins** (from *endo*, signifying "endogenous," and *orphin*, from *morphine*). The great similarity in structure among the peptides led researchers to conclude that there were several larger peptides, called propeptides (or precursor peptides), that are broken into smaller active opioids. Any confusion was resolved when molecular biologists found that there are four large propeptides and each is coded for by a separate gene. These large propeptides are called **prodynorphin** (254 amino acids), **pro-opiomelanocortin** or **POMC** (267 amino acids), **proenkephalin** (267 amino acids), and **pronociceptin/orphanin FQ** (180 amino acids). Each of the large propeptides manufactured in the soma must be processed by enzymes (called proteases) that are packaged in the Golgi apparatus along with them. These enzymes are responsible for chopping or cleaving the propeptides into individual peptide products that are stored in vesicles and are further processed as they are transported down the axon to be released at the synapse. Each of the large propeptides produces a number of biologically active opioid and non-opioid peptides (**FIGURE 11.9**). Some years later, Zadina and colleagues (1999) described a group of peptides with a distinct structure and distribution in the CNS. These peptides, called **endomorphins**, bind quite selectively to the μ-receptor and are as potent as morphine in relieving pain. Thus far, their propeptide has not been identified.

LOCALIZATION Mapping of the pathways utilizing the endogenous opioids was achieved by in situ hybridization to visualize propeptide mRNA, and immunohistochemistry was used to localize the propeptide itself (see Chapter 4). These propeptides are found in the brain, spinal cord, and peripheral autonomic nervous system, where their opioid products act as neurotransmitters and neuromodulators at specific synapses. The propeptides are concentrated in areas related to pain

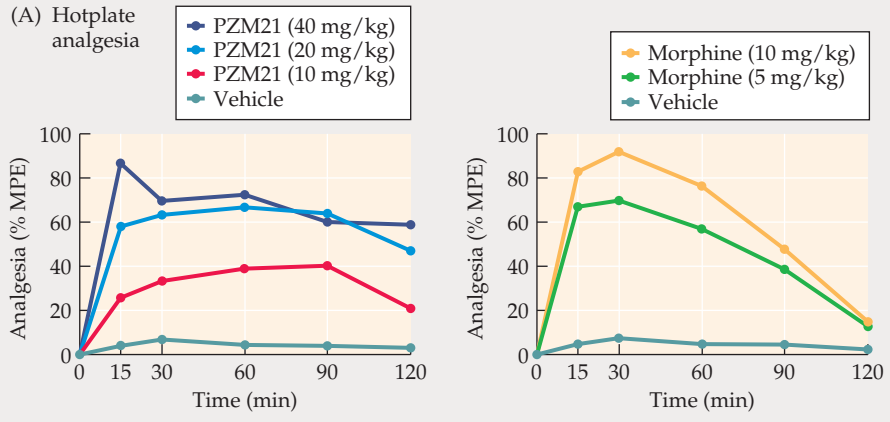
BOX 11.2 The Cutting Edge

Science in Action

Science and technology continue to build on past research to further the understanding of opioid transmission. In addition to labeling and localizing the receptors and cloning them to determine amino acid sequence, researchers now are using the latest methods in membrane protein crystallization. This method involves the solubilization, purification, and crystallization of receptor proteins to determine their three-dimensional structure with ultra-high resolution, down to individual atoms. Although images of the crystal structure of proteins had been achieved as early as the 1950s, the proteins crystallized were all water-soluble. However, proteins such as the signaling receptors coupled to G proteins (including the opioid receptors) are embedded in the phospholipid membrane and are poorly soluble in either water or oil. Crystallization was also made more difficult because G protein–coupled receptors move within the membrane when activated by the appropriate ligand (i.e., ligand binding causes a conformational change or movement in the receptor, which activates the G protein; see Chapter 3). Additional technical problems exist, but in 2011 Kobilka and his team (Rasmussen et al., 2011) developed a modified membrane protein crystallization technique to image the β_2-adrenergic receptor at just the precise instant when it was activated by a hormone and transferred the signal to the G protein. This extraordinary technological achievement and decades of earlier research to characterize the receptor led to the 2012 Nobel Prize in Chemistry for Brian Kobilka and Robert Lefkowitz (his mentor). To read more about the decades of research leading to this prize, read "The Nobel Prize in Chemistry 2012: Popular Information" on the Nobelprize.org website (Nobelprize.org, 2014).

Based on their work, modification of the crystallization technique was applied to the imaging of the opioid receptors. Since 2012, the crystal structures of all four opioid receptors have been determined. Despite the extensive chemical manipulation required, these engineered receptors, when expressed in cells in culture, still showed their ligand selectivity and affinity and were able to initiate appropriate intracellular functions. The four receptors have similar deep binding "pockets" located in the center that are open to the extracellular fluid. Details of ligand–receptor binding are discovered by creating mutations in selective amino acid residues. For example, mutating the charged amino acid asparagine (Asp) to a noncharged alternative amino acid prevents opioid activity. That suggests that the negatively charged Asp residue forms an ionic interaction with a positively charged group in the opioid ligands. The crystallized receptors, although similar, also have distinct molecular features, which are likely responsible for their selectivity for particular opioid agonists. Describing the molecular interactions of these receptors is beyond the scope of this chapter, but Cox (2013) and Shang and Filizola (2015) provide greater detail for those who are interested. Suffice it to say that with insight into the crystal structures of the receptors and detail of the mechanism of signal transduction, it is hoped that structure-based drug design may develop new pain-killing medications. These medications would interact with amino acid residues in a precise

(*Continued*)

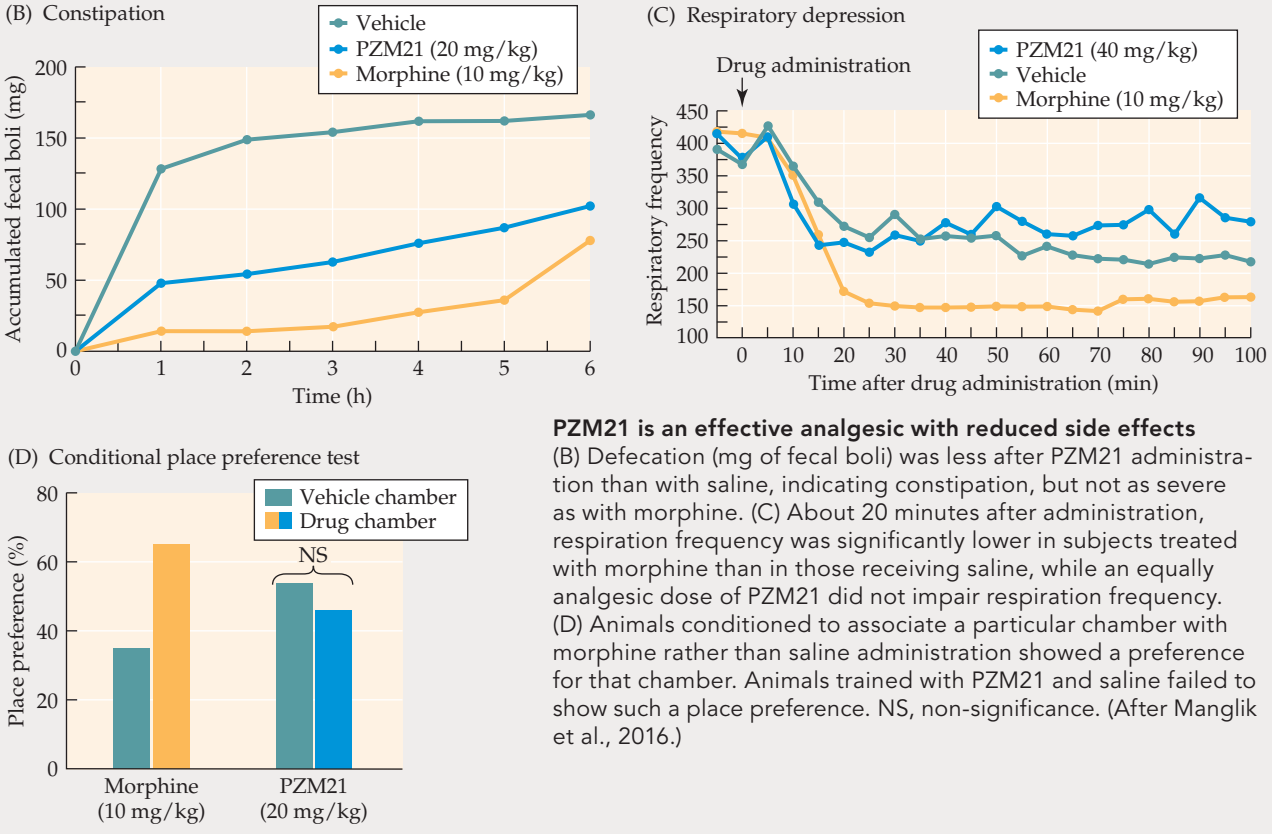


PZM21 is an effective analgesic with reduced side effects
(A) Dose-response curves for PZM21-induced analgesia (left) and morphine-induced analgesia (right). Latency to withdraw from a heated surface in the hot plate test is expressed as a percentage of the maximal possible effect (% MEP). The *x*-axis represents the time after subcutaneous administration of drug or saline (vehicle). The analgesic effect of 40 mg/kg of PZM21 was comparable to the effect of 10 mg/kg of morphine.

BOX 11.2 The Cutting Edge *(continued)*

fashion to produce distinct, predictable clinical benefits with fewer troublesome side effects.

Although it has taken a few years and the collaboration of a large number of international researchers specializing in different disciplines, one new custom-engineered drug ligand that acts at Kobilka's three-dimensional atomic structure of the μ-opioid receptor has been identified (Manglik et al., 2016). Using computer modeling techniques developed in the 1980s, the researchers evaluated ligand–receptor interactions for large libraries of over 3 million different molecules. They then used techniques in medicinal chemistry to enhance specificity and affinity of the new molecule to the μ-opioid receptor. The new ligand with the best fit, PZM21, was then preclinically tested in animal behavioral models (see Chapter 4). Dose-dependent PZM21 analgesia in the mouse hot plate test reached a maximal response of 87%, comparable to morphine's maximal effect of 92% (**Figure A**). The analgesic effects of the new molecule were absent in μ-opioid receptor knockout mice, demonstrating that PZM21 analgesia depends upon that receptor. The analgesic effects of PZM21 lasted longer than those of morphine, which would make it easier to administer clinically. Also, PZM21 had fewer significant side effects normally produced by morphine, specifically constipation and respiratory depression. **Figure B** shows that although PZM21 reduced defecation compared with controls, it had less of a constipating effect than morphine. Furthermore, while morphine greatly depressed the respiration rate, PZM21 was no different than vehicle in reducing respiration frequency (**Figure C**). The finding that PZM21 did not activate the dopaminergic reward pathway nor induce a conditioned place preference (**Figure D**) indicates that the molecule is unlikely to cause the typical opioid reinforcement and abuse. Although it would be premature to applaud the development of a new wonder drug, the science behind this discovery may mean that the structure-based approach used by these neuroscientists will ultimately help to develop a less hazardous pain control medication for those who are suffering.



PZM21 is an effective analgesic with reduced side effects
(B) Defecation (mg of fecal boli) was less after PZM21 administration than with saline, indicating constipation, but not as severe as with morphine. (C) About 20 minutes after administration, respiration frequency was significantly lower in subjects treated with morphine than in those receiving saline, while an equally analgesic dose of PZM21 did not impair respiration frequency. (D) Animals conditioned to associate a particular chamber with morphine rather than saline administration showed a preference for that chamber. Animals trained with PZM21 and saline failed to show such a place preference. NS, non-significance. (After Manglik et al., 2016.)

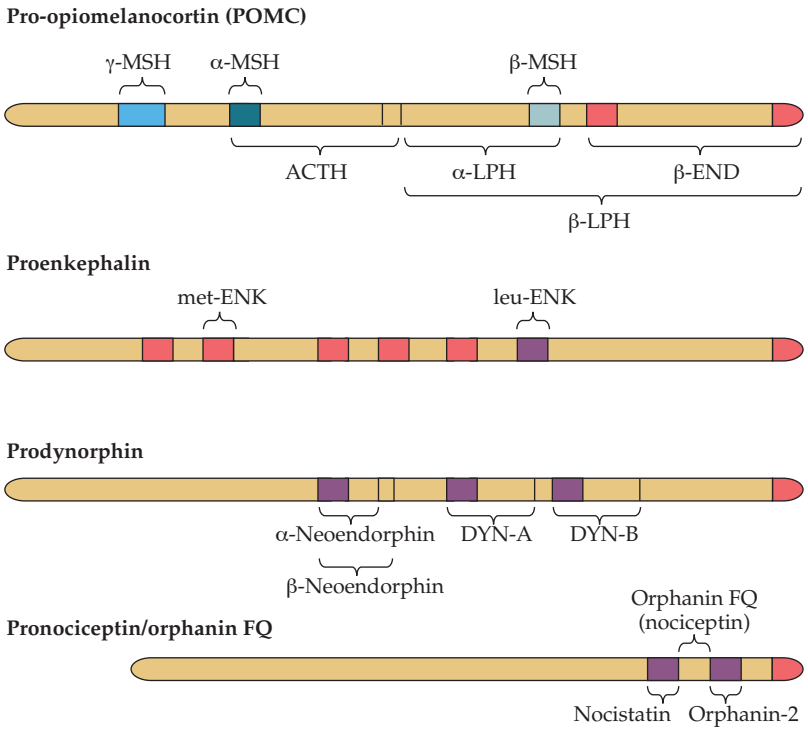


FIGURE 11.9 The four opioid propeptides and some of their possible products POMC is cleaved into β-endorphin (β-END) and a number of other peptides, including γ- and α-melanocyte-stimulating hormone (MSH), β-MSH, adrenocorticotropic hormone (ACTH), and several forms of lipotropin (LPH). Proenkephalin cleavage produces several copies of met- and leu-enkephalin (ENK). Prodynorphin contains α- and β-neoendorphin, as well as dynorphin (DYN) A and B. Pronociceptin/orphanin FQ cleavage produces one copy each of orphanin FQ, orphanin-2, and nocistatin. Tiny enkephalin peptides are also frequently found within the larger peptide fragments.

modulation and mood. In addition, POMC is found in particularly high concentration in the pituitary gland, which releases a variety of hormones in response to hypothalamic releasing factors. The hypothalamus releases corticotropin-releasing factor (CRF) in response to stress, which in turn increases adrenocorticotropic hormone (ACTH) release from the pituitary and ultimately glucocorticoids from the adrenal cortex (**FIGURE 11.10**). CRF also causes a rapid increase in POMC mRNA and subsequent increases in release of β-endorphin from the pituitary. A variety of stressors, such as painful foot shock, restraint, and swim stress, increase both CRF mRNA and POMC mRNA and induce analgesia that can be partially blocked by naloxone. Hence that stress-induced analgesia must be mediated in part by the release of endogenous opioids. The intimate relationship of these neuropeptides provides a physiological link between pain and stress regulation (Young, 1993). Overall, the widespread locations of the peptides strongly implicate them in many functions, including pain suppression, reward, motor coordination, endocrine function, feeding, body temperature and water regulation, and response to stress. **Web Box 11.2** describes some of the effects of opioids on feeding.

While some of the neurons containing the opioid propeptides have long projections, many more are small cells that form local circuits. Many of the peptides are co-localized with other neurotransmitters in the same neuron, including acetylcholine, GABA (γ–aminobutyric acid), serotonin, catecholamines, and other peptides. When peptides coexist with other neurotransmitters, they are likely to have a neuromodulatory role, that is, they modify the function of the neurotransmitter or produce changes in ion conductance and membrane potential.

Although we have four peptide families plus the endomorphins and three principal receptor subtypes, the peptides are not selective for a receptor type but show only a relative preference. The natural ligands for the δ-receptors are thought to be those derived from proenkephalin (enkephalins), and products from prodynorphin (dynorphin) are likely the natural κ-receptor agonists. The endomorphins bind preferentially to the μ-receptors, while POMC peptides (endorphins) bind readily to both μ- and δ-receptors. Since opioids do not bind to NOP-R, the receptor remained an "orphan" with no neuropeptide until two groups of researchers isolated a distinct peptide that one group called nociceptin because, in contrast to opioids, it *lowers* pain threshold. Since the other group called the same peptide orphanin FQ, it is now called nociceptin/orphanin FQ (N/OFQ). Pronociceptin/orphanin FQ and its peptides deserve a bit more discussion because although they are similar to the opioids, there are significant differences. The prohormone is widely distributed throughout the brain and spinal cord, with especially high concentrations in limbic regions. Although distribution of its peptides is different from that of the classical opioid peptides, in other instances they are found to be co-localized with the opioids. Significant evidence suggests that there is *reciprocal* modulation of N/OFQ neurons

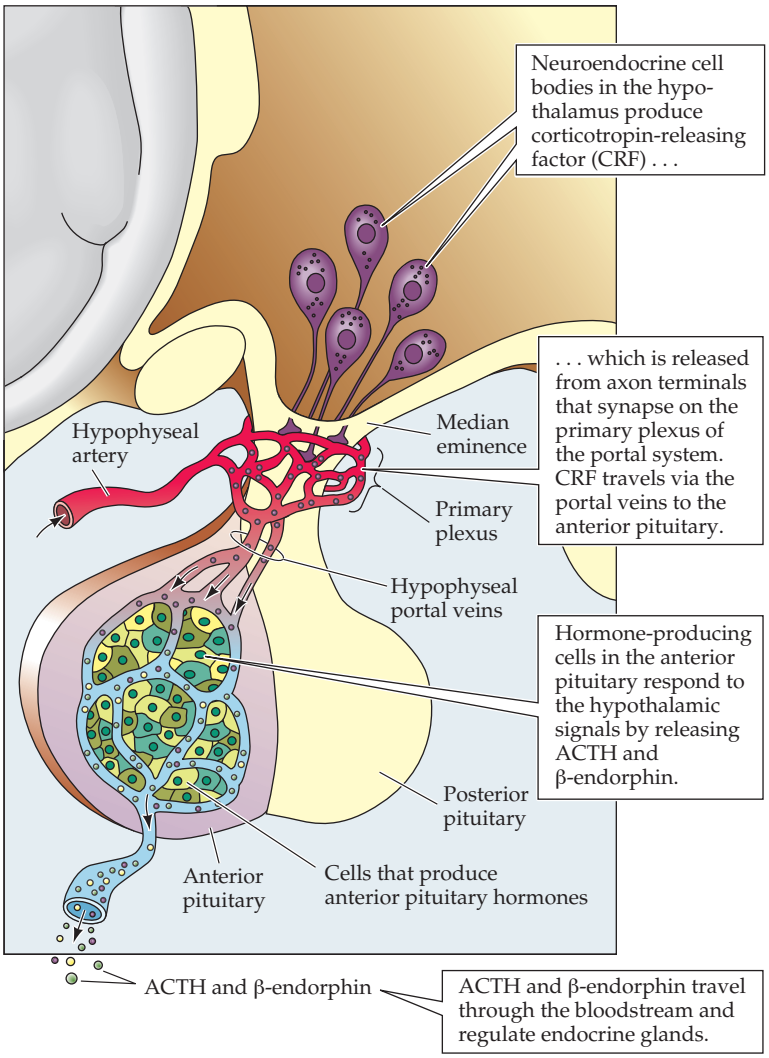


FIGURE 11.10 Hypothalamic control of ACTH and β-endorphin release
The hypothalamus releases CRF, which causes the anterior pituitary to secrete ACTH, which in turn acts on the adrenal gland to prepare the individual to deal with stress. CRF also influences β-endorphin synthesis and release from the pituitary in response to stress. Notice in Figure 11.9 that ACTH and β-endorphin come from the same propeptide, POMC.

and the classical opioids. Additionally, it is clear that although N/OFQ and the opioid peptides in some cases have similar effects, in other cases N/OFQ may cause effects opposite to those caused by the opioids. For example, although it is an analgesic at the spinal cord level, in supraspinal regions it is pronociceptive and has anti-opioid properties, including suppressing opioid-mediated analgesia. However, the complex action on analgesia is dependent on species, dose, assays, and pain modalities. For instance, it produces only analgesia in both spinal and supraspinal sites in non-human primates (reviewed by Kiguchi et al., 2016). Other effects include impairing motor performance, suppressing spatial learning, inducing feeding, and regulating stress-induced release of pituitary hormones. The peptide has many other functions and some that remain undiscovered. **TABLE 11.1** summarizes receptor subtype locations, functions, and preferences for endogenous opioids. For further

TABLE **11.1** Locations, Functions, and Endogenous Ligands for Opioid Receptor Subtypes

Receptor subtype	Endogenous ligands (prohormone sources)	Locations (most dense)	Functions
μ	Endomorphins (unknown), endorphins (POMC)	Thalamus, periaqueductal gray, raphe nuclei, spinal cord, striatum, brainstem, nucleus accumbens, amygdala, hippocampus	Analgesia, reinforcement, feeding, cardiovascular and respiratory depression, antitussive, vomiting, sensorimotor integration
δ	Enkephalin (proenkephalin), endorphins (POMC)	Neocortex, striatum, substantia nigra, nucleus accumbens, spinal cord, hippocampus, amygdala, hypothalamus	Analgesia, reinforcement, cognitive function, olfaction, motor integration
κ	Dynorphins (prodynorphin)	Pituitary, hypothalamus, amygdala, striatum, nucleus accumbens	Neuroendocrine function, water balance, feeding, temperature control, dysphoria, analgesia
NOP-R	Nociceptin/orphanin FQ (pronociceptin/orphanin FQ)	Cortex, amygdala, hypothalamus, hippocampus, periaqueductal gray, thalamus, substantia nigra, brainstem, spinal cord	Spinal analgesia, supraspinal pronociception, feeding, learning, motor function, neuroendocrine function

detail, you may turn to the thirty-eighth annual review of the behavioral effects of molecular, pharmacological, and genetic manipulations of the endogenous opioid system (Bodnar, 2017).

Opioid receptor–mediated cellular changes are inhibitory

You are already aware that each of the four opioid receptor types is linked to G proteins. You may recall from Chapter 3 that there are multiple forms of G proteins that have two principal actions. Some G proteins directly stimulate or inhibit the opening of ion channels (see Figure 3.14A), and others stimulate or inhibit enzymes to alter second-messenger production (see Figure 3.14B). Opioids and N/OFQ work by both of those mechanisms to open potassium (K^+) channels, close calcium (Ca^{2+}) channels, and inhibit adenylyl cyclase activity. The overall effects of the neuropeptides on nerve cell function include the reduction of membrane excitability and subsequent slowing of cell firing and inhibition of neurotransmitter release.

The neuropeptides reduce synaptic transmission in three principal ways: (1) by postsynaptic inhibition, (2) through axoaxonic inhibition, and (3) via presynaptic autoreceptors. First, opioid- and N/OFQ receptor–G protein opens K^+ channels, which increases K^+ conductance. Potassium exits the cell, forced by its concentration gradient, causing hyperpolarization. When the receptors are on the soma or dendrites of neurons, the hyperpolarization decreases the cell's firing rate (**FIGURE 11.11A**).

Second, opioids and N/OFQ also produce an inhibitory effect by closing voltage-gated Ca^{2+} channels. In this case (**FIGURE 11.11B**), receptors on the presynaptic terminal activate G proteins, which in turn close the Ca^{2+} channels. Reducing the amount of Ca^{2+} entering during an action potential proportionately decreases the amount of neurotransmitter released. For example, opioid-induced inhibition of norepinephrine and dopamine release has been found in many brain areas. As expected, this effect is prevented by the receptor antagonist naloxone. Note that naloxone does not antagonize N/OFQ because N/OFQ does not bind to classical opioid receptors. Likewise, NOP-R antagonists, such as SB-612,111, would not be expected to prevent opioid-mediated effects on Ca^{2+}

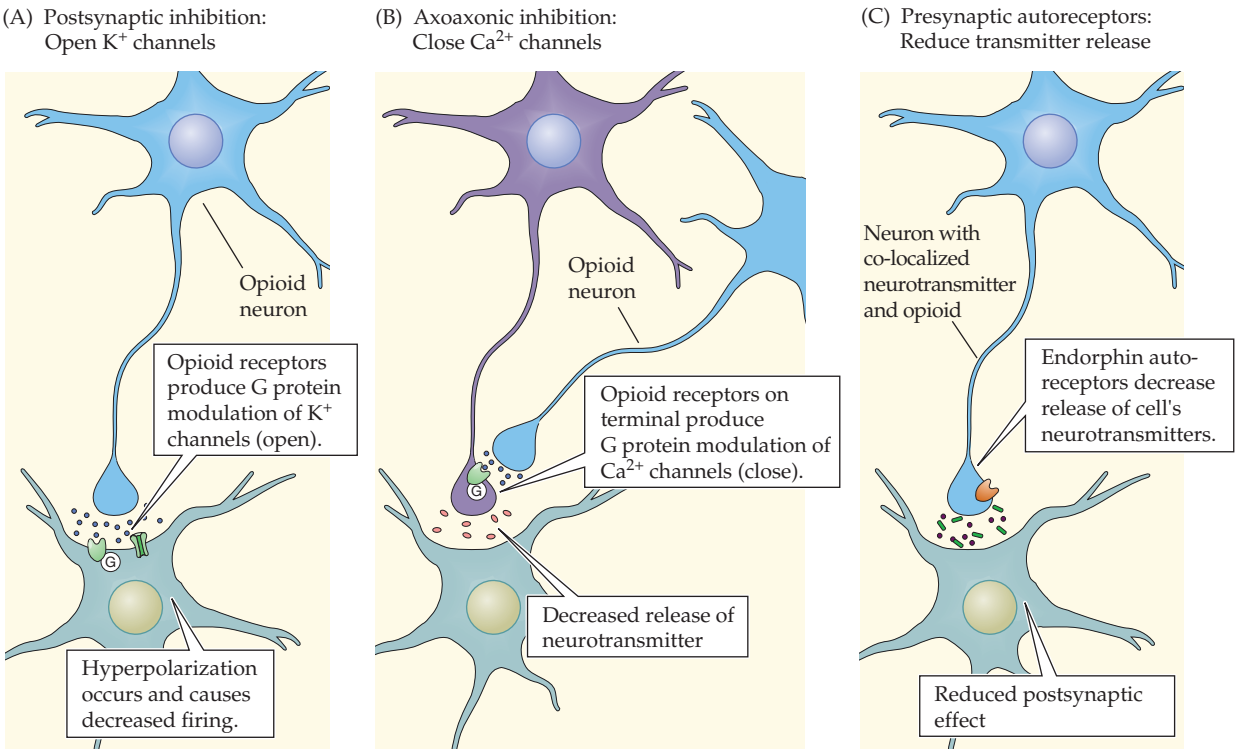


FIGURE 11.11 Inhibitory actions of endogenous opioids Opioids inhibit nerve activity in several ways. (A) Opioids bind to receptors that activate a G protein that opens K^+ channels to hyperpolarize the postsynaptic cells, thereby reducing the rate of firing. (B) Opioid receptors on nerve terminals (axoaxonic) activate G proteins that close Ca^{2+} channels, reducing the release of the neurotransmitter. (C) Presynaptic autoreceptors activate G proteins and reduce the release of a co-localized neurotransmitter. The mechanism may involve closing Ca^{2+} channels or opening K^+ channels that hyperpolarize the presynaptic cell.

channels. The inhibition of glutamate and substance P release in the spinal cord is of particular significance because those neurotransmitters are released from the afferent sensory neurons that transmit pain signals from the periphery into the CNS (see the section on opioids and pain).

Third, opioid autoreceptors also produce inhibitory effects. Somatodendritic autoreceptors hyperpolarize cells in the locus coeruleus by enhancing K^+ conductance and subsequently reducing cell firing (not shown in figure). Elsewhere, presynaptic autoreceptors reduce the release of co-localized neurotransmitters (**FIGURE 11.11C**). In summary, neuropeptide effects on both K^+ and Ca^{2+} channels produce inhibitory effects and reduce neurotransmitter release. These actions in the appropriate circuitry are ultimately responsible for the analgesic effects (DiChiara and North, 1992).

All four types of opioid receptor are also coupled to inhibitory G proteins (called G_i) that inhibit adenylyl cyclase, which normally synthesizes the second messenger cyclic adenosine monophosphate (cAMP). The reduced cAMP and subsequent decreased function of cAMP-dependent protein kinase may in part be responsible for opioid-induced ion channel changes; however, the immediate effects of the inhibition on cell function are not entirely clear. Nevertheless, the cAMP cascade has been implicated in chronic effects of opioids, including drug tolerance, dependence, and withdrawal. These topics are discussed in later sections.

Section Summary

- Opiates are natural narcotics derived from the opium poppy. Other narcotics are semisynthetic or totally synthetic.
- Minor differences in molecular structure determine whether the drugs are full agonists, partial agonists, or pure antagonists.
- Opioids relieve pain and produce drowsiness and sleep. Euphoria or dysphoria may occur. Opioids cause pinpoint pupils, vomiting, suppression of the cough reflex, drop in body temperature, reduced appetite, constipation, and a variety of hormonal effects. Respiratory and cardiac depression may also occur at higher doses.
- Repeated use produces tolerance to many but not all of the drugs' effects, as well as physical dependence.
- There are four opioid receptors (μ, δ, κ, and NOP-R), which are widely and unevenly distributed in the central and peripheral nervous systems. Opioids and opioid neuropeptides bind to the three classical receptors but not to NOP-R. Nociceptin/orphanin FQ is the neuropeptide that binds to NOP-R but not to the three classical receptors. Each of the receptors has been isolated and cloned and examined with membrane protein crystallization to visualize in ultra-high resolution their three-dimensional structures. All four are coupled to G proteins that induce metabotropic effects. The principal cellular activities include actions on ion channels (open K^+, close Ca^{2+}), which are responsible for cell hyperpolarization and inhibition of neurotransmitter release, and inhibition of adenylyl cyclase.
- Endogenous ligands for the opioid receptors are small peptides that are cleaved from larger propeptides manufactured in the soma. Molecular biology has shown four distinct propeptides (prodynorphin, POMC, proenkephalin, and pronociceptin/orphanin FQ), which produce a variety of opioid and non-opioid fragments. The propeptide for a fifth endogenous peptide endomorphin is not known.
- Endogenous opioids inhibit synaptic transmission by postsynaptic inhibition, by axoaxonic inhibition, and via presynaptic autoreceptors.
- The locations of these peptides in the brain, spinal cord, and pituitary implicate them in regulating pain, reward, stress response, water balance, feeding, body temperature, and endocrine function. Nociceptin/orphanin FQ acts at cellular and molecular levels much the same way as opioids but often produces different and opposing effects. See Table 11.1 for locations, functions, and endogenous ligands for opioid receptor subtypes.

Opioids and Pain

Although we all feel that we intuitively understand what pain is like, it is really far more complex than is generally believed. Since opioids are therapeutically best known as analgesics, a further discussion of pain and its neural circuitry is needed.

Pain is distinct from other sensory systems in that it can be caused by a variety of stimuli detected by several types of nociceptors (detectors of noxious stimuli). The nociceptors are networks of free nerve endings that are sensitive to intense pressure, extreme temperature including heat and cold, electrical impulses, cuts, chemical irritants, and inflammation. Pain varies not only in intensity but also in quality and may be described as "pricking," "stabbing," "burning," "aching," and so forth. Its perception is also highly subjective, and

no single stimulus will be described as painful by all individuals nor perhaps even by the same individual under different circumstances. Pain is modified by a number of factors, including strong emotion, environmental stimuli like stress, hypnosis, acupuncture, and opioid drugs.

Although we can get subjective reports of pain, quantification is difficult, particularly when analgesic drugs are tested. In the laboratory, when methods such as the application of sudden pressure, pinpricks, or stabs are used to induce pain, most analgesic drugs show ineffective or inconsistent analgesic effects. The failure of these drugs to show a significant reduction in pain is probably a result of the low emotional impact of those types of pain. More consistent results are obtained with the analgesics through the use of techniques that produce slowly developing or sustained pain. One technique used with humans is to stop blood flow to an exercising muscle with a tourniquet. With this method, the pain is slow in onset and is directly related to amount of exercise. Cutaneous pain in humans can be produced by the intradermal injection of various chemicals. A reliable method to test this kind of pain uses cantharidin to induce a blister, from which the outer layer of epidermis is removed to expose the blister base, on which small quantities of various agents can be applied for testing. Techniques that have been designed to produce more-intense or more-persistent pain are infrequently used because finding people willing to participate in such experiments is difficult. Animal testing is overall more reliable, yielding conditions that are comparable to pathological pain in humans. This may be because the human participant in the experimental setting realizes that the pain stimulus poses no real threat, whereas for the animal subject, all pain is potentially serious. Animal tests are described in Chapter 4.

The two components of pain have distinct features

Pain is often described as having several components. "First," or early, pain represents the immediate sensory component and signals the onset of a noxious stimulus and its precise location to cause immediate withdrawal and escape from the damaging stimulus. "Second," or late, pain has a strong emotional component, that is, the unpleasantness of the sensation. Adaptation occurs more slowly to the secondary component, so it attracts our attention in prolonged fashion to motivate behaviors that limit further damage and aid recovery. Late pain is less localized and is often accompanied by autonomic responses such as sweating, fall in blood pressure, or nausea. The separation of these two components can be clearly seen in the patient who after receiving morphine for persistent pain, describes the pain as just as intense as before treatment but much less aversive.

These distinct components of pain are in part explained by the types of neuron that carry the signals. Fibers called Aδ are larger in diameter and are myelinated, so they conduct action potentials more rapidly than the thin and unmyelinated C fibers. The difference in speed explains why when you smash your finger in the car door, you first experience a sharp pain that is well localized but brief, followed by a dull aching that is a prolonged reminder of the damage your body has experienced. These neurons have their cell bodies in the dorsal root ganglia and terminate in the gray matter of the dorsal horn of the spinal cord, ending on projection neurons that transmit pain signals to higher brain centers (**FIGURE 11.12**).

A second distinction between the two components of pain is their route and final destination in the brain. Early pain is transmitted from the spinal cord via the spinothalamic tract to the posteroventrolateral (PVL) nucleus of the thalamus before going directly to the primary and then secondary somatosensory cortex. The primary somatosensory cortex provides sensory discrimination of pain, while the secondary cortex is involved in the recognition of pain and memory of past pain. Late pain also goes to the thalamus, but in addition it gives off collaterals to a variety of limbic structures such as the hypothalamus and amygdala, as well as the anterior cingulate cortex. The anterior cingulate has a role in pain affect, attention, and motor responses (Rainville, 2002).

Recently researchers were able to demonstrate the temporal relationship between pain-evoked cortical activation and reported pain in humans. Ploner and colleagues (2002) subjected individuals to brief painful laser stimuli and continuously monitored the study participants' subjective pain ratings while simultaneously recording faint magnetic fields on the surface of the skull using magnetoencephalography (MEG). Although MEG is somewhat inaccurate in precisely locating brain activity, it is excellent at showing the neural changes over very small units of time (from one millisecond to another). In this way, Ploner could trace a wave of brain activity from its origin to sequential brain areas during processing (**FIGURE 11.13A**). When the cortical activation was superimposed on magnetic resonance images (**FIGURE 11.13B**), they showed that first pain (pain recognition), identified by participants' ratings, was temporally related to activation of the primary somatosensory cortex, whereas second pain (identified by participants' ratings of unpleasantness) was strongly associated with anterior cingulate activation. Both types of pain were associated with neural activity in the secondary somatosensory cortex.

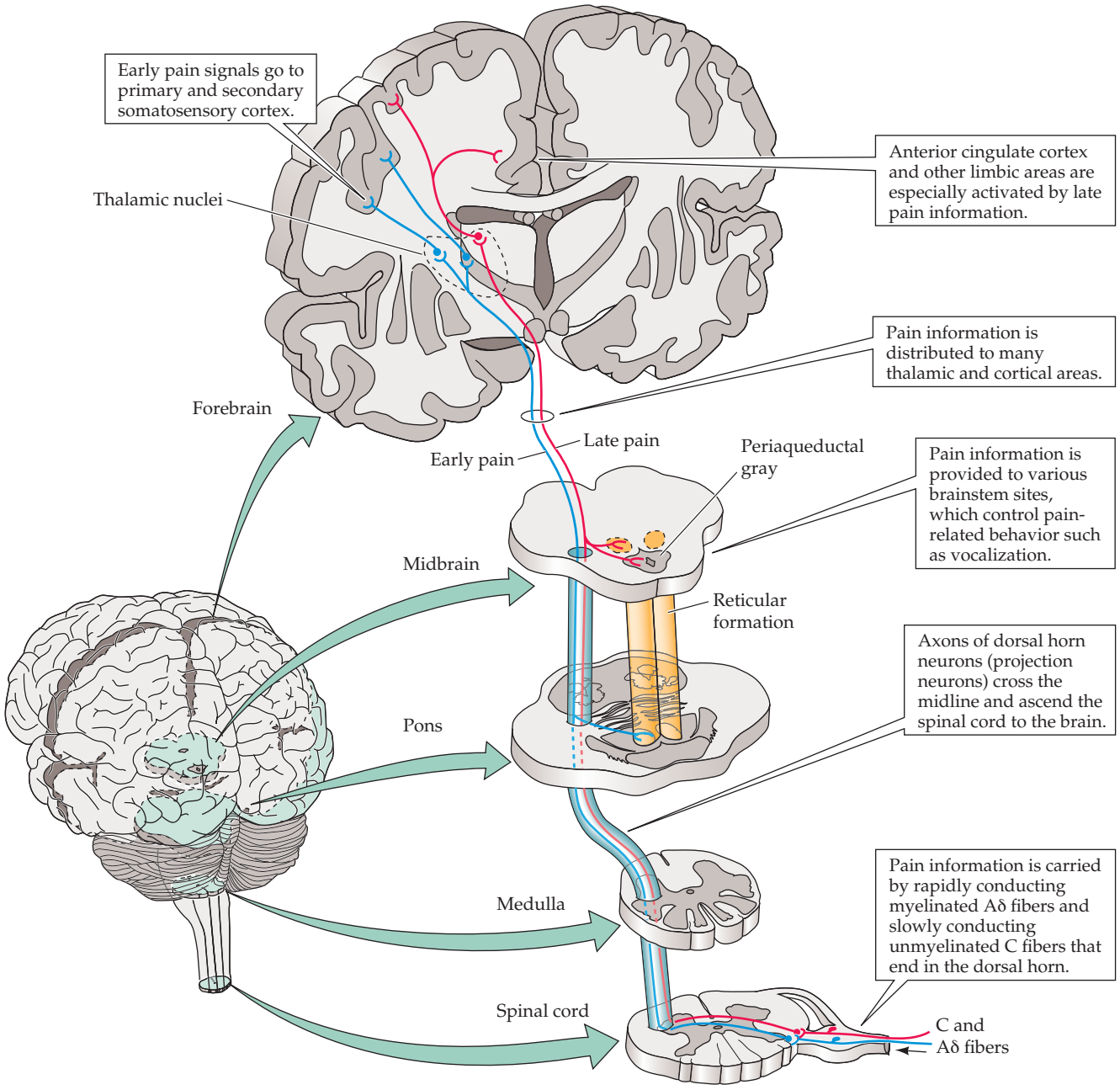


FIGURE 11.12 Ascending pain pathways Sensory neurons (Aδ and C fibers) activated by noxious stimuli enter the dorsal horn of the spinal cord. Dorsal horn neurons travel up the spinal cord on the contralateral side and ultimately reach various nuclei in the thalamus. Neurons transmitting "early" (first pain) end first in the primary somatosensory cortex for well-localized sensory discrimination, before the information is transferred to the secondary somatosensory cortex, where pain recognition occurs. The slower-conducting neurons transmit information (second pain) to a variety of limbic areas, including the anterior cingulate cortex, which is important for the emotional or suffering aspect of pain.

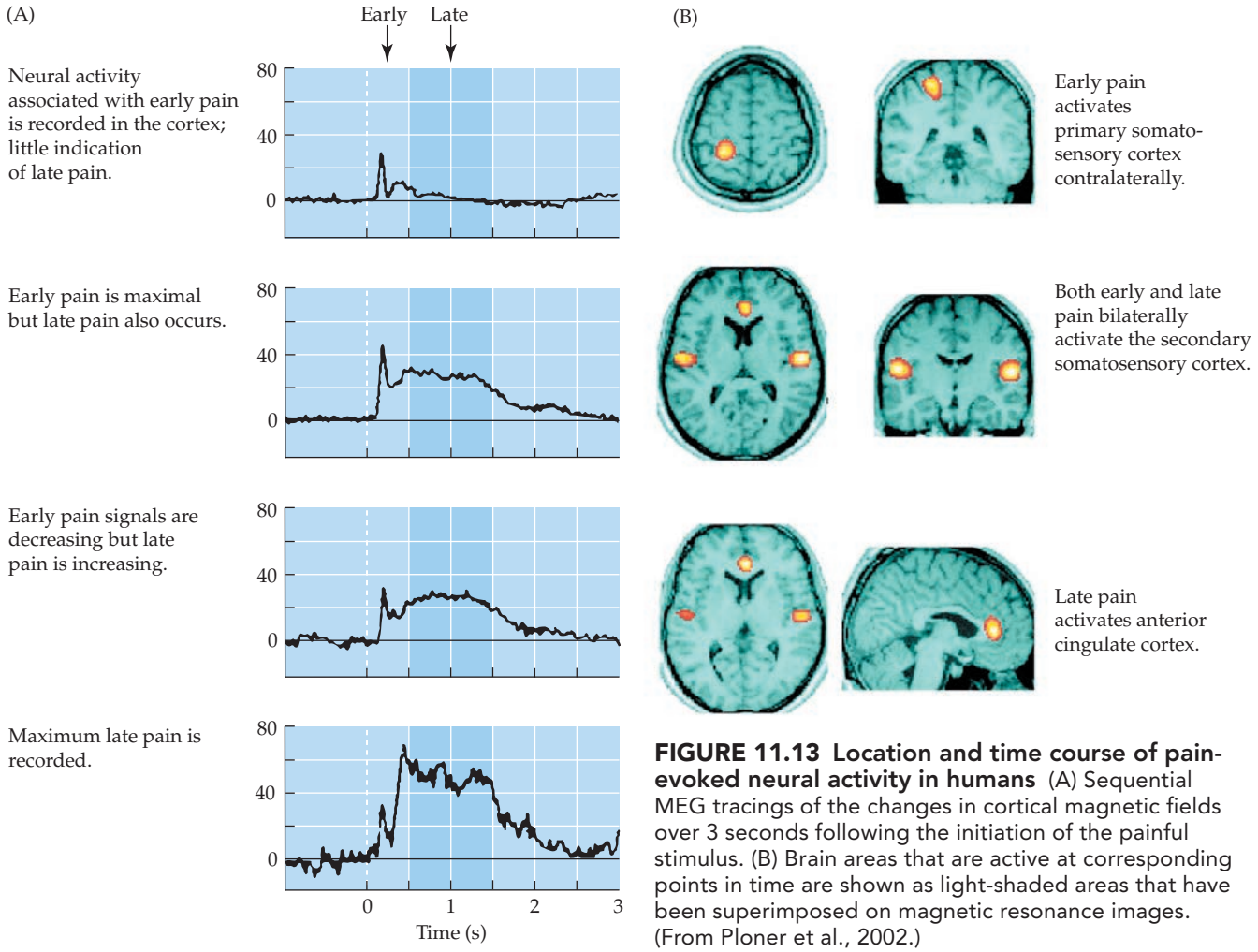


FIGURE 11.13 Location and time course of pain-evoked neural activity in humans (A) Sequential MEG tracings of the changes in cortical magnetic fields over 3 seconds following the initiation of the painful stimulus. (B) Brain areas that are active at corresponding points in time are shown as light-shaded areas that have been superimposed on magnetic resonance images. (From Ploner et al., 2002.)

Opioids inhibit pain transmission at spinal and supraspinal levels

By binding to opioid receptors, morphine and other opioid drugs mimic the inhibitory action of the endogenous opioids at many stages of pain transmission within the spinal cord and brain. To simplify, we can say that opioids regulate pain in three ways:

1. Within the spinal cord by small inhibitory interneurons
2. By two significant descending pathways originating in the periaqueductal gray (PAG)
3. At many higher brain sites, which explains opioid effects on emotional and hormonal aspects of the pain response

As you know, information about pain, from either the surface or deep within the body cavity, is carried by neurons from the body into the spinal cord. Some of these primary afferent neurons end directly on projection neurons that transmit pain signals to higher brain centers (e.g., first to the thalamus and then to the somatosensory cortex) (**FIGURE 11.14A**). Others end on small excitatory interneurons (i.e., short neurons within the spinal cord) that in turn synapse onto the projection neurons (**FIGURE 11.14B**).

Opioids reduce the transmission of pain signals at the spinal cord in two ways. First, small inhibitory **spinal interneurons** release endorphins that inhibit the activation of the spinal projection neurons (**FIGURE 11.14C**). Morphine can act directly on the same opioid receptors on the spinal projection neurons to inhibit the transmission of the pain signal to higher brain centers that normally allow us to become aware of the sensory experience. Second, endorphins regulate several modulatory pathways that descend from the brain to inhibit spinal cord pain transmission, either by directly inhibiting the projection neuron (Figure 11.14A) or the excitatory interneuron (Figure

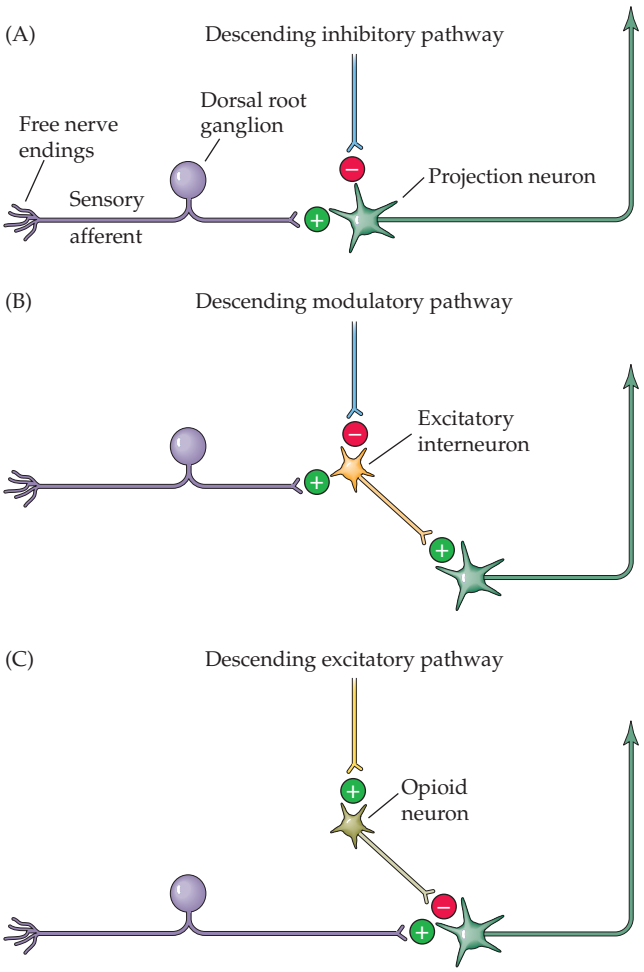


FIGURE 11.14 Pain transmission in the spinal cord
Descending modulatory neurons modify transmission via (A) inhibition of the projection neuron, (B) inhibition of an excitatory interneuron, and (C) excitation of an inhibitory opioid interneuron. Opioid drugs can influence the activity of the descending pathways and can act directly on opioid receptors in the spinal cord.

11.14B), or by exciting the inhibitory opioid neuron (Figure 11.14C). These **descending modulatory pathways** (**FIGURE 11.15**) begin in the midbrain and modify the pain information carried by spinal cord neurons.

The most important descending pathways begin in the PAG. The PAG is a brain area rich in endogenous opioid peptides and high concentrations of opioid receptors, particularly μ and κ. Local electrical stimulation of the PAG produces analgesia but no change in the ability to detect temperature, touch, or pressure. Treatment of chronic pain in human patients by electrical stimulation of the PAG is frequently successful, although tolerance occurs with repeated use, and cross-tolerance (see Chapter 1) with injected morphine also occurs. This phenomenon suggests that electrical stimulation releases a morphine-like substance onto the same postsynaptic receptor sites occupied by exogenous morphine. Partial blockade of stimulation-induced analgesia with the specific opioid antagonist naloxone further supports that idea.

The neurons beginning in the PAG end on cells in the medulla, including the serotonergic cell bodies of the nucleus of the raphe. Microinjection of opioids into the raphe produces significant analgesia. The serotonergic neurons descend into the spinal cord to inhibit cell firing there and in that way reduce pain transmission. Similarly, other cells originating in the PAG terminate in the brainstem in an area close to the locus coeruleus, an important cluster of noradrenergic cell bodies that also send axons to the spinal cord to modulate pain conduction. In both cases, these descending pathways are activated when opioids inhibit an inhibitory GABA "brake." Furthermore, neurotoxic lesions of the descending serotonergic and noradrenergic cells prevent systemic morphine-induced analgesia. Therefore, there are at least two major pathways that descend to the spinal cord to inhibit the projection of pain information to higher brain centers. However, the inhibitory action is direct in some cases, while at other times the inhibition occurs by action on small spinal interneurons (as seen in Figure 11.15).

In summary, opioids modulate pain directly in the spinal cord and by regulating the descending pain inhibitory pathways ending in the spinal cord. In addition, significant opioid action occurs in other **supraspinal** (above the spinal cord) locations, including higher sensory areas and limbic structures, as well as the hypothalamus and the medial thalamus. A high concentration of endogenous opioids and the presence of opioid receptors suggest that these areas may be responsible for the emotional component of pain, as well as for autonomic and neuroendocrine responses. In one PET study, the endogenous activation of the μ-opioid system was evaluated during sustained pain induced by injection of hypertonic saline into the massiter muscle, which was compared with reaction to placebo injection in the same study participants. Zubieta and colleagues (2001) found a significant negative correlation between μ-opioid activity (measured as displacement of [^{11}C]carfentanil from μ-receptors) in the nucleus accumbens, amygdala, and thalamus and reported *sensory* pain scores (**FIGURE 11.16**). That is, the greater the μ-opioid activation, the lower was the individual's sensory pain score. The PAG also showed increased μ-receptor displacement, although it was not significant. When the scores on the *affective* component of pain were evaluated, increased μ-opioid activity was found in the bilateral anterior cingulate cortex, thalamus, and nucleus accumbens. These results indicate that endogenous μ-opioids modulate both the sensory and emotional components of pain

and that morphine and other opioids likewise act at these sites. The existence of multiple circuits carrying pain information demonstrates the redundancy and diffuse nature of pain transmission, which reflects its tremendous evolutionary significance for survival. Stein (2016) reviews receptor subtypes, opioid receptor ligands, and central and peripheral mechanisms of opioid analgesia.

Other forms of pain control depend on opioids

ACUPUNCTURE The discovery of the endogenous opioids led to a dramatic increase in research into the mechanisms of the ancient Chinese method for pain relief called *acupuncture*. Acupuncture involves inserting a metallic needle into the skin to reach deep structures, such as muscles and tendons. Rhythmic movement of the needle or application of mild electrical current reduces pain perception. Beginning in the late 1950s, scientific studies using modern technology were initiated in China to determine the physiological basis of acupuncture-induced analgesia. Although naloxone was shown to reduce acupuncture-induced analgesia in human dentally evoked pain, it became clear that its antagonistic effect depended on the characteristics of the acupuncture treatment, which may be mediated by distinct opioid peptides. For instance, a look at electrically induced analgesia shows that the κ-receptor antagonist MR 2266 blocked the analgesia induced by 100 Hz electroacupuncture, but not that induced by 2 Hz. In contrast the δ-opioid antagonist ICI 174864 blocked electroacupuncture-induced analgesia from 2 Hz, but not that induced by 100 Hz. Further support for the selectivity comes from the finding that 2 Hz acupuncture develops cross-tolerance with δ- but not κ-agonists, while 100 Hz acupuncture shows cross-tolerance to κ-agonists but not δ-agonists. More direct measurements demonstrated that 2 Hz electroacupuncture caused significant elevations in the proenkephalin peptide, whose cleavage

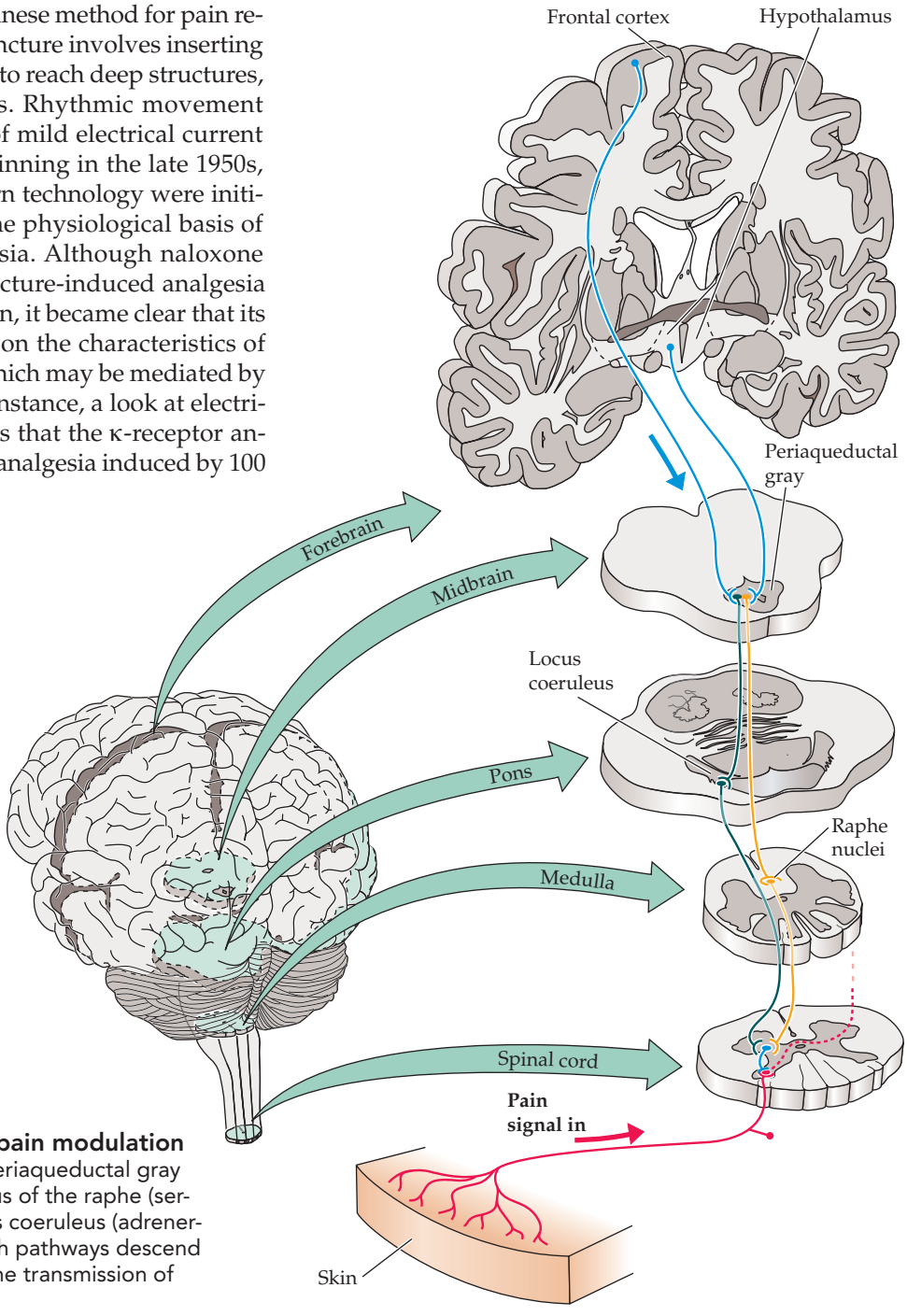


FIGURE 11.15 Descending pain modulation pathways Neurons from the periaqueductal gray descend to the brainstem nucleus of the raphe (serotonergic neurons) and the locus coeruleus (adrenergic neurons). The neurons in both pathways descend to the spinal cord to modulate the transmission of the pain signal at that level.

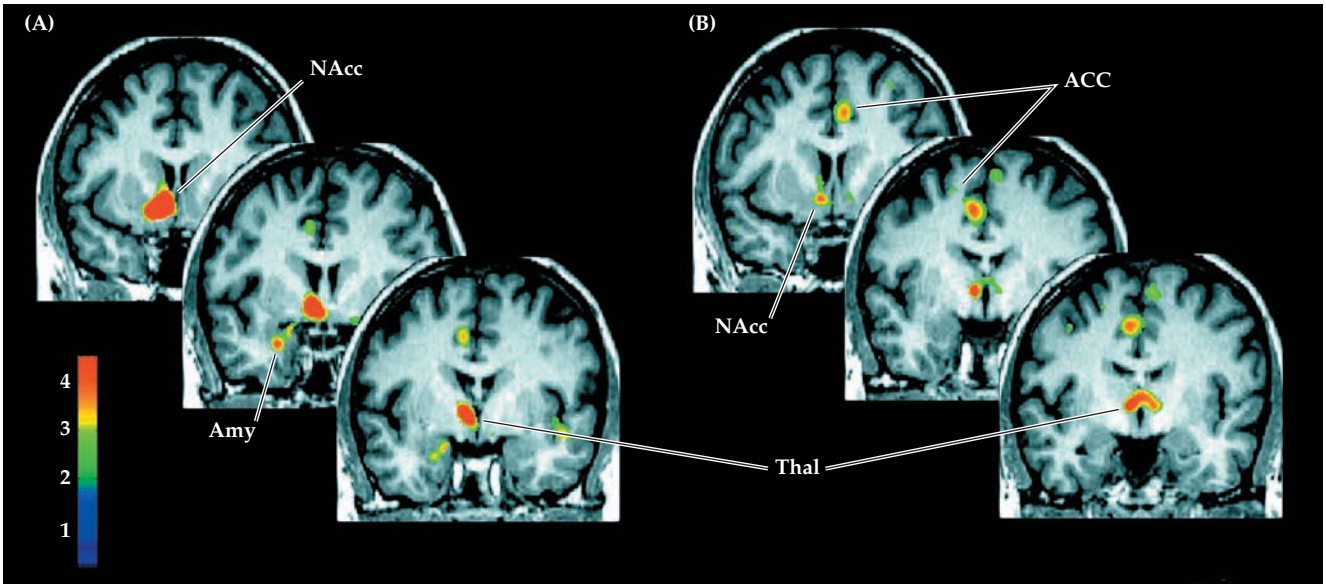


FIGURE 11.16 PET scans showing endogenous opioid activation during sustained pain In study participants injected with [^{11}C]carfentanil, endogenous opioid action is shown by the ability of the endogenous opioid to displace the radiolabeled μ-receptor ligand from the receptors. Pain was induced by continuous infusion of hypertonic saline in the masseter muscle and was compared with a control condition of isotonic saline infusion. (A) Activation of the μ-opioid system in the nucleus accumbens (NAcc), amygdala (Amy), and anterior thalamus (Thal) after the participants were exposed to the prolonged noxious stimulus. The warmer colors indicate greater activation. These increases were negatively correlated with the participants' *sensory* scores of pain. (B) μ-Opioid activity in the bilateral anterior cingulate cortex (ACC) and anterior thalamus and unilaterally in the NAcc. These increases were negatively correlated with pain *affect* scores. Therefore, the greater the μ-opioid activation in these areas, the lower was the emotional component of pain. Areas in red indicate greatest activity. (Courtesy of Dr. Jon-Kar Zubieta.)

products would be expected to act at δ-receptors, and 100 Hz raised CSF levels of dynorphin, which acts most robustly on κ-receptors (Han, 2004).

Because acupuncture releases endogenous opioids that you now realize have widespread effects in the brain and the gastrointestinal system, it is likely that it will be used effectively to treat other medical problems beyond pain (Cabýoglu et al., 2006). Electroacupuncture has been found to enhance the immune response and reduce gastric acid secretion. Since it activates the satiety center in the hypothalamus, it is potentially useful in treating obesity. Interestingly it has also been tested as a means to reduce the unpleasant signs of morphine withdrawal (see later in this chapter).

DUAL INHIBITION OF PEPTIDASES A different physiological approach to achieving antinociception involves enhancing the effects of endogenous enkephalin by inhibiting the two peptidase enzymes that degrade it (Noble and Roques, 2007). Microdialysis studies in rats showed that the dual inhibitors such as RB-101, RB-120, RB-3007, and others increase the extracellular levels of enkephalin in vivo in numerous brain regions. These drugs are good examples of prodrugs (see Chapter 1) because their lipophilic nature allows rapid passage of the blood–brain barrier into the brain, where a bond between the two inhibitors is biologically broken, making them active. Although inhibiting either one of the two peptidases produces only weak analgesic effects, inhibiting both produces far more robust dose-dependent effects in the hot plate, tail flick, and other animal tests of analgesia. The relatively long-lasting antinociceptive effects following intraperitoneal (IP), IV, or oral (PO) administration are blocked by naloxone. The importance of enkephalin to the pharmacological action of the dual inhibitors is further demonstrated by the lack of analgesic effect in mutant mice lacking proenkephalin. Which opioid receptor is involved is somewhat unclear because the analgesic effect is mediated by μ-receptors or both μ- and δ-receptors, depending on the nature of the pain (thermal, chemical, mechanical, or inflammatory) used in animal testing. The mechanism of action has clinical significance because it may explain a synergistic effect of a dual inhibitor and morphine. Research shows that a subanalgesic dose of RB-101 significantly potentiates a subanalgesic dose of morphine. Being able to use low doses of the two drugs for pain control would prevent the unwanted side effects caused by high doses of either one. One possible explanation for the synergism is that low doses of morphine increase the release of enkephalins that is further enhanced by the peptidase inhibition.

Of further importance is that because completely inhibiting the peptidases with high doses of dual inhibitors never achieves the maximum analgesic effect of morphine, it is assumed that endogenous enkephalin

does not saturate opioid receptors and overstimulate them. This may be part of the reason that the dual inhibitors avoid some of the major disadvantages of morphine treatment. At doses that produce significant analgesia, the dual inhibitors do not depress either the rate or volume of respiration and induce only a partial tolerance to the analgesic effect. Furthermore, following chronic administration, naloxone administration fails to induce a morphine-like withdrawal syndrome, hence the risk of physical dependence is minimal. Low abuse potential is suggested by studies showing that animals rarely develop conditioned place preference or altered intracranial electrical self-stimulation behavior (see Chapter 4). Additionally, animals do not distinguish the dual inhibitors from saline in drug discrimination tests; nor do morphine-trained animals generalize to the dual inhibitors, which indicates that their interoceptive cues are different. These characteristics make the dual inhibitors tempting targets for future clinical trials once toxicology and safety studies have been completed.

GENE THERAPY On the basis of strong preclinical evidence using a variety of rodent models of pain, a small clinical trial of gene therapy in patients with intractable pain from terminal cancer was undertaken (Fink et al., 2011). This study used the cold sore–causing herpes simplex virus to act as the gene transfer vector because this virus is taken up from the skin by sensory nerve endings and transported along their axons to the nuclei in the cell bodies located in the corresponding dorsal root ganglia. The virus was modified so it could not replicate and was engineered to contain the gene coding for human proenkephalin. The newly synthesized proenkephalin would be expected to be packaged in vesicles, spliced into multiple enkephalin peptides, and ultimately released by the sensory nerve terminals in the dorsal horn of the spinal cord to inhibit pain signal conduction (see Figures 11.12 and 11.14). Earlier studies of the technique using laboratory animals showed significant analgesic effects that were blocked by pretreatment with naloxone, demonstrating the importance of opioid receptors. Additionally, the analgesic effect was additive with morphine and shifted the dose–response curve for morphine to the left, which indicates that less morphine was needed to achieve the same level of analgesia. Of potential importance is that the vector-mediated analgesia occurred in animals that were tolerant to the effects of morphine, quite possibly because the analgesic effect was mediated by δ-opioid receptors, while the morphine-induced analgesia depends primarily on μ-opioid receptors.

This clinical trial included only 10 patients, all of whom had moderate to severe pain that was not eliminated by at least 200 mg of morphine. After receiving 10 injections in a single session into the skin where pain was localized, they were evaluated seven times over a 28-day follow-up and monthly thereafter for 4 months. Although there were reports of mild and transient side effects such as elevation of body temperature, no serious adverse effects were reported throughout the period of evaluation. Despite the small number of patients and the lack of a placebo group, the researchers found an apparent dose response. The lowest dose produced no pain relief, while the higher doses reduced pain to 50% of pretreatment levels initially and continued to reduce pain to 20% over several weeks. These encouraging results are being expanded into a larger phase II randomized, double-blind, placebo-controlled trial using similar patient populations.

DUAL μ-RECEPTOR/NOP-R AGONISTS An exciting new approach has produced an agent that is highly effective in reducing pain without many of the common narcotic side effects. The focus has been on the nociceptin/orphanin receptor (NOP-R) because agonists for the receptor produce analgesia but are not reinforcing and can reduce the reinforcing effects of μ-receptor agonists. Medicinal chemists have identified ligands that bind to both μ-receptors and NOP-R and produced analgesia in rodent models. One of particular interest is BU08028. Ding and colleagues (2016) performed extensive experiments on BU08028 in 12 rhesus monkeys because, like humans, these animals display opioid-induced abuse liability and respiratory depression. For that reason, their findings with the monkeys, compared with rodent studies, are more likely to translate to humans. The rationale for using the dual μ-receptor/NOP-R agonist is that the NOP-R stimulation will reduce some of the pain so less μ-receptor stimulation will be needed and side effects will be correspondingly reduced. Ding's group measured analgesia by placing the monkeys' shaved tails in hot (120°F) water and measuring latency to withdraw. They found dose-dependent analgesia that at 0.01 mg/kg produced the maximum possible effect. Further, a single injection lasted up to 30 hours, which is an extremely long duration of action. The fact that both the opioid receptor antagonist naltrexone and the NOP-R antagonist J-113397 shifted the dose-response curve to the right to the same extent indicates that the two receptors were equally responsible for the analgesia. After 3 days of morphine treatment alone, naltrexone precipitated measurable withdrawal signs. In contrast, when naltrexone and J-113397 were administered to animals following 3 days of BU08028, no withdrawal was evident, suggesting lack of physical dependence. In the drug self-administration breaking point test, both the opioids remifentanil and buprenorphine were readily self-administered, with remifentanil being significantly more reinforcing than buprenorphine. In contrast, self-administration of BU08028 was not significantly different from saline self-administration, indicating little reinforcement and

no abuse potential. In evaluation of multiple respiratory functions, BU08028 at maximum analgesic dose and doses 10 times higher did not decrease any of the measures, nor did it alter cardiovascular function (e.g., bradycardia, hypotension, or electrocardiogram parameters). In sum, this study provides evidence that the dual μ-receptor/NOP-R agonist BU08028 is highly effective as an analgesic in non-human primates and has a wider therapeutic window than morphine because it produces less respiratory depression and cardiovascular effects. Furthermore, physical dependence and abuse potential are minimal. Further testing in non-human primates is needed at higher doses and for longer periods of time to pave the way toward clinical trials with human patients. This pharmacological approach and the structure-based drug development described in Box 11.2 are promising advances in the search for safer painkillers (briefly summarized by Perlman, 2017).

Section Summary

- Nociceptors are free nerve endings that are sensitive to a variety of pain stimuli.
- Pain has two components. First pain is the immediate sensory component carried by myelinated Aδ neurons and transmitted via the spinothalamic tract to the PVL nucleus of the thalamus before projecting to primary, then secondary, somatosensory cortex. Second pain is the emotional component carried by C fibers and transmitted to the thalamus with collaterals to limbic areas, including the anterior cingulate.
- Opioids (both endogenous and exogenous) act at spinal and supraspinal levels to relieve pain. Endorphin neurons in the spinal cord decrease the conduction of pain signals from the spinal cord to higher brain centers. Descending neurons from the periaqueductal gray activate pathways from the locus coeruleus (noradrenergic) and nucleus of the raphe (serotonergic) that impede pain signals in the spinal cord. Opioid receptors in the neocortex and limbic regions modulate the emotional component of pain to relieve the sense of suffering.
- Analgesic effects of electroacupuncture depend on opioids. Acupuncture induced by 100 Hz is blocked by κ-receptor antagonists, shows cross-tolerance to κ-agonists, and increases CSF levels of dynorphin. Acupuncture induced by 2 Hz is antagonized by δ-receptor blockers, shows cross-tolerance to δ-agonists, and elevates proenkephalin.
- Analgesia can be produced by drugs that inhibit both of the enkephalin degrading enzymes. Elevating endogenous enkephalin has fewer side effects than morphine: less respiratory depression, partial tolerance, and low abuse potential.
- A gene therapy clinical trial showed that the gene coding for proenkephalin carried by a herpes simplex viral vector reduced pain in patients with cancer with minimal side effects.
- Dual μ-receptor/NOP-R agonists produce analgesia without respiratory depression, cardiovascular effects, physical dependence, or abuse liability.

Opioid Reinforcement, Tolerance, and Dependence

Although the opioids are the best pain-reducing drugs presently available, their use continues to be problematic because of the potential for abuse. The drugs in this class are highly reinforcing, and despite strict legal controls, they sometimes wind up in the hands of individuals who abuse them. Furthermore, chronic use leads to tolerance and ultimately to physical dependence, but keep in mind that most chronic pain patients receiving opioids do not develop opioid use disorder or abuse. In order to learn more about how medical patients become chronic drug users, multiple studies have been done recently looking at both acute pain and emergency room visits as well as chronic pain conditions. Barnett and colleagues (2017) examined the records of almost 400,000 older individuals on Medicare over several years who had visited emergency rooms in multiple hospitals with similar types of injury/pain. Opioids are frequently prescribed by emergency room physicians because they are highly effective and act rapidly for treatment of pain from broken bones and other severe acute pains. What the researchers found was that those patients who were treated by "high-intensity" opioid-prescribing physicians (those with a high prescription rate) were significantly more likely to develop long-term opioid use (180 days or more in the next year) than those patients who were treated by "low-intensity" opioid prescribers. Since clear guidelines do not exist, ER physicians base their decisions on past experience, which varies between physicians, so there are examples of both excessive treatment and insufficient treatment within the same hospital. This study was of particular interest because it involved just a short-term exposure to opioid use, so most of the long-term use must also have involved securing subsequent prescriptions from primary care physicians outside the hospital setting. Of course, the dangers especially for older individuals are the side effects of constipation, sleepiness or

TABLE 11.2 Risk Factors for Opioid Misuse

Family history of substance abuse
Personal history of substance abuse
Young age
History of criminal activity and/or legal problems including DUIs[a]
Regular contact with high-risk people or high-risk environments
Problems with past employers, family members, and friends
Risk-taking or thrill-seeking behavior
Heavy tobacco use
History of severe depression or anxiety
Psychosocial stressors
Prior drug and/or alcohol rehabilitation

Source: After Jamison and Mao, 2015.
[a] DUI, driving under the influence.

confusion, impaired balance leading to falls, and opioid use disorder. Unfortunately, at this point there is no quantitative measure to help physicians identify individuals who will experience adequate analgesia but few side effects and will have low potential abuse liability (Bruehl et al., 2013), although there are multiple risk assessment tools available (see Jamison and Mao, 2015). **TABLE 11.2** provides some of the identified risk factors for opioid misuse. Perhaps in the future, genetic testing will provide information regarding the probability of opioid misuse in a given individual as well as predicting effective pain relief.

Obvious candidate genes for predicting abuse potential involve the receptors and propeptides for opioid transmission, and they have been most extensively studied. Researchers look for gene variants that associate with the disorder of interest. Among the most replicated findings involves a single-nucleotide polymorphism (SNP) of the gene coding for the μ-receptor. Altering just a single nucleotide changes the identity of one of the amino acids in the protein coded by the gene and modifies function of the protein. In the case of opioid function the SNP identified reduces mRNA expression, leading to reduced μ-receptor levels and also altered β-endorphin binding affinity. It also alters the hypothalamic–pituitary–adrenal axis stress response. Multiple studies report an association of that SNP with significantly increased vulnerability to heroin addiction and also overdose severity. These findings are encouraging, but since opioids modify both dopamine (DA) in the reward pathway and serotonin (5-HT) function that alters mood, components of those transmitter systems, including receptors, transporters, and synthesizing enzymes, are also being investigated. Discussing the genetic contribution to addiction potential is beyond the scope of this chapter, but Reed and colleagues (2014) describe the genetics of addiction, considering not only opioid, DA, and 5-HT function, but also GABA, norepinephrine, glutamate, and neuromodulators.

A second focus of research into the precursor to chronic drug use has been on patients with chronic pain conditions. About 25% of the U.S. general population suffers from some chronic pain condition, including arthritis, back pain, inflammatory pain, diabetic neuropathy, and residual effects following accidents. It is clear that many people require opioids for severe, acute pain or pain associated with malignant cancer, but a recent meta-analysis suggests that opioids are much less effective for chronic non-cancer pain and that some nonpharmacological methods, such as physical therapy and cognitive therapy, may be equally useful for some patients (Reinecke et al., 2015). The analysis compared non-opioid analgesics, weak opioids, strong opioids, physical therapy, and cognitive therapy against placebo. To be optimum the treatment must not just relieve pain, but also increase physical functioning, quality of sleep, and quality of life with minimal side effects. In the study, statistical averages showed not much difference among treatments regarding pain control, although opioids improved sleep quality but not quality of life. Also, opioids showed the greatest number of adverse effects. Physical therapy and cognitive therapy produced no significant effects on quality of life, sleep quality, or physical function. Although averaging the data showed little difference, each patient responded differently, so individual treatment strategies using a combination of approaches is recommended.

The first nonbinding federal guidelines from the CDC for pain regulation are aimed at reducing the increase in opioid misuse and deaths that often begin with prescription drugs. The CDC recommendation is that physicians suggest the use of ibuprofen and aspirin for their patients rather than prescribing opioids unless necessary, and with opioids they should limit the duration of treatment. Of course, the over-the-counter drugs have their own sets of side effects, which prohibit their use for some patients, and they may or may not provide the extent of pain relief needed. Physicians are trained to relieve their patients' pain and suffering, so some are uncomfortable monitoring their patients' drug use. Additionally, many resent following a formula for treatment when each patient is different and many are in severe and constant pain. Intense chronic pain conditions severely limit normal daily activities and the ability to work and are responsible for both stress and depression. Nevertheless, some state medical boards are trying to limit the number of opioid doses per month for any given individual. Some of these limits are especially hard on

the older population, who are more likely to suffer from the chronic pain of arthritis, diabetes and shingles neuropathy, cancer, and multiple surgeries, and yet they are typically less likely to misuse or abuse opioids than the younger population. However, it must be said that because of their slower metabolism, they are more likely to suffer from side effects. A second issue to be addressed is that of cost. Although treatments other than the opioids are available, including acupuncture, hypnosis, chiropractic manipulation, physical therapy, therapeutic massage, yoga, cognitive behavioral therapy, and mindfulness meditation, and each can be effective for a given individual, these alternative treatments can be quite expensive and are rarely covered by insurance, including Medicare. Hence if the government wants physicians to reduce opioid prescribing, they may have to expand insurance coverage for alternative approaches to treating pain.

Animal testing shows significant reinforcing properties

Experimental techniques used to demonstrate the reinforcement value of opioids are described in Chapter 4. Intracerebral electrical self-stimulation allows subjects to press a lever to self-administer a weak electrical current to certain brain areas that constitute central reward pathways. When the animal presses the lever, electrical activation causes release of neurotransmitters from the nerve terminals in the region, which in turn mediate a rewarding effect. The fact that morphine and other opioids lower the electrical current threshold for self-stimulation indicates that the drugs enhance the brain reward mechanism.

When the drug self-administration technique is used, one striking finding is that the reinforcement value and the pattern of opioid use in animals are quite similar to those seen in humans. Self-injection gradually increases over time until the animals self-administer a stable and apparently optimal amount of drug. The ability of animals to maintain a stable blood level is demonstrated by pretreatment with morphine, codeine, or meperidine, which subsequently reduces IV self-administration of morphine. In contrast, when some receptors are blocked with naloxone, the self-administration rate increases and matches that seen during morphine abstinence. It is evident from these studies that the animals learn to regulate with some accuracy the amount of morphine that they require. Dose–response curves can be used to compare the relative potencies of opioid drug reinforcement (Woods et al., 1993).

The endogenous opioid β-endorphin is also self-administered, which strongly suggests that it mediates opioid reinforcement. Beta-endorphin self-administration is blocked by either μ- or δ-receptor antagonists. Thus, both types of receptor are involved in reward processes. In contrast, κ-agonists fail to produce self-injection and may induce aversive states, leading to avoidance behavior (Shippenberg, 1993).

Dopaminergic and nondopaminergic components contribute to opioid reinforcement

Two important methods are used to identify the neurobiology of opioid reinforcement. In one, self-administration of opioid ligands microinjected into discrete brain areas is evaluated. In the second, selective lesions are used to identify the brain areas and neurotransmitter pathways that eliminate opioid-induced reinforcement.

Microinjection studies from many laboratories demonstrate the contribution of the dopaminergic mesolimbic pathway to opioid reinforcement. This pathway originates in the ventral tegmental area (VTA) of the midbrain and projects to limbic areas, including the nucleus accumbens (NAcc). Return to Figure 5.9 to review the important dopamine (DA) pathways in the brain. Self-administration of morphine or endogenous peptides occurs when the microcannula is implanted near the dopamine cell bodies within the VTA. Intra-VTA microinjection of morphine or selective μ-agonists also produces conditioned place preference and reduces the threshold for intracranial electrical self-stimulation. Each of these results argues for a direct action of opioids on central reward mechanisms served by the mesolimbic pathway.

But what exactly happens to the cells in the VTA in the presence of opioids? Both systemic opioids and opioids microinjected into the VTA increase dopaminergic cell firing, which subsequently increases the release of dopamine within the NAcc. Intraventricular β-endorphin produces similar enhancements of neuronal firing. In contrast, κ-agonists produce the opposite effects on mesolimbic neurons and reduce dopaminergic neuronal activity and subsequent DA turnover (release and metabolism). Since microinjected κ-agonists produce conditioned place *aversions*, it seems possible that the mesolimbic dopamine system may mediate aversive effects of opioids, as well as their reinforcing properties (Shippenberg et al., 1991).

A model of the opposing effects of opioid neurons on mesolimbic dopaminergic cells is shown in **FIGURE 11.17**. Beta-endorphin and opioid drugs seem to increase VTA cell firing by inhibiting the inhibitory GABA cells found in the VTA. They can decrease the release of GABA by opening K^+ channels or reducing Ca^{2+} influx on GABA terminals. This inhibition of inhibitory neurons leads to increased firing and greater DA release in the NAcc. The endogenous peptide dynorphin, which acts on κ-receptors on the terminals of the DA neurons, can reduce the release of DA by similar mechanisms, causing dysphoria.

How sure are we that mesolimbic DA is really important? DA receptor antagonists are seen to block the reinforcing effects of opioids when evaluated by each

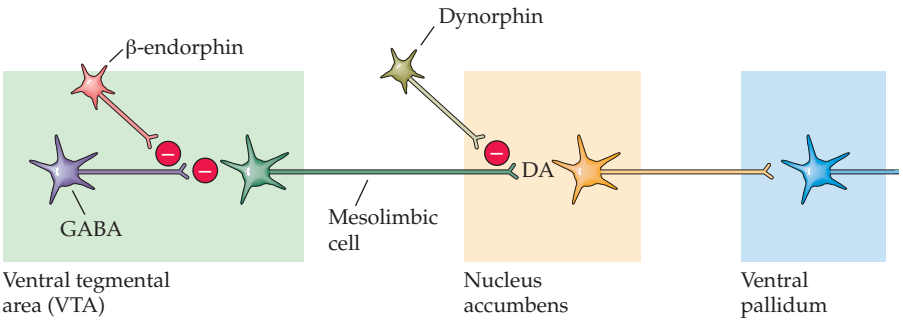


FIGURE 11.17 Model of the effects of opioids on mesolimbic dopaminergic cells The β-endorphin cell has an inhibitory effect on the normally inhibitory GABAergic cell, allowing the firing rate of the mesolimbic dopaminergic cells to increase and the cells to release more dopamine (DA) in the nucleus accumbens. In contrast, the axoaxonic dynorphin cell inhibits the release of dopamine from the mesolimbic cell by preventing calcium entry.

of the three standard behavioral measures. However, inducing lesions of dopaminergic neurons with the neurotoxin 6-hydroxydopamine (6-OHDA) reduces (but does *not* abolish) the reinforcement value. The fact that heroin self-administration is only *partially* reduced rather than eliminated by the lesion certainly suggests that other brain areas and other neurotransmitters in addition to DA are also involved. Although these studies clearly support earlier results showing that increased mesolimbic firing is a common link in the actions of many self-administered drugs, including ethanol, nicotine, and psychostimulants (see Chapter 9), opioids do not have to release mesolimbic DA to be reinforcing (Gerrits and Vanree, 1996).

Further, the mesolimbic pathway may be more specifically involved in the salience (or significance) of events to an individual. What we mean is that the dopaminergic cell firing may tell an organism when some event is important or meaningful, regardless of whether it is appetitive (positive) or aversive (negative). We would therefore have incentive (or motivation) to approach (in the case of a reinforcer) or avoid (in the case of a noxious event) a particular significant stimulus. This idea has been developed in the "incentive sensitization" hypothesis of addiction as described in Box 9.2 (see the next section).

Long-term opioid use produces tolerance, sensitization, and dependence

You have just read about the acutely rewarding effects of opioids, which increase the likelihood that the drug will be used again. Chronic use subsequently leads to neuroadaptive changes in the nervous system, which are responsible for tolerance, sensitization, and dependence.

Tolerance (see Chapter 1) refers to the diminishing effects of a drug with repeated use, and it occurs for all of the opioids, including the endorphins. Although tolerance to the opioids develops quite rapidly, tolerance does not occur for all of the pharmacological effects to the same extent or at the same rate. For example, tolerance to the analgesic effect occurs relatively rapidly, but the constipating effects and the pinpoint pupils persist even after prolonged opioid use.

Cross-tolerance among the opioids also exists. For this reason, when tolerance develops to one opioid drug, other chemically related drugs also show a reduced effectiveness. For instance, following chronic heroin use, treatment with codeine will elicit a smaller-than-normal response even if the individual has never used codeine before. Since we now know that at least four types of opioid receptors exist, we might wonder whether the receptor subtype plays a role in cross-tolerance. Indeed, it seems that selective agonists for the μ-receptor reduce the effectiveness of other μ-receptor agonists, but they only minimally reduce κ-agonist activity. Likewise, repeated exposure to κ-agonists diminishes the effects of other κ-agonists but not μ-agonists.

As is true for many drugs, several mechanisms are responsible for the development of tolerance to the opioids. An increased rate of metabolism with repeated use (metabolic tolerance) is responsible for some small portion of opioid tolerance. Classical conditioning processes also contribute to this phenomenon. However, most tolerance is based on changes in nerve cells that compensate for the presence of chronic opioids (pharmacodynamic tolerance). The cell mechanisms are discussed in more detail in the section on neurobiological adaptation and rebound.

Under some circumstances, repeated exposure to opioids produces **sensitization**. Sensitization refers to the increase in drug effects that occurs with repeated administration. In their incentive sensitization hypothesis Robinson and Berridge (2001) propose that in the case of substance abuse, the motivation (incentive) to approach, better called *craving* or desire for the drug, undergoes sensitization. Meanwhile, the neural mechanism responsible for the high, or liking of the drug, remains unchanged or decreases as tolerance develops over repeated administration. Both the decrease in liking and the increase in craving lead to further drug taking and may explain the intense compulsion to use a drug that no longer produces pleasurable effects.

The third consequence of chronic opioid use is the occurrence of **physical dependence** (see Chapter 9), which is a neuroadaptive state that occurs in response

TABLE 11.3 Acute Effects of Opioids and Rebound Withdrawal Symptoms

Acute action	Withdrawal sign
Analgesia	Pain and irritability
Respiratory depression	Panting and yawning
Euphoria	Dysphoria and depression
Relaxation and sleep	Restlessness and insomnia
Tranquilization	Fearfulness and hostility
Decreased blood pressure	Increased blood pressure
Constipation	Diarrhea
Pupil constriction	Pupil dilation
Hypothermia	Hyperthermia
Drying of secretions	Tearing, runny nose
Reduced sex drive	Spontaneous ejaculation
Flushed and warm skin	Chilliness and "gooseflesh"

to the long-term occupation of opioid receptors. Because the adaptive mechanism produces effects that oppose those of the opioid, when the drug is no longer present, cell function not only returns to normal but overshoots basal levels. The effects of drug withdrawal are *rebound* in nature and are demonstrated by the occurrence of a pattern of physical disturbances called the **withdrawal** or **abstinence syndrome**. Since opioids in general depress CNS function, we consider opioid withdrawal to be rebound hyperactivity (**TABLE 11.3**). You already know that opioid effects are due to drug action at various receptors in a variety of locations in the CNS and elsewhere in the body, so it should not be a surprise to learn that the abstinence signs reflect a loss of inhibitory opioid action at all of those same receptors as blood levels of the drug gradually decline. Withdrawal can also be produced by administering an opioid antagonist that competes with the drug molecules for the receptors and thus functionally mimics the termination of drug use. Note, however, that the withdrawal following antagonist administration is far more severe than that following drug cessation, because the opioid receptors are more rapidly deprived of opioid.

Opioid withdrawal is not considered life threatening, but the symptoms are extremely unpleasant and include pain and dysphoria, restlessness, and fearfulness, as well as several symptoms that are flu-like in nature. How severe the symptoms are and how long they last depend on a number of factors: the particular drug used, as well as the dose, frequency, and duration of drug use and the health and personality of the addict. To give an example, morphine withdrawal symptoms generally peak 36 to 48 hours after the last administration and disappear within 7 to 10 days. In contrast,

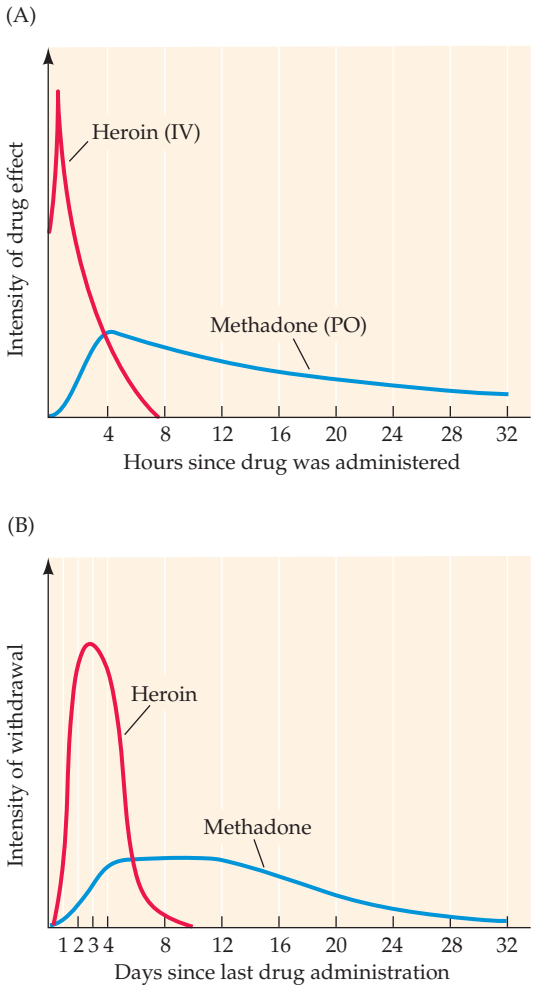


FIGURE 11.18 Relationship between acute effects and withdrawal (A) Time course showing the intensity and duration of the acute effects of IV heroin and oral (PO) methadone, and (B) the corresponding intensity and duration of withdrawal after chronic drug treatment.

methadone, which has a more gradual onset of action and is longer lasting, has a withdrawal syndrome that does not abruptly peak but increases to a gradual maximum after several days and decreases gradually over several weeks. Abstinence for the very long-acting opioid buprenorphine is even more prolonged, but as is true for all of the longer-lasting opioids, the withdrawal signs are milder (**FIGURE 11.18**). From this, you should conclude that the longer the duration of action of the opioid, the more prolonged is the abstinence syndrome but the lower is the intensity of the syndrome. At the point when abstinence signs end, the user is considered to be **detoxified**.

Readministering the opioid any time during withdrawal will dramatically eliminate all the symptoms. In addition, administering any other opioid drug will stop or reduce the withdrawal symptoms because these agents show **cross dependence**. This characteristic plays an important part in drug abuse treatment and is discussed further later in the chapter.

It may be surprising to learn that although physical dependence commonly occurs following chronic opioid use, it does not necessarily lead to abuse or opioid use disorder. Patients treated with opioids for protracted pain (e.g., postsurgical or cancer-related pain) show both tolerance and physical dependence, although withdrawal signs can be minimized by gradually reducing the dose when pain relief is no longer needed. However, most patients treated with opioids do not show addictive behaviors, such as craving and compulsive drug seeking. Nevertheless, as discussed earlier, there is a great deal of concern regarding opioid prescribing because of the sudden rash of overdose deaths especially among young people. Unfortunately, current fears of drug diversion, opioid use disorder, and overdose deaths may prevent many individuals from receiving the relief from severe pain that they require. Failure to use adequate painkilling treatment produces much more suffering and subsequently much slower healing than is warranted. Transdermal patches and patient-controlled drug delivery systems in the hospital setting are drug administration techniques that provide more humane control of pain and more effective recovery. Although effective relief from pain is vital to patients to enhance the quality of life, when opioids are diverted to illicit use, serious societal problems occur. **BOX 11.3** describes the recent epidemic of abuse of oxycodone, fentanyl, and other opioids.

BOX 11.3 Of Special Interest

The Opioid Epidemic

The startling increase in opioid-related overdose deaths has prompted a reexamination of our approach to treating chronic pain. A large proportion of opioid addicts initiated their abuse by taking prescription painkillers following surgery, recovery from a broken bone, or dental surgery. Among the most common prescription painkillers is oxycodone. Oxycodone is a semisynthetic opioid that works in a manner similar to morphine. The short-acting formulation Percodan is used to treat acute pain, while the long-acting time-release version OxyContin is used for cancer or musculoskeletal pain that is chronic and moderate to severe. These drugs have never been found to be better than morphine, which remains the gold standard for pain relief. Nor are they free from morphine-like side effects and the danger of abuse or fatal respiratory depression, especially when combined with CNS depressants such as alcohol. Oxycodone is classified by the DEA as a Schedule II drug because although it is used medically, it also has high abuse potential.

OxyContin became a popular street drug in the 1990s because it produces a heroin-like euphoria when crushed to eliminate the time-release mechanism. It is then snorted, ingested, or dissolved and injected. The fact that OxyContin readily dissolves in water encourages the injection of the drug, which increases the subjective effects but also increases the dangers of overdose and medical problems associated with needle use.

It became a problem because it was relatively easy to access with fake or tampered prescriptions, with theft from pharmacies or family members, or via the internet from other countries. Unfortunately, the high markup of street value prices compared with legal prices provided financial incentive to divert drugs into the black market. There are unfortunate stories of the drug being purchased in small amounts from those with prescriptions who need the money to pay for their medication or to supplement their Social Security income. Occasionally, unconscionable physicians and pharmacists are found running OxyContin "pill mills" that increase the availability of the drug to those without medical need. "Doctor shopping" (visiting multiple doctors with complaints of pain) became highly visible when the radio commentator Rush Limbaugh admitted his addiction to the drug.

The initial warning of the increase in OxyContin abuse came from Maine, but abuse rapidly expanded down the East Coast, hitting rural Appalachia and the Ohio Valley particularly hard. The spread of OxyContin use was paralleled by a dramatic increase in arrests for theft in those areas as well as overdose fatalities. As OxyContin abuse spread further, some officials called it a national epidemic, and that prompted efforts by state and federal government agencies, physicians' groups, and manufacturers to formulate a solution to the problem.

Unfortunately, more recent epidemics of opioid abuse have developed and spread across the country, the most recent being the surge in fentanyl abuse. Fentanyl is also a semisynthetic opioid and is 50 times more potent than heroin and 100 times more potent than morphine (**Figure A**). For the addicted opioid user it provides "higher highs" at lower costs because drug cartels, especially in Mexico, have been able to manufacture it more cheaply than heroin. Carfentanyl is a legal synthetic drug used primarily as a large animal tranquilizer in veterinary medicine. It has no practical use in humans but is

(*Continued*)

BOX 11.3 Of Special Interest (*continued*)

(A)

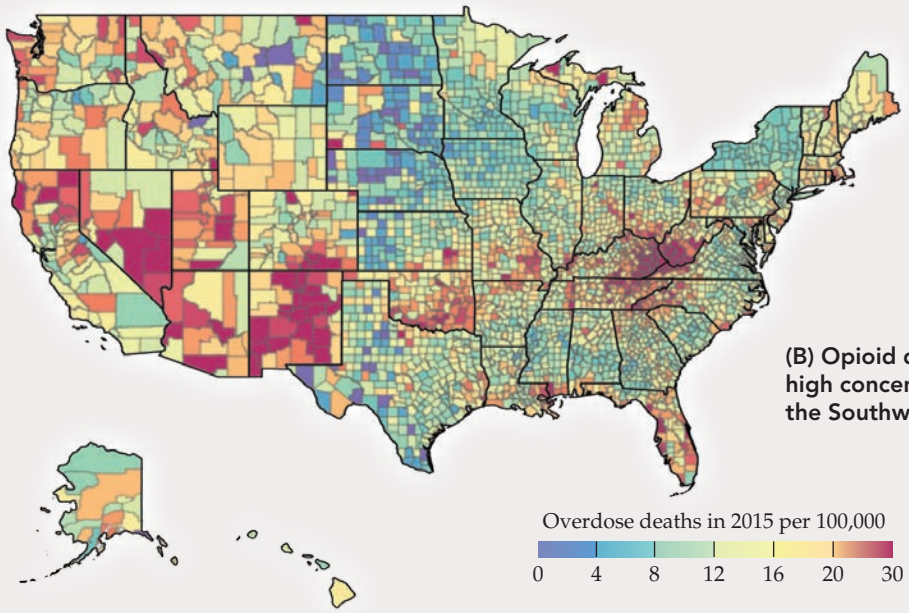
(A) Comparison of the doses of heroin and fentanyl that are fatal to an average-sized adult male (Courtesy NH State Police Forensic Lab.)

potentially highly lethal since it is 100 times more potent than fentanyl. Since it works very rapidly, the opportunity to treat overdoses with naloxone is reduced (see Box 11.1), and because of its potency, multiple applications of naloxone are required. Because it is cheaper to produce, it is currently mixed with heroin or prescription painkillers, which increases the euphoria but has contributed to a surge in overdose fatalities, since users frequently are unaware of the combination (King, 2016).

In fact, 61% of drug overdose deaths in 2014 involved opioids. Since 2000, overdose deaths involving opioids have almost tripled (see figure in Box 11.1). Just between 2013 and 2014 the overdose death rates increased for opioid pain relievers by 9%, for heroin by 26%, and for synthetic opioids such as fentanyl by 80%. The increases occurred for both males and females and spanned all age groups (Rudd et al., 2016) in many parts of the country. The highest numbers of fatalities were concentrated in Appalachia, particularly West Virginia and Kentucky, and in the Southwest, particularly New Mexico, Utah, and Nevada (see **Figure B**). Since past misuse of prescription pain relievers is the strongest risk fact for heroin use and abuse, more rigorous controls over prescription practices have been called for (discussed previously in the text). In addition to professional education, the creation of a national pharmacy database to identify abusers, and tamper-resistant prescription pads, manufacturers have developed several opioid formulations to discourage abuse. One (Remoxy) is a slow-release oxycodone that is surrounded by a hard gelatin capsule that prevents tampering or the removal of the drug with a needle. The second (Acurox with niacin) is an immediate-release oxycodone that also contains niacin that produces flushing and other unpleasant effects if the drug is taken in higher than prescribed doses. It comes as a gel to prevent use by inhalation.

Unfortunately, the problem does not stop there. Designer drugs (novel, often minor, variants of better-known drugs) that are developed become killers before they are identified by chemical formula, which is required before they can be classed as illegal. Most seem to be coming from Chinese labs that access the formulas from drug patent literature, make minor modifications, and then sell the drugs online. Among these are fentanyl variants such as furanyl fentanyl and acetyl fentanyl but also krokodil (see chapter opener), kratom, W-18, and U-47700.

(B)



(B) Opioid deaths are in especially high concentration in Appalachia and the Southwest (Rossen et al., 2017.)

Several brain areas contribute to the opioid abstinence syndrome

The signs of withdrawal represent the rebound hyperactivity in many different systems, including the gastrointestinal tract, the autonomic nervous system, and many sites within the brain and spinal cord. To identify which of the many brain areas are involved in the appearance of the particular signs of abstinence, an animal model is used. Pellets of opioid drugs are implanted under the skin so that the subcutaneous administration produces significant blood levels of drug over a week or longer. After the animals have become physically dependent, selective intracerebral injection of an opioid antagonist produces distinctive and easily quantifiable signs of withdrawal. Withdrawal signs in rodents include jumping, rearing, "wet dog" shakes, and increased locomotor behavior. Intracerebral injection of opioid antagonists into specific brain areas can help to identify which sites produce particular signs of abstinence. On the basis of these measures, no single brain area has been found to precipitate the entire withdrawal syndrome, but the locus coeruleus and the PAG are particularly sensitive to the antagonist in terms of precipitating withdrawal. As you will see in the next section, the locus coeruleus has become a neurochemical model for dependence.

In Chapter 9, you learned that the NAcc is a limbic structure that is particularly important for the reinforcement value of many abused substances. For this reason, it is somewhat surprising that microinjection of opioid antagonists into this area is not very effective in eliciting bodily signs of withdrawal in a dependent animal. However, Koob and coworkers (1992) have suggested that the NAcc may be important in the aversive stimulus effects or motivational aspect of opioid withdrawal. This conclusion was based on a series of experiments in which opioid-dependent rats experienced naloxone-precipitated withdrawal in a novel environment. Under such conditions, the animals develop a place aversion for the novel location and remain in an adjacent compartment (see Figure 4.8). Koob and colleagues were interested in finding out which brain area, when microinjected with antagonist, is responsible for the place aversion. They found that the areas most sensitive to low doses of antagonist are the NAcc, followed by the amygdala and the PAG. In conclusion, the brain areas implicated in the physiological response to opioid withdrawal are the PAG and the locus coeruleus, which may also mediate withdrawal-induced anxiety, while the NAcc is likely responsible for the aversive qualities of withdrawal, as well as some of the positive-reinforcing values of opioid use.

Neurobiological adaptation and rebound constitute tolerance and withdrawal

The classic hypothesis of opioid tolerance and dependence was first developed by Himmelsbach (1943) and is shown in **FIGURE 11.19A**. He suggested that acute administration of morphine disrupts the organism's homeostasis but that repeated administration of the drug initiates an adaptive mechanism that compensates for

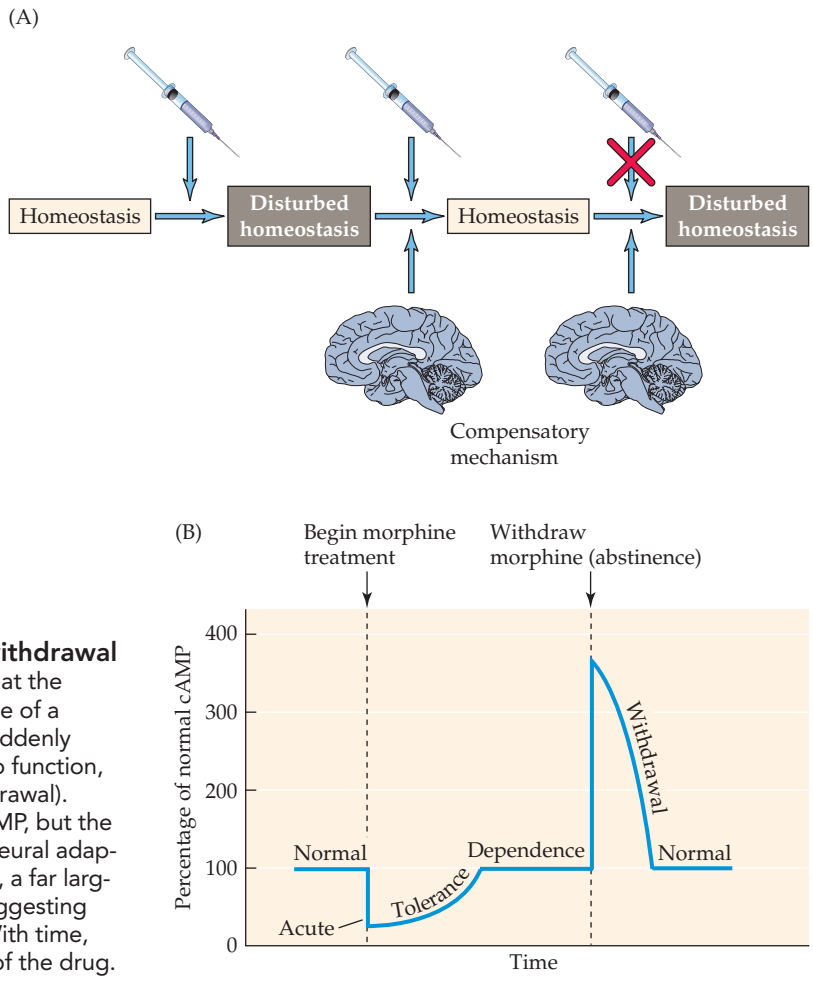


FIGURE 11.19 Model of tolerance and withdrawal
(A) Himmelsbach's theoretical model suggests that the nervous system adapts to the disturbing presence of a drug, so tolerance develops, but if the drug is suddenly withdrawn, the adaptive mechanism continues to function, causing a rebound in physiological effects (withdrawal). (B) Morphine acutely inhibits the synthesis of cAMP, but the effect becomes less as tolerance develops and neural adaptation occurs. If morphine is suddenly withdrawn, a far larger than normal amount of cAMP is produced, suggesting that the adaptive mechanism is still operating. With time, the cells once again adapt, now to the absence of the drug.

the original effects and returns the organism to the normal homeostasis. At this point, tolerance to the drug has occurred, since the same dose of morphine no longer produces the original disturbance. When morphine administration is abruptly stopped, the drug's effects on the body are terminated, but the adaptive mechanism remains active and overcompensates. The subsequent disruption of homeostasis is the withdrawal syndrome.

Although the Himmelsbach model was entirely theoretical, in the mid-1970s a physiological correlate was described by Sharma and coworkers (1975). They used cells with opioid receptors and maintained them in cell culture. They found that the acute administration of morphine caused an inhibition of adenylyl cyclase, the enzyme that manufactures cAMP (**FIGURE 11.19B**). Himmelsbach would call this stage "disturbed homeostasis." However, when the cells were kept in the morphine solution for 2 days, they showed tolerance to the drug's inhibitory effect. That is, after 2 days, they had levels of cAMP equal to control cells that had not been exposed to morphine. Apparently, the adaptive mechanism proposed by Himmelsbach became effective. When the opioid was abruptly removed from the cell culture solution or naloxone was added, the concentration of cAMP rose significantly above control levels because the adaptive mechanism was still operating, although the drug's inhibitory effect was gone. This rebound in cAMP levels corresponds with the withdrawal phenomenon and clearly represents disturbed homeostasis again. Other parts of the cAMP system, such as cAMP-dependent protein kinase A (PKA) and phosphorylated neuronal proteins, are also up-regulated by the chronic use of opioids.

The relationship of cAMP to neural activity and the withdrawal syndrome is suggested by the parallel time course of changes in those three factors. Nestler and coworkers (1994) examined the electrophysiological effects of morphine on cells in the locus coeruleus. The acute effect of opioids acting at μ-receptors consists of hyperpolarization and reduced rate of firing. Repeated exposure to opioids produced a gradual increase in firing rates of locus coeruleus cells as tolerance developed. Administration of an opioid antagonist after chronic opioid treatment induced a significant rise in firing rate to levels well above pretreatment levels, reflecting a rebound withdrawal that gradually returned to normal. A similar time course occurred for the overshoot of cAMP synthesis, the up-regulation of PKA and protein phosphorylation, and their return to control levels. Behavioral manifestations of abstinence also declined over the same 72-hour period. It is of interest that in subsequent research, Punch and colleagues (1997) showed that intra–locus coeruleus infusion of a PKA inhibitor attenuated several prominent behavioral signs of morphine withdrawal in dependent animals. Conversely activating PKA produced quasi-withdrawal symptoms in nondependent drug-naive animals.

Despite the evidence for the importance of cAMP signaling to tolerance and withdrawal, a lot of research strategies, including animal models, cell culture, or pharmacological manipulations to disrupt tolerance development, have found a variety of cellular mechanisms potentially responsible in addition to cAMP. In fact, it is likely that multiple mechanisms are responsible for tolerance (reviewed by Williams, 2013). It has been suggested that the very rapid process of desensitization, as shown in vitro with just a few minutes of exposure to agonists, is likely due to uncoupling between the opioid receptor and G protein signaling. However, development of tolerance over longer periods of drug administration may be due to loss of receptors. Such down-regulation of receptors has been shown in some brain regions, but not others. Additionally, down-regulation occurs for some opioids, such as etorphine, but chronic morphine does not alter receptor number in most brain regions. Hence down-regulation is not a likely explanation for morphine tolerance. Another possibility is that receptors are internalized, that is, their location is altered, moving from the plasma membrane to other internal cellular compartments. Finally, a role for learning in tolerance development is another potential factor (see below).

Environmental cues have a role in tolerance, drug abuse, and relapse

We have already alluded to the idea that environmental factors can be classically conditioned to parts of the drug experience (see the section on behavioral tolerance in Chapter 1). Conditioning theory has also been applied to the development of tolerance to opioids. Siegel and Ramos (2002) and Tiffany et al. (1992) propose that narcotic tolerance is in part the result of the learning of an association between the effects of the drug and the environmental cues that reliably precede the drug effects. Several experiments have shown that after repeated drug administration in a particular environment, the animal begins to show anticipatory physiological responses when it is in that same situation. Thus, it is argued that tolerance to the analgesic effects of morphine results because environmental cues regularly paired with drug administration begin to elicit the compensatory response of hyperalgesia, which diminishes the analgesic effect of the drug. Some have suggested this mechanism as the basis for some of the drug overdose fatalities among addicts. One would have to assume that an addict who has developed significant tolerance to his drug of choice in his standard environment might find that his tolerance is much less if he uses the drug in a novel situation. His usual dose may then be enough to produce overdose.

Environmental factors can also clearly have a role in portions of the drug experience. For example, if euphoria

is associated with certain stimuli such as the camaraderie of the drug-using subculture, drug acquisition activities, or drug injection rituals, those aspects of the environment will act as secondary reinforcers, strengthening the drug-taking behavior. What this means is that many components of drug-taking behavior become so closely associated with the drug-induced euphoria and sense of well-being that they are in themselves reinforcing. This association can be used to explain the unusual behavior of the "needle freak" who can inject any substance and achieve some measure of the "high" associated with drug taking. Childress et al. (1999) reported changes in limbic system neural activity as measured by PET scans of increased cerebral blood flow in drug users who were merely exposed to drug cues that increase their craving. The brain areas activated were similar to those activated by the drug (in this case cocaine). Increased metabolic activity in the amygdala and the anterior cingulate (**FIGURE 11.20**) during cue-induced craving suggests the importance of emotional memory (amygdala) and emotional expectation (anterior cingulate) to the conditioning. Since these regions are both connected to the nucleus accumbens and are activated during drug exposure, it is reasonable to suggest that they help the individual learn the signals that are linked to rewarding events. When these cues, acting as secondary reinforcers, are present, they may act as **triggers** that promote drug taking because they remind the individual of how the drug feels. Cue-induced craving and its neural mechanism have become an important focus of research because they are most closely associated with the compulsive drug-seeking behavior that characterizes drug abuse.

Objective (respiration rate, skin temperature, heart rate) and subjective elements of narcotic withdrawal symptoms can be experimentally conditioned to environmental stimuli in humans as well (Childress et al., 1986). The high rate of relapse among detoxified addicts may be due to the conditioned abstinence syndrome in the old environment. O'Brien (1993) and others have presented reports of addicts who describe withdrawal symptoms when they visit areas of prior drug use even years after the withdrawal syndrome has ended. These findings have convinced many researchers that learning is a critical factor in opioid use disorder (**Web Box 11.3**). Under what circumstance individuals develop drug-enhancing associations or drug-opposing responses is not clear, but the mesolimbic DA pathway may be involved in both (Self and Nestler, 1995).

Treatment Programs for Opioid Use Disorder

Treating opioid use disorder requires understanding the multiple contributors to the problem. Treatment clearly depends on more than eliminating the drug from the body (detoxification), since the relapse rate for detoxified addicts is very high. Ultimately, a host of behavioral and social factors must be identified and altered for a successful outcome.

Most drug treatment programs utilize a biopsychosocial model as the basis for therapy. Models in this category take into account the multidimensional nature of chronic drug use:

- The physiological effects of the drug on nervous system functioning, as when the opioids activate the mesolimbic reward pathway
- The psychological status of the individual and her unique neurochemical makeup and history of drug use
- The environmental factors that provide salient cues for drug taking and powerful secondary reinforcement

Detoxification is the first step in the therapeutic process

Detoxification, or the elimination of the abused drug from the body, can be assisted or unassisted. Unassisted detoxification is often referred to as going "cold turkey," and many opioid abusers experience withdrawal symptoms on a fairly regular basis because

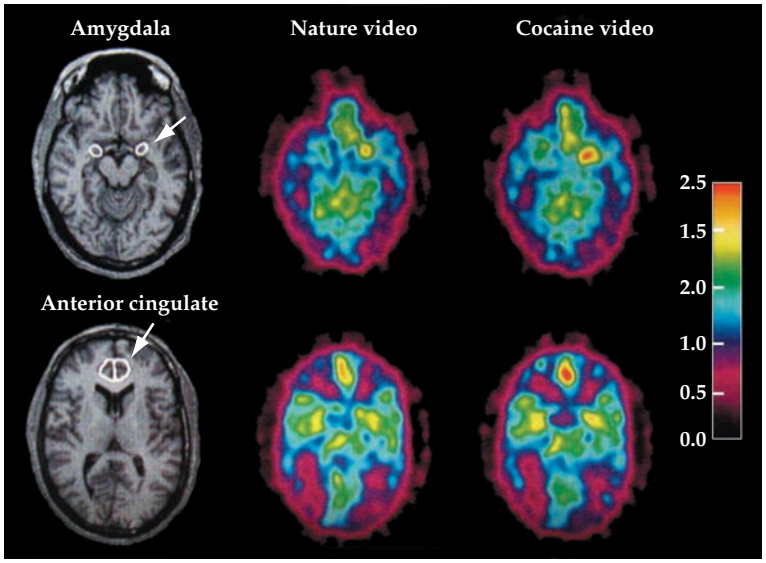


FIGURE 11.20 PET scans of cerebral blood flow Blood flow in a cocaine addict differed during exposure to a non-drug-related video (nature video) and exposure to a cocaine-related video containing many cues. Areas with the greatest activity are shown in red. Activity in the amygdala and anterior cingulate is significantly increased during the cocaine video. (From Childress et al., 1999.)

they have difficulty securing more drug. Alternatively, detoxification may be assisted by the administration of a long-acting opioid drug, such as **methadone**, which reduces the symptoms to a comfortable level. The dose of methadone is gradually reduced over a period of 5 to 7 days until it can be terminated with only mild symptoms. Sometimes the α_2-adrenergic agonist **clonidine** is used in this stage. Clonidine acts on noradrenergic autoreceptors to reduce norepinephrine activity. Since the noradrenergic neurons in the locus coeruleus are inhibited by opioids and the cells increase firing during withdrawal (see Nestler et al., 1994, described earlier), clonidine-induced inhibition of firing reverses this hyperexcitable state. The drug seems to relieve the chills, tearing, yawning, stomach cramps, sweating, and muscle aches that are associated with the activity of the locus coeruleus but does not reduce the remaining withdrawal symptoms nor the subjective discomfort and craving (Gold, 1989; O'Brien, 1993). Unfortunately, clonidine itself has side effects including insomnia, dry mouth, sedation, joint pain, and dizziness. For these reasons, it is not very popular with addicts, who much prefer detoxification with an opioid.

An ultrarapid detoxification that is completed in a few hours or over several days requires that withdrawal be initiated with opioid antagonists while the addict is treated with clonidine, a benzodiazepine, or general anesthesia. This method is considered by many to be ineffective because it does not produce improved abstinence rates and is associated with potentially life-threatening events.

Rather than pharmacological intervention to prevent withdrawal, it is possible to use electroacupuncture (EA) instead. Using morphine-dependent rats, G. B. Wang and colleagues (2011) showed that 30 minutes of 100 Hz EA delivered 12 hours after the last morphine administration significantly reduced the classic signs of rodent withdrawal. They found an apparent "dose-dependent" effect in that a single EA treatment had a small effect, two sessions 11 hours apart had a significant effect, and four administrations had still greater effects on 4 out of 5 withdrawal signs measured. Perhaps even more interesting was the finding that EA restored the low levels of prodynorphin mRNA to normal in the spinal cord, hypothalamus, and PAG, which suggests that withdrawal is suppressed by increasing dynorphin synthesis. EA has been used in China to treat both opioid withdrawal and relapse for a number of years, but more recently, more controlled studies with animals and human addicts have been conducted. Cui et al. (2008) review that evidence.

Treatment goals and programs rely on pharmacological support and counseling

Treatment for heroin use disorder offers several options and may be selected on the basis of availability in a given location, cost, or personal preference. Description and evaluation of treatment options is provided by Connery (2015). Most programs begin with detoxification before starting intensive treatment on either an inpatient or an outpatient basis. Prolonged periods of follow-up care and supplementary services are advantageous in preventing relapse.

METHADONE MAINTENANCE The most commonly used treatment method for heroin use disorder is the **methadone maintenance program**. Originally developed by Dole and Nyswander (1965), the program involves the long-term substitution of one opioid drug for another. The rationale for the program is that by having his craving relieved, the addict is able to redirect his energy away from the activities needed to secure the drug toward more productive behaviors such as education or job training.

A large number of studies over many years have shown that when compared with any other treatment method, methadone maintenance has been the most effective in reducing heroin and other illicit drug use and has at least doubled the rate of abstinence compared with psychosocial treatment. The percentage of patients who remained abstinent has been reported to be as high as 90% among those who continued in the program for the recommended duration, while among those who dropped out, the abstinence rate fell to 12% (California Society of Addiction Medicine, 2011). In addition, those addicts involved in the program showed significantly lower rates of criminal activity, HIV infection, and mortality, while showing greater involvement in becoming self-supporting (Bertschy, 1995). For these reasons, it has been estimated that methadone treatment programs save taxpayers $12 for every $1 spent (California Society of Addiction Medicine, 2011). Prolonged involvement with the program is expected, and in many cases methadone is used in a chronic fashion, just as one would treat a diabetic with insulin. Alternatively, the individual may be gradually weaned from the drug support, but in these cases relapse rates are higher.

Among the hazards of methadone maintenance is the significant risk for accidental overdosing of the addict at the start of the treatment. Overdosing is a common event because it is difficult to determine the precise level of the individual's tolerance, making it difficult to choose the appropriate starting dose. There is also a large variation in required effective dose among individuals. Because methadone has such a long half-life, blood levels gradually increase for up to 5 days at the same dose (see steady state plasma levels in Chapter 1). Hence an initial ineffective dose can become toxic. Careful monitoring of the patient and dose adjustments must occur daily during the first 2 weeks and less frequently thereafter. In the future, pharmacogenetics may make it easier to determine the appropriate daily maintenance dose. For

example, particular gene variants of the metabolizing cytochrome P450 enzyme (CYP2B6) are associated with lower methadone dose requirements because individuals with those gene variants metabolize the drug more slowly, so their blood levels are higher for a given dose. Other gene variants of the transporter protein that is responsible for moving methadone across the blood–brain barrier have also been identified. Clearly differences in how much drug reaches the brain can help to explain the significant variability among individuals in their treatment needs. For more detail, see Reed et al. (2014).

Methadone was chosen for use in opioid drug treatment programs for several reasons. First, cross dependence with morphine or heroin means that it can prevent the more severe withdrawal associated with the abused drug. Second, the cross-tolerance that develops to repeated methadone use means that the normal euphoric effects of heroin are reduced or prevented. If an addict uses the illicit drug but gets little or no "rush," continued drug use should be less likely. Unfortunately, the tolerance can be overcome by high (and expensive) doses of heroin. In addition, methadone itself can produce a "rush" of euphoria if it is injected intravenously, which can lead to illegal diversion and trafficking of the drug. For this reason, most programs require *supervised* daily administration of *oral* methadone. The oral administration of methadone is a third important factor in the popularity of methadone maintenance because although little or no euphoria occurs with oral administration, the drug is fully effective in relieving craving for opioids. Craving is believed to be an important motive for relapse. In addition, oral administration reduces the use of the needle by the addict and the ritual surrounding its use. It also eliminates the danger of disease due to unsterile injection techniques. The spread of infectious diseases such as hepatitis and HIV is also reduced by eliminating the need to share contaminated needles.

Fourth, methadone is relatively long-acting, which produces a more constant blood level of drug such that the individual experiences fewer extremes of drug effect. A more even blood level produces a more stable daily experience and also normalizes body functions such as hormone secretion. Methadone is needed only once a day to prevent methadone withdrawal for 24 to 36 hours. The time course of drug action means daily contact and interaction with clinical staff who can provide behavioral therapy, group and family counseling, and support in education or job training. In addition, medical care can be provided. Of particular significance are the prenatal care for pregnant addicts and treatment of diseases, such as HIV, hepatitis, and syphilis, that are common among addicted pregnant females. Additionally, nutritional status is much improved, which leads to increased birth weight of infants born to mothers enrolled in the methadone program. However, since methadone passes the placental barrier like other opioids, the infant at delivery will sometimes show withdrawal signs, including tremors, twitching, seizures, vomiting, diarrhea, and poor feeding. These symptoms are treated by low doses of opioids, which are then tapered down until no drug is needed.

Fifth, methadone is considered medically safe even with long-term use and does not interfere with daily activities. Unfortunately, some side effects do not diminish with repeated use, so constipation, excessive sweating, reduced sex drive, and sexual dysfunction may persist during treatment for some individuals. It is noteworthy that long-term use of any opioid drug has few damaging effects on organ systems. The greatest dangers stem from poor living conditions, including inadequate diet, lack of medical care, and homelessness; dangerous and unlawful behaviors required to secure the drugs; and potentially fatal side effects of using contaminated needles or impure sources of drug. A description of clinical treatment guidelines can be found at www.cpso.on.ca.

BUPRENORPHINE MAINTENANCE Another opioid, **buprenorphine** (**Buprenex**), is an opioid partial agonist and is used in the same manner as methadone. Because it has a high affinity but low efficacy at the μ-opioid receptor, as well as antagonist activity at the κ-receptor, it has weaker opioid effects and is less likely to result in overdose or respiratory depression. It produces similar treatment results but has a longer duration of action and so produces more stable physiological effects and an extremely mild withdrawal syndrome.

The longer duration of action also means less frequent administration (one to three times a week), which significantly reduces the costs of the program and gives an extra measure of freedom to the opioid abuser who needs daily clinic visits for methadone. Fewer clinic visits also tends to improve the relationship with members of the surrounding community, who often object to high rates of drug user visits to their neighborhood. Buprenorphine is the only opioid substitute that can be prescribed in a physician's office that is not part of a federally regulated opioid treatment program. It is hoped that greater use of this drug will reduce costs and make more treatment facilities available. In fact, the U.S. Department of Health and Human Services (2016) is trying to expand access to agonist-assisted treatment by enabling nurse practitioners and physician assistants to take the 24-hour training session required in order to prescribe buprenorphine. Although initially they can prescribe for a limit of 30 patients, after 1 year they can prescribe for up to 100 addicts. The increase in access to medication-assisted treatment is part of the government's efforts to stem the opioid addiction crisis, along with changing opioid prescription practices and increasing availability of naloxone to reverse opioid overdoses. Another government website provides information on

buprenorphine treatment, side effects, and safety and has links to other resources (SAMHSA, 2016).

In addition, because buprenorphine does not produce more than a mild euphoria when taken as directed, the drug abuser can get a supply of the drug rather than just a single dose. To further reduce its potential for IV use, buprenorphine is available in a sublingual formulation (Suboxone) that also contains the antagonist naloxone. When taken sublingually, the buprenorphine is absorbed but the naloxone is not. If the tablet is crushed and injected intravenously in an effort to experience the euphoria, the naloxone blocks buprenorphine's effects. However, Suboxone can still be abused if crushed and snorted. Unfortunately, there has been an increase in law enforcement seizures of the drug that has been diverted to individuals without a prescription, and emergency department visits related to nonmedical use of buprenorphine rose almost fivefold from 2006 to 2011 (Crane, 2015) (**FIGURE 11.21**). Although buprenorphine is less likely to lead to overdose than methadone, in combination with CNS depressants, it can lead to respiratory depression and death. The newest approach to buprenorphine treatment is an implantable option (Probuphine) in which several matchstick-size rods are implanted under the skin and deliver buprenorphine at a continuous rate for 6 months. The depot administration enhances compliance with the drug treatment regimen because the addict does not have to remember to take a pill every day. It also means the pills cannot be lost or stolen. Finally, the implant clearly prevents diversion of drug and its abuse and protects against accidental ingestion by children. An additional advantage is that the sustained delivery avoids the peaks and troughs in blood level that occur with oral medications, so there is less risk of sudden craving. In the initial trials individuals on this sustained delivery method were much more likely to avoid illicit opioids than those using the pill form (FDA, 2016).

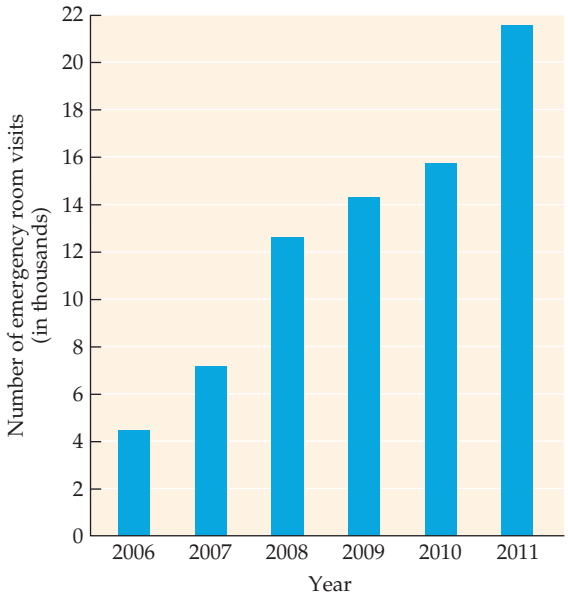


FIGURE 11.21 U.S. emergency department visits related to nonmedical use of buprenorphine The number of visits, including those in which buprenorphine was the direct cause or a contributing factor, rose from 2006 to 2011. (After Crane, 2015).

Although during pregnancy complete abstinence of opioid use would be ideal, abrupt withdrawal could be hazardous to the fetus. Since relapse is common after withdrawal, repeated intoxication and withdrawal causes significant fetal distress that can cause miscarriage, impaired fetal growth, low birth weight that is a risk factor for later developmental delay, or premature birth. Maintenance treatment with methadone or buprenorphine is preferable to heroin use, and when combined with prenatal care, it produces better outcomes. When compared in a meta-analysis, researchers found that buprenorphine compared with methadone maintenance was associated with lower risk of preterm birth, higher birth weight, and larger head circumference. There did not seem to be any difference in congenital abnormalities, although most studies did not do follow-up evaluating through the first year of life (Zedler et al., 2016).

Research comparing the two drug maintenance approaches shows that the neonatal abstinence syndrome is also milder than that after methadone maintenance and that buprenorphine-exposed infants require significantly less morphine to treat the withdrawal. Because the withdrawal is less stressful, recovery is more rapid and the infants spend about half as much time in the hospital as methadone-exposed infants. Although there were no lasting differences (over the first 30 days) between the two groups of infants, the methadone-exposed infants were initially more stressed and excitable, were unable to self-regulate or manage sleep and wakeful periods, and showed difficulty with handling. These kinds of neurobehavioral differences may pose greater challenges as the infant transitions home, which may be an at-risk environment (Coyle et al., 2012).

Although buprenorphine maintenance seems to have advantages for pregnant addicts, the developmental consequences of prenatal buprenorphine have not been fully determined in humans. In rats, pre- and postnatal exposure to buprenorphine produced dose-dependent effects on several proteins needed for myelin formation, smaller-diameter myelinated axons, and reduced thickness of the myelin in the corpus callosum (Sanchez et al., 2008). More recently Hung and colleagues (2013) administered buprenorphine to pregnant rats starting at 7 days gestation for 14 days and studied the weanlings at 21 days of age. The animals

were tested in the open field test, Morris water maze, forced swim test, and tail suspension test (see Chapter 4) and also were subjected to neurochemical analyses. Those young animals exposed to high doses of buprenorphine (but not their mothers) showed behavioral signs of depression in the forced swim test and tail suspension test that could be reversed with the antidepressant drug imipramine. Anatomically, both brain and body mass were reduced. Neurochemically, there were reductions in plasma 5-HT and the neurotrophic factor BDNF. In addition, in cortical tissues there was reduced activation of the transcription factor CREB, which is important for expression of BDNF. These signaling molecules have long been associated with clinical depression (see Chapter 18). Further studies should be conducted to determine which opioid receptor is involved and whether these changes persist over the long term.

In summary, compared with psychological treatments alone or managed tapering off of opiate use, long-term maintenance with opioid agonists produces better treatment outcomes as measured by days of illicit opiate use. Also, those individuals on the opioid agonists were more persistent in follow-up medication and psychological therapies. Meta-analyses suggest that there is no difference in efficacy associated with methadone compared with buprenorphine (Nielsen et al., 2017). Maintenance therapy is more problematic during pregnancy.

USE OF NARCOTIC ANTAGONISTS We have already described the utility of naloxone in reversing the effects of opioid toxicity. Antagonists also represent a component of some drug abuse treatment programs. After detoxification, antagonist treatment will block the effects of any self-administered opioid. Naltrexone (Trexan) is the most commonly used because it has a longer duration of action than naloxone and is effective when taken orally. It also has fewer side effects than cyclazocine, which may produce irritability, delusions, hallucinations, and motor incoordination. Nalmefene (Revex) is a newer pure opioid antagonist and is similar to naltrexone but more potent and longer-lasting.

This method is effective for addicts who are highly motivated, have strong family support, and are involved in careers (e.g., addicted medical personnel). Reliable patients have taken naltrexone for 5 to 10 years without relapse to drug-taking behavior and with minimal adverse effects on appetite, sexual behavior, or endocrine function (O'Brien, 1993). Unfortunately, this method appeals to only about 10% of the addicted population, because a great deal of motivation is needed to voluntarily substitute an antagonist for a drug with highly reinforcing properties. Since craving for the drug is not eliminated, most less-motivated addicts stop antagonist treatment and return to drug use. Only about 27% of addicts in these treatment programs complete a 12-week preliminary session (Osborn et al., 1986).

VACCINES FOR ADDICTION TREATMENT The rationale for using vaccines for the treatment of opiate abuse is that abused substances must enter the brain to exert their behavioral effects. A vaccine would produce antibodies in the individual that would bind to the drug molecules in the blood circulation and prevent entry into the brain. Although it is not feasible to vaccinate the general population against every abused drug, vaccines might be useful in treating existing abuse of specific drugs. One such vaccine for opioids recognizes heroin and its active metabolites, 6-acetylmorphine and morphine (Stowe et al., 2011). This vaccine prevents the self-administration and analgesic effects of heroin in rats. Clinical trials with humans still need to be conducted.

COUNSELING SERVICES Most often, addicts benefit from a **multidimensional approach** that includes a combination of detoxification, pharmacological support, and group or individual counseling. Counseling frequently is used to help addicts identify the environmental cues that trigger relapse for the individual. Having identified his "triggers," the addict must then design a behavioral response to those cues to prevent relapse. Furthermore, job training, educational counseling, and family therapy may be useful. Based on a model program for alcohol abuse treatment, Narcotics Anonymous is another option for motivated drug abusers to achieve drug abstinence.

Section Summary

- Predicting which patients receiving opioids for pain will become chronic drug users is difficult. Physicians struggle with the dilemma of needing to treat serious pain and yet follow new CDC guidelines that want to limit opioid prescriptions because of the rising number of opioid fatalities.
- Animal studies show that opioids have reinforcing effects in self-administration paradigms, in conditioned place preference, and in reducing the threshold for electrical self-stimulation of the brain.
- Opioid drugs inhibit inhibitory GABA cells, increasing mesolimbic cell firing and DA release in the NAcc. Both dopaminergic and nondopaminergic mechanisms are necessary for the reinforcing effects.

- Opioid drugs demonstrate tolerance to many of the drug effects and cross-tolerance with other drugs in the same class, as well as with endogenous opioids.
- Prolonged use produces physical dependence, which is characterized by a classic rebound withdrawal syndrome that includes many flu-like symptoms, insomnia, depression, and irritability. Cross dependence means that any drug in the opioid family can abruptly stop withdrawal symptoms.
- A model of the physiological mechanism for tolerance and dependence is the compensatory response of cells in the locus coeruleus to the acute inhibition of adenylyl cyclase. Tolerance may also be due to uncoupling of the receptor from G protein, down-regulation of receptors, or receptor internalization.
- Classical conditioning of environmental cues associated with components of drug use is important in the development of tolerance and in maintaining the drug habit. Conditioned craving is significant in producing relapse in the detoxified addict.
- Detoxification is the first step in drug abuse treatment.
- Opioid abuse treatment programs include the substitution of another opioid, such as methadone or buprenorphine, for the abused drug. These substitutes, when given orally, produce no euphoria but eliminate craving for heroin and reduce exposure to HIV and hepatitis. The long-acting opioids stabilize the physiological effects and encourage contact with support staff. Methadone has a mild withdrawal. There are few damaging effects on organs with prolonged use.
- Compared with methadone, buprenorphine is longer acting, has a milder withdrawal and neonatal withdrawal, and can be prescribed in a physician's office. When combined with naloxone, abuse potential is reduced. An implantable device to administer buprenorphine has multiple advantages over the pill form of the drug.
- Maintenance treatment with methadone or buprenorphine is preferable to heroin use in pregnant addicts. Buprenorphine and methadone are equally effective in reducing heroin use but buprenorphine may have advantages in fetal outcome.
- Opioid antagonists are effective in blocking the opioid receptors so that self-administered narcotics have no effect.
- In rats, heroin vaccine binds to drug molecules, which prevents entry to the CNS and reduces self-administration of opioids.
- The most successful treatment approaches are multidimensional ones.

STUDY QUESTIONS

1. How do differences in molecular structure determine opioid effects?
2. Describe opioid effects on the CNS and gut.
3. Briefly discuss the localization and function of the four opioid receptor subtypes.
4. What are the classic neuropeptides that bind to opioid receptors. Describe how they are synthesized. How is nociceptin/orphanin FQ different?
5. Describe the cellular mechanisms responsible for opioid-induced inhibition.
6. Compare early and late pain.
7. Describe the three ways that opioids inhibit pain transmission in the spinal cord. How does opioid action in higher brain centers modulate pain transmission?
8. What is the mechanism of action of acupuncture? Dual inhibition of peptidases? Gene therapy? Dual receptor agonists?
9. Describe the research on emergency room patients and patients with chronic pain that helps to explain how medical patients become chronic drug users. Why do physicians find that treating pain is so difficult?
10. Provide evidence for opioid reinforcement, and describe the neurochemical basis.
11. Define *tolerance, cross-tolerance, sensitization, physical dependence*, and *cross dependence*. Be sure to tell how they apply to opioid use.
12. Which brain areas are responsible for opioid withdrawal signs? What is the role of the nucleus accumbens?
13. Describe the Himmelsbach model of tolerance and withdrawal. Provide several experimental findings in support of the model.
14. How do environmental cues influence tolerance? Drug abuse? Relapse?
15. Describe several ways that detoxification can be achieved.
16. What are the advantages of using methadone maintenance treatment for opioid use disorder?
17. Compare methadone and buprenorphine maintenance. What are the potential disadvantages of these methods?
18. Beyond opioid maintenance treatment, what other options are available to the opioid abuser?

Go to the *Psychopharmacology* Companion Website at oup-arc.com/access/meyer-3e for animations, web boxes, flashcards, and other study aids.

CHAPTER 12

Cocaine can be taken anywhere, from crack house to dorm room. (Christopher Morris-Corbis/Getty Images.)

Psychomotor Stimulants: Cocaine, Amphetamine, and Related Drugs

"THE PSYCHIC EFFECT ... CONSISTS OF EXHILARATION AND LASTING EUPHORIA, which does not differ in any way from the normal euphoria of a healthy person….One senses an increase of self-control and feels more vigorous and more capable of work….Long-lasting, intensive mental or physical work can be performed without fatigue; it is as though the need for food and sleep, which otherwise makes itself felt peremptorily at certain times of the day, were completely banished…. Opinion is unanimous that the euphoria … is not followed by any feeling of lassitude or other state of depression."

Any substance having the marvelous properties just described should be a boon to humankind. What is this miracle drug, then? The answer, unfortunately, is cocaine, and the nearly rapturous description quoted above comes from the writings of Sigmund Freud (*Über Coca*; reprinted in Byck, 1974).

Cocaine, **amphetamine**, and related compounds belong to a class of drugs called **psychomotor stimulants**. This term refers to the marked sensorimotor activation that occurs in response to drug administration. Indeed, psychomotor stimulants are characterized by their ability to increase alertness, heighten arousal, and cause behavioral excitement. This chapter considers the behavioral and physiological effects of these stimulants, their mechanisms of action, and their potential for producing abuse and dependence. Chapter 13 covers nicotine and caffeine, two less potent but more widely used stimulants. ■

Cocaine

Background and History

Cocaine is an alkaloid found in the leaves of the shrub *Erythroxylon coca*. The coca shrub is native to South America and is primarily cultivated in the northern and central Andes Mountains extending from Colombia into Peru and Bolivia (**FIGURE 12.1**). The latest data indicate that 92% of the cocaine entering the United States comes from Colombia (National Drug Threat Assessment, DEA, 2017). The inhabitants of these coca-growing regions consume cocaine by chewing the leaves—a practice thought to have begun at least 2000 and perhaps as many as 5000 years ago, according to archeological evidence. Because cocaine is a weak base, coca chewers also include some lime or ash to make the pH of the saliva more alkaline (**FIGURE 12.2**). This decreases ionization of the cocaine and promotes absorption across the mucous membranes of the oral cavity.

Coca chewing was an important feature of ceremonial or religious occasions in the Incan civilization, and use of the drug was ordinarily restricted to the ruling classes up to the time of the Spanish conquest. After the fall of the Incan empire, coca chewing became more widespread and commonplace, and there are even reports that coca was used as a medium of exchange. Over time, many Spanish missionaries and churchmen argued that coca chewing was idolatrous and interfered with conversion of the natives to Catholicism. The practice was consequently discouraged and even banned in some areas. The Spaniards soon discovered, however, that without the stimulating and hunger-reducing effects of coca, Incan workers lacked the endurance necessary to work long hours in the mines and fields at high altitudes and with little food. Thus coca cultivation and chewing were restored with the blessing of the Spanish rulers and the church.

Although coca leaves were brought back to Europe, coca chewing never caught on, possibly owing to degradation of the active ingredient during the long sea voyage. But travelers to the New World had occasion to sample the leaf, and several came back with glowing reports of its beneficial effects. By the late 1850s, German chemists had isolated pure cocaine and had characterized it chemically. Over the next 30 years, cocaine became tremendously popular as many scientists and physicians of the time lauded its properties. A chemist named Angelo Mariani concocted an infamous mixture of cocaine and wine called *Vin Mariani*, which was awarded a Vatican gold medal by Pope Leo XIII and was consumed by many other notables including Queen Victoria of England, Thomas Edison, and Ulysses S. Grant (Wielenga and Gilchrist, 2013). Another famous cocaine user was Sigmund Freud. In 1885, Freud published the monograph *Über Coca* ("On Coca"), which extolled the drug's virtues and recommended its use in the treatment of alcoholism, morphine addiction, depression, digestive disorders, and a variety of other ailments. Freud also performed the

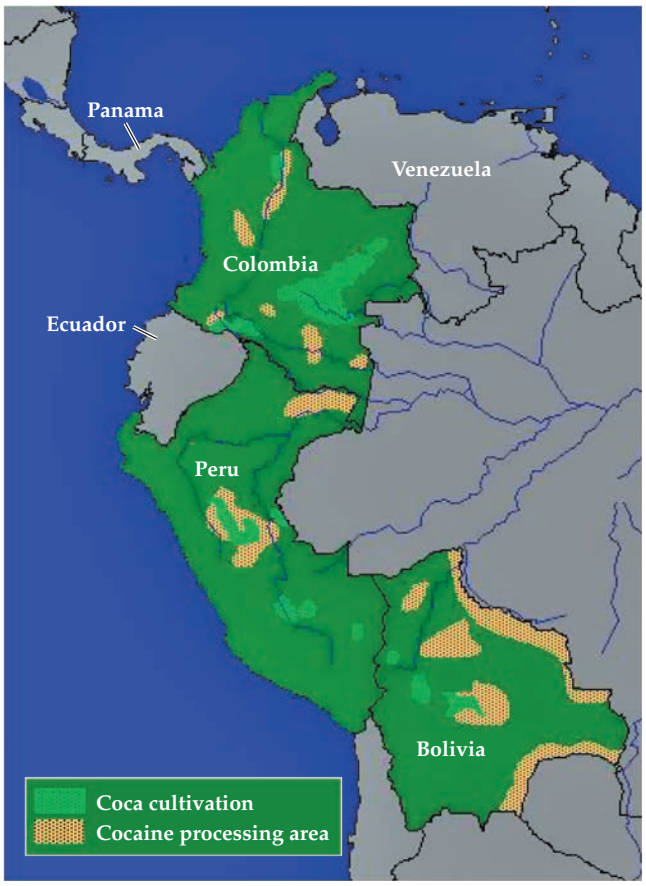


FIGURE 12.1 Map of principal coca-growing regions of South America (Courtesy of Rosemary Mosher and Michael Steinberg.)

FIGURE 12.2 Coca chewing is still practiced by some Bolivian miners Note the miner's bulging cheek, which is filled with coca leaves. The feelings of increased energy and reduced hunger resulting from cocaine consumption helps the user to work long hours in the mines. (© Hemis/Alamy Stock Photo.)

first recorded psychopharmacological experiments on cocaine and published the results in a paper entitled "Contribution to the Knowledge of the Effect of Cocaine." In his last written comments about cocaine in 1887, Freud acknowledged its dangers when used to treat morphine addiction, although he continued to maintain that the drug was nonaddictive under other circumstances.[1] Others, however, were more perceptive. The harshest critic was the German psychiatrist A. Erlenmeyer, who labeled cocaine the "third scourge of the human race," after alcohol and opium.

Despite the warning signs emanating from Europe, cocaine's popularity grew in the United States during the late nineteenth and early twentieth centuries. By 1885, Parke, Davis & Co., an early pharmaceutical firm, was manufacturing 15 different forms of cocaine and coca, including cigarettes, cheroots (a type of cigar), and inhalants. One year later, a pharmacist from Georgia named John Pemberton introduced a new beverage, "Coca-Cola," which contained cocaine from coca leaves and caffeine from cola nuts.[2] Coca-Cola and similar concoctions were marketed as suitable alternatives to alcoholic drinks because of the growing strength of the alcohol temperance movement at that time (**FIGURE 12.3**). Cocaine-containing tooth drops were even given to infants to relieve the discomfort of teething (the local anesthetic effects of cocaine are discussed further in the section on mechanisms of action). Not surprisingly, widespread cocaine abuse began to spread across the United States until President Taft declared cocaine to be "public enemy number one" in 1910. Congress then passed the 1914 Harrison Narcotic Act prohibiting the inclusion of cocaine (as well as opium) in over-the-counter medicines and specifying other restrictions on its import and sale (see Chapter 9). Subsequent state and federal laws, of course, placed even tighter regulations on cocaine distribution and use.

From the 1920s to the 1960s, cocaine use continued primarily among a relatively small group of avant-garde artists, musicians, and other performers. Beginning in the 1970s, however, two successive waves of increasing cocaine use were seen in the United States. The first involved an escalation of cocaine use by snorting or intravenous (IV) injection, whereas the more recent epidemic of cocaine use has been driven by the smoking of "crack" cocaine.

According to estimates derived from the 2016 National Survey on Drug Use and Health, over 1.9 million people age 12 or older (0.7% of the population) were

FIGURE 12.3 Coca-Cola ad from the 1890s

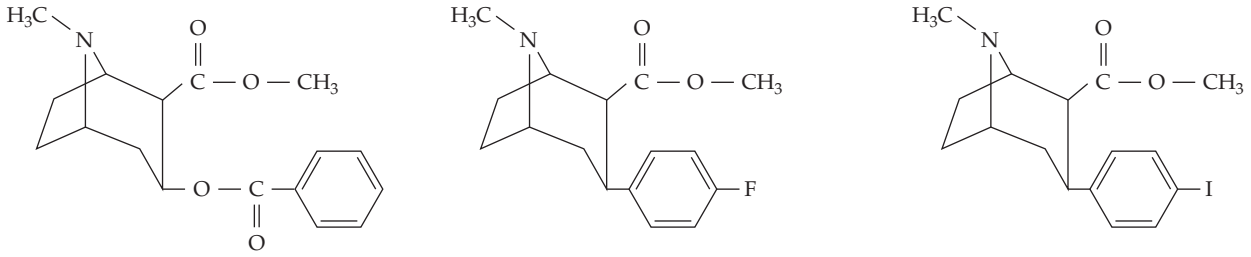
current users of cocaine at the time of the survey (Substance Abuse and Mental Health Administration, 2017). By "current user," we mean that the individual had used cocaine at least once during the previous month. Of these current users, approximately 900,000 people met criteria for abuse of or dependence on cocaine. During 2016, more than one million people age 12 or older used cocaine for the first time. On the basis of past experience, we know that some of those individuals will subsequently begin to abuse and in some cases will become dependent on the drug, whereas others will not. Although we know some of the psychosocial and environmental characteristics that contribute to differences between those groups, it would be of great value to know more about the genetic and molecular determinants that may also contribute to vulnerability to cocaine abuse and dependence.

Basic Pharmacology of Cocaine

FIGURE 12.4 presents the chemical structure of alkaloidal cocaine, which is its naturally occurring form. The molecule contains two rings: the six-carbon phenyl ring shown on the right and the unusual nitrogen (N)-containing ring shown on the left. Both are necessary for the drug's biological activity. Other features of the molecule have been manipulated with interesting results. For example, Figure 12.4 also depicts the structures of two synthetic cocaine-like drugs: WIN 35,428 (also known as CFT) and RTI-55 (also called β-CIT). Notice that both compounds lack the ester (–O–CO–) linkage between the rings, and both possess a halogen (fluorine [F] or iodine [I]) atom on the phenyl ring. WIN

[1]In fairness to Freud, it should be noted that he normally took cocaine orally—a route of administration with less abuse potential than IV injection, smoking, or even snorting.

[2]Although cocaine itself was removed from the product in 1903, Coca-Cola continues to contain a non-narcotic extract from the coca leaf that is regularly prepared by a chemical company in the United States (Goldstein et al., 2009).



Cocaine **WIN 35,428 (CFT)** **RTI-55 (β-CIT)**

FIGURE 12.4 Chemical structures of cocaine, WIN 35,428, and RTI-55

35,428 and RTI-55 are more potent than cocaine, and if available on the street, they presumably would be highly addictive. Fortunately, they are used only experimentally, primarily for in vitro studies on brain tissue.

Coca leaves contain between 0.6% and 1.8% cocaine. Initial extraction of the leaves results in a coca paste containing about 80% cocaine. The alkaloid is then converted to hydrochloride (HCl) salt and is crystallized. Cocaine HCl is readily water soluble and thus can be taken orally (as in *Vin Mariani*), intranasally (snorting), or by IV injection. One disadvantage of cocaine HCl is its vulnerability to heat-induced breakdown, thereby preventing it from being smoked. However, the hydrochloride salt can be transformed back into cocaine freebase by two different methods. The method developed first was to dissolve cocaine HCl in water, add an alkaline solution such as ammonia, and then extract the resulting cocaine base with an organic solvent, typically ether. The term **freebasing** refers to smoking cocaine that was obtained in this manner. However, because ether is highly flammable and explosive, a certain danger is involved, not only in this method of preparing cocaine freebase but even in smoking it, since an ether residue may still be present if one is not careful. In the early 1980s, it was discovered that cocaine base could be made more safely by mixing dissolved cocaine HCl with baking soda, heating the mixture, and then drying it. Chunks of the dried, hardened mixture are known on the street as **crack** (so named because of the popping sounds produced when the chunks are heated), or "rock" cocaine. Such chunks are generally sold inexpensively in small amounts sufficient for only one or two doses (**FIGURE 12.5**). The cocaine that ends up in a user's bloodstream is the same substance regardless of whether its initial form was the hydrochloride salt or the freebase. However, the heat involved in smoking crack produces unique chemical products that can be detected in the urine and used by forensic chemists to verify crack use (Dinis-Oliveira, 2015).

Different routes of consumption yield somewhat different patterns and levels of plasma cocaine. Extremely rapid absorption occurs with both IV injection and smoking. Hence, typical single doses taken by these routes yield rather high concentrations of circulating cocaine, and even higher values can be attained with multiple doses that mimic the pattern of a cocaine "binge" (see the section on cocaine abuse later in the chapter). Absorption is somewhat slower following oral administration or snorting; consequently, these routes yield lower cocaine levels. **FIGURE 12.6** compares the time course of plasma cocaine concentrations as a function of route of administration. It is interesting to note that a few hours of coca leaf chewing delivers enough drug to mimic the plasma concentrations produced by a modest dose taken either orally or intranasally.

Although researchers normally measure blood levels of drugs in a peripheral vein such as the antecubital vein located in the arm, even more important for a psychoactive drug is the level present in the brain. Cocaine is sufficiently lipophilic (fat soluble) that it passes readily through the blood–brain barrier (see Chapter 1). This property particularly comes into play when cocaine is smoked, because it results in exposure of the brain to a very large surge in drug levels not reflected in peripheral venous concentrations. Rapid entry into the brain is believed to be an important factor in the strong addictive properties of crack cocaine.

FIGURE 12.5 Crystals of crack cocaine (Courtesy of the U.S. DEA.)

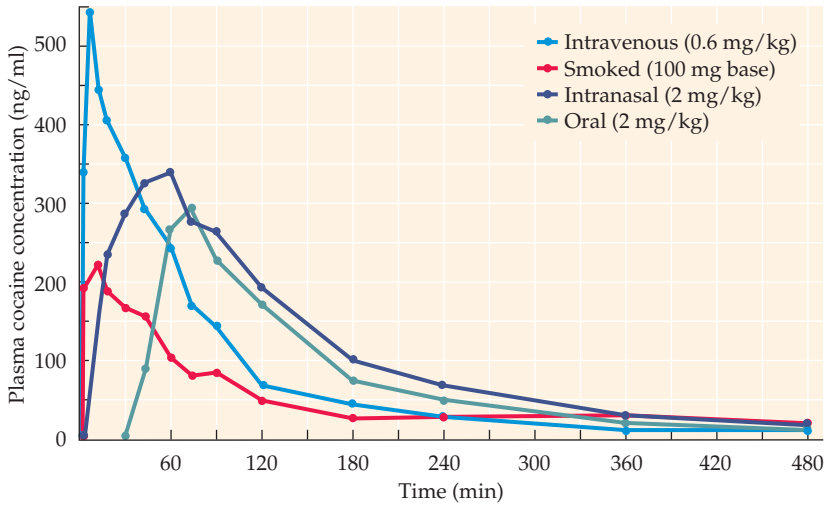


FIGURE 12.6 Time course of plasma cocaine concentrations following different routes of administration
Each curve shows the mean for 10 human participants. For cocaine freebase smoking, participants were allowed only one to three puffs of the vapor from 100 mg of drug heated in a flask. The peak plasma concentrations produced under these conditions probably underestimate the levels occurring from recreational use. (After Jones, 1990.)

Once absorbed into the circulation, cocaine is rapidly broken down by enzymes found in the bloodstream and the liver. It is also rapidly eliminated, with a half-life ranging from about 0.5 to 1.5 hours. For this reason, the subjective high produced by a single IV or smoked dose of cocaine may last only about 30 minutes. However, the breakdown products of cocaine persist in the body for a longer period of time. For example, in a heavy cocaine user, the major metabolite **benzoylecgonine** can be detected in the urine for several days following the last dose.

Alcohol or other depressant drugs are sometimes taken along with cocaine to "take the edge off" the extreme arousal produced by cocaine alone. By studying the combined effects of cocaine and alcohol, researchers have discovered an unexpected and potentially important interaction between these two compounds. When taken together, cocaine and alcohol (ethanol) produce a unique metabolite called **cocaethylene**. This substance not only has biological activity similar to that of cocaine itself, but it has a longer half-life. As we shall see later, cocaine can exert toxic effects on the heart and other organs. Such toxicity may be exacerbated in individuals consuming large amounts of cocaine and alcohol together (Dinis-Oliveira, 2015).

Mechanisms of Cocaine Action

Cocaine is a complex drug because it interacts with several neurotransmitter systems. Most of the behavioral and physiological actions of cocaine can be explained by its ability to block the reuptake of three monoamine neurotransmitters: the two catecholamines dopamine (DA) and norepinephrine (NE), and also serotonin (5-HT). In earlier chapters, we learned that these transmitters are cleared from the synaptic cleft by membrane proteins called *transporters*. Cocaine binds to these transporters and inhibits their function, thereby leading to increased neurotransmitter levels in the synaptic cleft and a corresponding increase in transmission at the affected synapses. Cocaine does not affect all monoamine transporters equally. In vitro binding studies have shown that cocaine has higher affinity for the 5-HT and NE transporters than for the DA transporter. Nevertheless, blockade of DA reuptake is considered to be most important for cocaine's stimulating, reinforcing, and addictive properties. Indeed, many drugs used in the treatment of depression block the 5-HT and/or the NE transporter (see Chapter 18). Yet these agents do not have the strong arousing effect produced by cocaine in nondepressed individuals, nor do they have any abuse potential. Nevertheless, 5-HT and NE must also be taken into account, as alterations in the DA system do not explain all of cocaine's effects.

Binding of cocaine to the DA transporter (DAT) and the resulting effect on synaptic DA are shown in **FIGURE 12.7**. Depending on the route of administration, this effect can occur very rapidly. Indeed, Yorgason et al. (2011) reported that intravenously administered cocaine in rats begins to block DA uptake within 5 seconds, with peak inhibition occurring approximately 30 seconds postinjection. Yet, inhibition of DA uptake is not the only mechanism by which cocaine elevates extracellular DA. Administration of the drug also increases the frequency of transient (brief) release events (Stuber et al., 2005; Covey et al., 2014). It is not yet clear how this enhancement of DA transients occurs, but one possibility is related to connections from the prefrontal cortex (PFC) to the midbrain ventral tegmental area (VTA). The VTA is the major site of dopaminergic neurons that project to the nucleus accumbens (NAcc), a projection known as the mesolimbic DA pathway (see Chapter 5). As will be discussed in the next section of the chapter, the VTA-to-NAcc pathway is critical for the behavioral actions of psychostimulants. A number of years ago, Carboni and coworkers surprisingly reported that cocaine and amphetamine

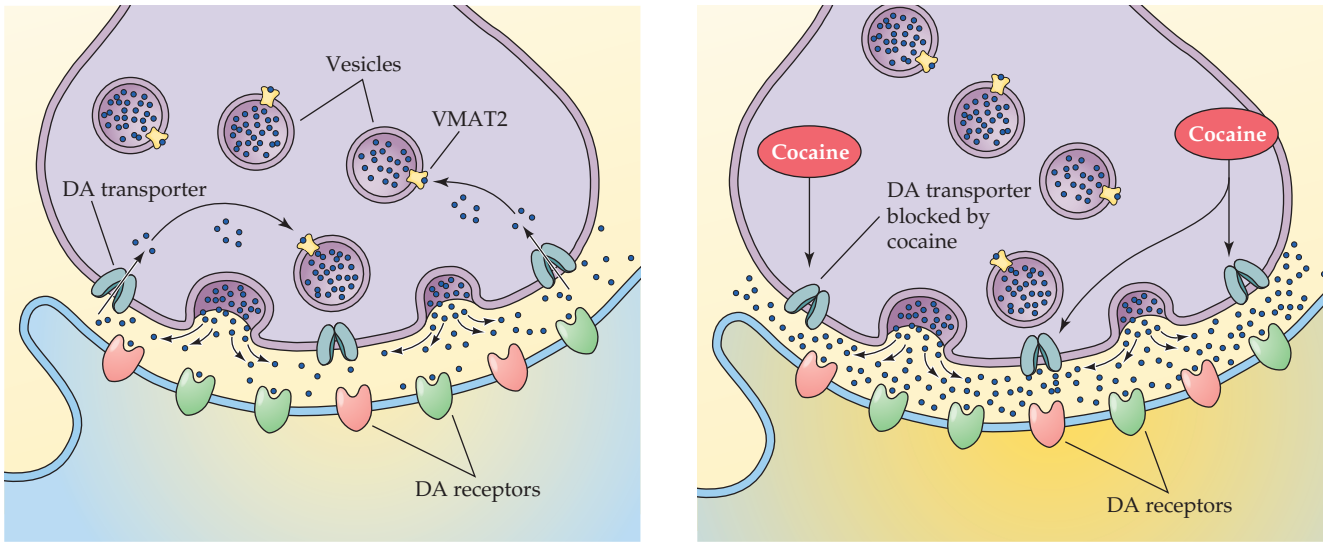


FIGURE 12.7 Mechanisms of cocaine action
Cocaine increases synaptic DA levels by binding to the plasma membrane DA transporter and blocking reuptake of the neurotransmitter. A similar process occurs at serotonergic and noradrenergic synapses because of cocaine's inhibition of 5-HT and NE reuptake. VMAT2, vesicular monoamine transporter 2.

could increase extracellular DA in the NAcc in knockout mice lacking the DA transporter (Carboni et al., 2001). How could this occur when there was no DA transporter for these drugs to block? One hypothesis is based on the model shown in **FIGURE 12.8** and summarized in dela Peña et al. (2015). Glutamatergic pyramidal neurons in the PFC provide an excitatory input to the VTA. In turn, the pyramidal neurons are stimulated by NE released from a locus coeruleus noradrenergic innervation and mediated by excitatory α_1-adrenoceptors. Cocaine blocks NE uptake by the noradrenergic neurons, thereby elevating extracellular NE levels in the PFC, activating the postsynaptic α_1-receptors, and stimulating pyramidal neuron firing. Subsequently, the projection from the PFC to the VTA either directly (through synaptic contacts on the dopaminergic neurons themselves) or indirectly (through action on other neurons synapsing on the dopaminergic cells) alters the firing of the VTA neurons to produce the previously mentioned transient release events. Overall, this model points out that cocaine's actions go beyond those mediated directly by DA uptake blockade, and it is consistent with evidence for a significant role of NE and adrenergic receptors both in psychostimulant-induced DA release and in the behavioral actions of psychostimulants (Schmidt and Weinshenker, 2014).

Many different compounds block DA uptake, yet only some of them produce arousing, euphoric, and behaviorally activating effects

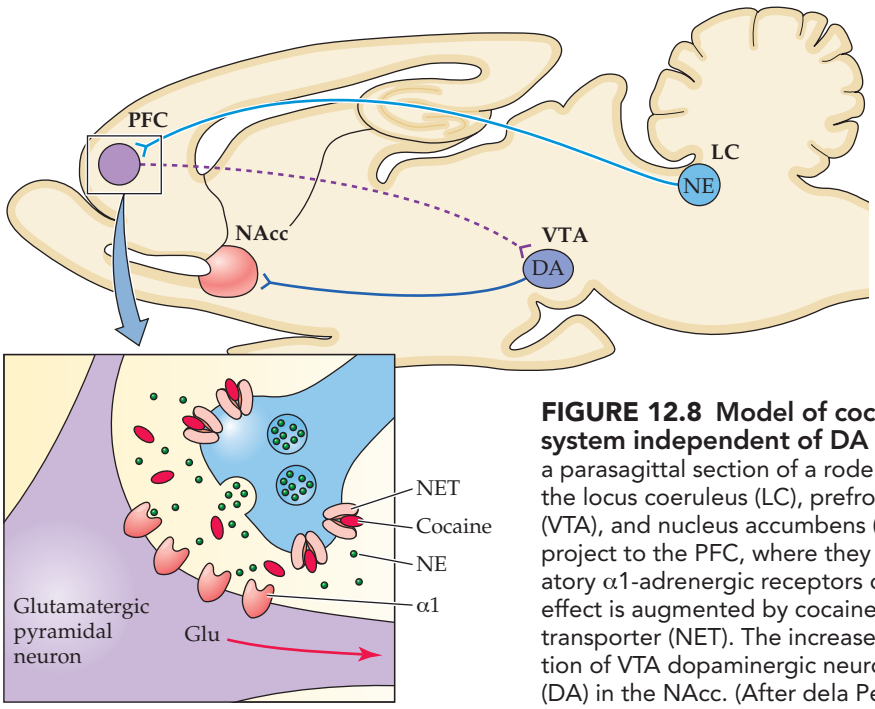


FIGURE 12.8 Model of cocaine stimulation of the dopaminergic system independent of DA transporter blockade This depiction of a parasagittal section of a rodent brain illustrates a circuit that includes the locus coeruleus (LC), prefrontal cortex (PFC), ventral tegmental area (VTA), and nucleus accumbens (NAcc). Noradrenergic fibers from the LC project to the PFC, where they release norepinephrine (NE) onto excitatory α1-adrenergic receptors on glutamatergic pyramidal neurons. This effect is augmented by cocaine's ability to block NE reuptake by the NE transporter (NET). The increased glutamatergic activity causes stimulation of VTA dopaminergic neurons and elevated release of dopamine (DA) in the NAcc. (After dela Peña et al., 2015.)

similar to those of cocaine. For example, the stimulant methylphenidate exerts a similar behavioral profile to cocaine (see later in the chapter for more information on this compound), but the DA uptake blockers mazindol, nomifensine, vanoxerine (also known as GBR 12909), and GBR 12935 do not produce euphoria and lack cocaine's abuse potential. This discrepancy has plagued researchers for many years, and although a number of explanations have been proposed, no consensus has yet been reached. Numerous studies have shown that DA uptake inhibitors interact in different ways with the transporter protein, and one possibility is that these interactions may help explain why so-called atypical (i.e., non-cocaine-like) DA uptake inhibitors have different behavioral effects than cocaine (Schmitt et al., 2013; Reith et al., 2015). A related hypothesis offered by Heal et al. (2014) proposes that cocaine and methylphenidate act on the transporter to cause DA release, in contrast to atypical inhibitors that do not. We shall see later in the chapter that amphetamine, methamphetamine, and related drugs operate by yet a third mechanism of action: those drugs are both transporter blockers and *substrates* for the transporter, which means that they are carried across the membrane into the cell. None of the previously mentioned uptake inhibitors, whether cocaine, methylphenidate, or the atypical inhibitors, is a substrate for the DA transporter. Because of this dual action, amphetamine-type compounds elevate extracellular DA concentrations to a much greater extent than cocaine.

Finally, relatively high concentrations of cocaine cause an inhibition of voltage-gated sodium (Na^+) channels in nerve cell axons. As these channels are necessary for neurons to generate action potentials, this action of cocaine causes a block of nerve conduction. Thus, when cocaine is applied locally to a tissue, it acts as a local anesthetic by preventing transmission of nerve signals along sensory nerves. Indeed, two synthetic local anesthetics that are widely used in medical and dental practice, procaine (Novocaine) and lidocaine (Xylocaine), were developed from the structure of cocaine.

Section Summary

- Cocaine is an alkaloid derived from the leaves of the shrub *Erythroxylon coca*, which is indigenous to the northern and central Andes Mountains of South America.
- Although the peoples of that region have been chewing coca leaves for perhaps 5000 years, cocaine use did not become popular in Western cultures until after the pure compound was isolated in the late 1850s. Freud was one of many notable cocaine users in nineteenth-century Europe.
- In the United States, cocaine was a constituent of numerous popular beverages and over-the-counter pharmaceutical products in the late nineteenth and early twentieth centuries until its nonprescription use was banned by the Harrison Narcotic Act in 1914. Cocaine then went "underground" until the 1970s, at which time the first of two waves of increased cocaine use began in this country.
- Recent household survey data indicate that about 1.9 million people in the United States are current users of cocaine, although not all of these individuals are dependent on the drug.
- Cocaine HCl is water soluble and therefore can be taken orally, intranasally, or by IV injection. On the other hand, cocaine base (including crack cocaine) is the chemical form most suitable for smoking.
- The most rapid absorption and distribution of cocaine occur following IV injection and smoking, which may account for the highly addictive properties of these routes of consumption.
- One of the main cocaine metabolites is benzoylecgonine, whereas a compound called cocaethylene is also formed when alcohol is ingested along with cocaine.
- At typical doses, cocaine acts mainly to block synaptic uptake of DA, 5-HT, and NE by binding to their respective membrane transporters. This enhances transmission at monoaminergic synapses by increasing the synaptic concentrations of each transmitter. However, inhibition of DA uptake is most important for cocaine's behavioral effects and abuse potential.
- A second mechanism by which cocaine enhances dopaminergic transmission is an increased frequency of transient DA release events. This effect may be mediated by cocaine's inhibition of NE uptake in the PFC, causing an α_1-adrenoceptor-mediated stimulation of glutamatergic pyramidal neurons that project to the VTA.
- Not all DA uptake inhibitors share cocaine's euphoric effects and abuse potential. Such atypical uptake inhibitors seem to interact with the transporter protein in a different manner than cocaine.
- At higher concentrations, cocaine also blocks voltage-gated Na^+ channels, which leads to a local anesthetic effect.

Acute Behavioral and Physiological Effects of Cocaine

Cocaine is used and abused for the "high" and the "rush" produced by the drug. The pleasurable feelings associated with the "high" serve as a reinforcer, causing

many users to use the drug repeatedly. In this section, we will discuss the behavioral and physiological effects of cocaine and the neurochemical mechanisms underlying these effects.

Cocaine stimulates mood and behavior

Typical aspects of the cocaine "high" are feelings of exhilaration and euphoria, a sense of well-being, enhanced alertness, heightened energy and diminished fatigue, and great self-confidence. Taken by IV injection or by smoking, cocaine also produces a brief "rush," described by some users as involving a sense of great pleasure and power and by others as being like an intense orgasm. At low and moderate doses, cocaine often increases sociability and talkativeness. There are also reports of heightened sexual interest and performance under cocaine's influence, although the drug's legendary ability to enhance sexual prowess is highly exaggerated. Cocaine can apparently also increase aggressive behavior, which suggests that some of the street violence associated with cocaine might be attributable to a direct effect of the drug.

The major behavioral and subjective effects of cocaine and other psychostimulants are summarized in **TABLE 12.1**. Effects listed in the "mild to moderate" category are generally produced by single, low to moderate doses of cocaine either in naive individuals or in users who have not yet progressed to heavy, chronic patterns of drug intake. "Severe" effects are most likely to be seen with high-dose use, particularly in individuals with long-standing patterns of chronic intake. It is easy to see that many of the positive characteristics of cocaine that may contribute to its powerful reinforcing properties become negative or aversive with escalation of dose and duration. Some of these aversive effects (e.g., irritability) are present in most high-dose users, whereas others mainly occur in cases of cocaine-induced psychosis (e.g., incoherence or delusions; see the discussion on health consequences later in the chapter).

Cocaine and other psychomotor stimulants also cause profound behavioral activation in rats, mice, and other animals used in psychopharmacological studies. At low doses, such activation takes the form of increased locomotion, rearing, and mild sniffing behavior. As the dose is increased, these behaviors are replaced by **focused stereotypies** (repetitive, seemingly aimless behaviors performed in a relatively invariant manner) confined to a small area of the cage floor. Psychostimulant stereotypies vary according to species and other factors. In rats and mice, one observes intense sniffing, continuous head and limb movements, and licking and biting. Humans using large amounts of cocaine occasionally also exhibit motor stereotypies such as repetitive picking and scratching.

All species tested thus far readily learn to self-administer cocaine intravenously. Marilyn Carroll and colleagues (1990) were also able to train monkeys to smoke cocaine freebase, a procedure that has led to a number of follow-up studies on the mechanisms underlying self-administration via this route (e.g., Campbell et al., 1999). Furthermore, unlimited access to cocaine can lead to heavy self-administration, gradual debilitation of the animals, and a high rate of mortality. These findings underscore the drug's powerful reinforcing properties in animals and its high abuse potential in humans. On the other hand, it is important to recognize that such compulsivity is not observed under all circumstances. When cocaine is pitted against an alternative reinforcer under controlled laboratory conditions, preference for the drug

TABLE **12.1** Mild to Moderate versus Severe Behavioral and Subjective Effects of Cocaine and Other Psychostimulants in Humans*

Mild to moderate effects	Severe effects
Mood amplification; both euphoria and dysphoria	Irritability, hostility, anxiety, fear, withdrawal
Heightened energy	Extreme energy or exhaustion
Sleep disturbance, insomnia	Total insomnia
Motor excitement, restlessness	Compulsive motor stereotypies
Talkativeness, pressure of speech	Rambling, incoherent speech
Hyperactive ideation	Disjointed flight of ideas
Increased sexual interest	Decreased sexual interest
Anger, verbal aggression	Possible extreme violence
Mild to moderate anorexia	Total anorexia
Inflated self-esteem	Delusions of grandiosity

Source: From Post and Contel, 1983.
*The actual effects observed show individual variability and depend on the dose, route of administration, pattern and duration of use, and environmental context.

in both monkeys and humans depends on the relative magnitude of each reinforcer and other experimental conditions (**Web Box 12.1**).

Animals can also learn to discriminate cocaine from vehicle treatment. Subjects initially trained on cocaine readily generalize to amphetamine, indicating a fundamental similarity in the cue properties of these two drugs. In contrast, there is much less generalization to caffeine, which is a weaker stimulant and which exerts its behavioral effects by a different mechanism than cocaine or amphetamine (see Chapter 13).

Cocaine's physiological effects are mediated by the sympathetic nervous system

Cocaine is considered a **sympathomimetic** drug, which means that it produces symptoms of sympathetic nervous system activation. The physiological consequences of acute cocaine administration include tachycardia (increased heart rate), vasoconstriction (narrowing of blood vessels) and hypertension (increased blood pressure), and hyperthermia (elevated body temperature). At low doses, these physiological changes are usually not harmful to the individual. High doses of cocaine, however, can be toxic or even fatal. Cocaine intoxication syndrome produced by an overdose of the drug is characterized by extreme tachycardia and hypertension, tachypnea (rapid breathing), hyperthermia and diaphoresis (excessive sweating), and various psychological/behavioral symptoms that can include agitation, mania, paranoia, and a state of delirium (Zimmerman, 2012). Other potential adverse consequences of heavy cocaine use are seizures, heart failure, stroke, and intracranial hemorrhage (Treadwell and Robinson, 2007). Research using mice showed that even in the absence of an actual hemorrhage, the vasoconstrictive effects of cocaine can significantly interrupt blood flow to the brain and that this phenomenon is exacerbated with repeated drug dosing (Ren et al., 2012).

Dopamine is important for many effects of cocaine and other psychostimulants

Several neurotransmitters, including 5-HT (Müller and Huston, 2006) and NE (Sofuoglu and Sewell, 2009; Schmidt and Weinshenker, 2014), contribute to the behavioral responses of animals to cocaine, amphetamine, and related psychostimulant drugs. However, as mentioned earlier, there is overwhelming evidence that DA plays the most important role in mediating these responses (Zhu and Reith, 2008). Of special relevance are the dopaminergic projections from the midbrain (substantia nigra and VTA) to the striatum and NAcc, which were first described in Chapter 5.

Early studies used microinjection and lesion methods to investigate the involvement of these DA systems in the behavioral effects of psychostimulants. The results of these studies are summarized in **TABLE 12.2**. For example, psychostimulants elicit a locomotor response when microinjected directly into the NAcc. Injection into the striatum instead leads to a pattern of stereotyped behavior. Another approach has been to lesion DA nerve terminals in either the accumbens or the striatum using the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA). Such lesions cause a profound reduction of both DA and its transporter in the affected area, thereby preventing activation of dopaminergic transmission by psychostimulants. 6-OHDA lesions of the NAcc blunt psychostimulant-induced locomotion, whereas similar lesions of the striatum antagonize the stereotypies associated with higher drug doses.

The mesolimbic DA pathway to the NAcc also plays a key role in the reinforcing effects of cocaine and amphetamine in animals. Thus 6-OHDA lesions of the accumbens reduce the reinforcing properties of systemically administered cocaine or amphetamine. Moreover, rats will self-administer amphetamine and cocaine directly into the NAcc, although for unknown reasons cocaine self-administration to this area is much weaker. Genetic knockout mice can provide yet another

TABLE 12.2 Dopaminergic Projections to the Striatum and Nucleus Accumbens: Role in Psychostimulant-Induced Behaviors in Animals

Experimental manipulation	Brain area	Behavioral effect
Psychostimulant microinjection	Nucleus accumbens	Increased locomotor behavior
Psychostimulant microinjection	Striatum	Increased stereotyped behaviors
6-Hydroxydopamine (6-OHDA) lesion	Nucleus accumbens	Decreased locomotor response following systemic administration of a low-dose psychostimulant
6-OHDA lesion	Striatum	Decreased stereotyped behaviors following systemic administration of a high-dose psychostimulant
6-OHDA lesion	Nucleus accumbens	Decreased reinforcing effectiveness of systemically administered psychostimulants
Amphetamine microinjection	Nucleus accumbens	Reinforcing to the animal
Cocaine microinjection	Nucleus accumbens	Reinforcing to the animal

approach to understanding the neurochemical mechanisms of cocaine action. We saw in Chapter 5 that mutant mice lacking DAT fail to show hyperactivity following psychostimulant treatment. Most investigators expected that the rewarding effects of psychostimulants would similarly be lost in the absence of DAT. Although initial studies suggested that the rewarding and reinforcing effects of cocaine were still present in DAT knockout mice (Rocha et al., 1998; Sora et al., 1998), these findings were disputed in later studies. For example, Thomsen and coworkers (2009) ingeniously engineered a mutant DAT that was still functional for DA (i.e., was able to take up DA from the extracellular fluid) but was insensitive to cocaine. When these researchers tested mice homozygous for this mutant DAT on their responsiveness to IV cocaine, they found that the mice failed to self-administer the drug (above the levels of saline self-administration), in contrast to heterozygous mutants and wild-type mice (**FIGURE 12.9**).

Nucleus accumbens DA has also been implicated in cocaine reward by using paradigms that test for **drug-seeking behavior** as a model of relapse in previously abstinent individuals. Such paradigms typically train animals to self-administer cocaine intravenously and then extinguish the operant response (e.g., pressing a lever) by substituting saline for cocaine over a number of trials. Many studies have shown that following extinction, one can provoke a reinstatement of responding (i.e., drug seeking) by administering a single priming dose of cocaine to the animals through the IV catheter, despite the fact that the resumption of responding continues to result in the delivery of the saline vehicle instead of the drug. More relevant to the present discussion, two separate studies demonstrated that a reinstatement of cocaine-seeking behavior in previously extinguished rats could be stimulated by microinjection of either a D_1 or a D_2 DA receptor agonist directly into the NAcc (Bachtell et al., 2005; Schmidt et al., 2006). If these findings can be applied to human cocaine users, they suggest that DA acting within the NAcc may play an important role in the urge to take cocaine in dependent users who are attempting to maintain abstinence from their drug use.

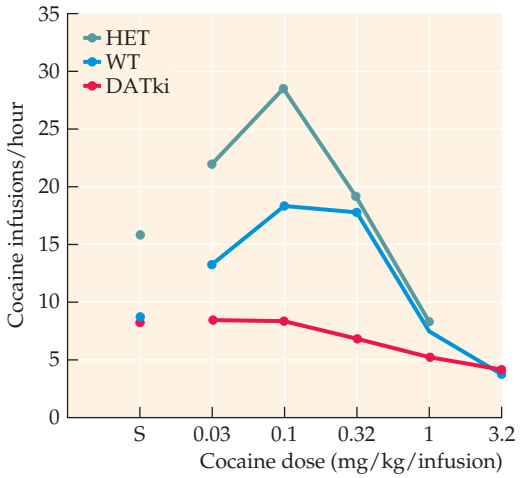


FIGURE 12.9 Lack of cocaine self-administration in mutant mice expressing a cocaine-insensitive DAT Mice were genetically engineered with a DAT "knockin" gene that produced a protein that transported DA across the cell membrane but was not blocked by cocaine. The graph shows the number of drug infusions per hour in wild-type (WT), homozygous DAT knockin (DATki), and heterozygous knockin (HET) mice. Note that the WT and HET groups showed significant dose-dependent cocaine self-administration that was abolished in the DATki group. The S data show the number of infusions obtained by each group when saline was substituted for cocaine. (After Thomsen et al., 2009.)

All of the studies described above used experimental manipulations such as drug microinjection, brain lesions, or genetic engineering to produce mutant strains of animals. An ingenious program of research by Zahniser and her coworkers has used the different approach of correlating naturally occurring behavioral responses to cocaine with individual differences in neurochemistry (reviewed in Yamamoto et al., 2013). The researchers study behavioral responses such as locomotor activation in outbred Sprague-Dawley rats given a moderate dose of cocaine (e.g., 10 mg/kg by intraperitoneal [IP] injection). Partly because the animals are not genetically identical, this dose of the drug produces a range of response magnitudes. A median split is then performed to produce two groups: high cocaine responder (HCR) rats (upper 50%) and low cocaine responder (LCR) rats (lower 50%). Results from a typical experiment measuring cocaine-induced locomotor activity are shown in **FIGURE 12.10A**. During the first 90 minutes of testing, activity of all three groups (saline control, HCR, and LCR) decreased as the animals became habituated to the previously novel activity chamber. We can see that after the drug injection at the 90-minute point, the HCR group displayed a sustained increase in locomotor activity, whereas the LCR group showed only a brief increase due to the mild stress of handling and IP injection, which was also shown by the saline controls. Note that the assignment of animals to the HCR and LCR groups was made by performing the median split *after* the behavioral data had been collected. Zahniser's group was interested in ascertaining whether there were any measurable neurochemical differences, especially in the dopaminergic system, between the HCR and LCR animals that could account for their differential sensitivity to cocaine. The research showed that cocaine inhibited DA clearance in the NAcc and dorsal striatum much more effectively in the HCR than the LCR rats (Yamamoto et al., 2013). In vitro binding studies found that this difference was related to LCR rats

expressing a greater number of DA transporters in both areas than HCR rats. **FIGURE 12.10B** illustrates how this difference accounts for the differential sensitivity to cocaine. As shown in the figure, LCR and HCR rats have differing numbers of DA transporters but, presumably because of adaptive processes, the two groups show similar levels of extracellular DA and similar locomotor activity under drug-free conditions (first 90 minutes in Figure 12.10A). However, after administration of cocaine, the lower transporter expression in the HCR animals causes more effective inhibition of DA uptake, leading to elevated extracellular DA, greater intracellular signaling, and increased behavioral activation. These findings have significant clinical relevance, as humans also have a naturally occurring variation in DA transporter expression. Accordingly, the intensity of an individual's initial reactions to cocaine might differ depending on such expression, and this difference could affect their desire to continue using the drug or not.

Brain imaging has revealed the neural mechanisms of psychostimulant action in humans

Given the critical role of DA in the activating, rewarding, and reinforcing effects of psychostimulants, are the mood-altering properties of these compounds in human users dependent on the same neurochemical system? Since researchers have not discovered any people with genetic deletions of DAT, other experimental approaches are needed to address this question. One of the most exciting approaches has been to use brain imaging techniques such as positron emission tomography (PET) (see Chapter 4). For example, a research team headed by Nora Volkow, the current director of the National Institute on Drug Abuse, has used PET imaging to estimate DAT occupancy by behaviorally active doses of either cocaine or methylphenidate. Methylphenidate, a stimulant that is commonly used to

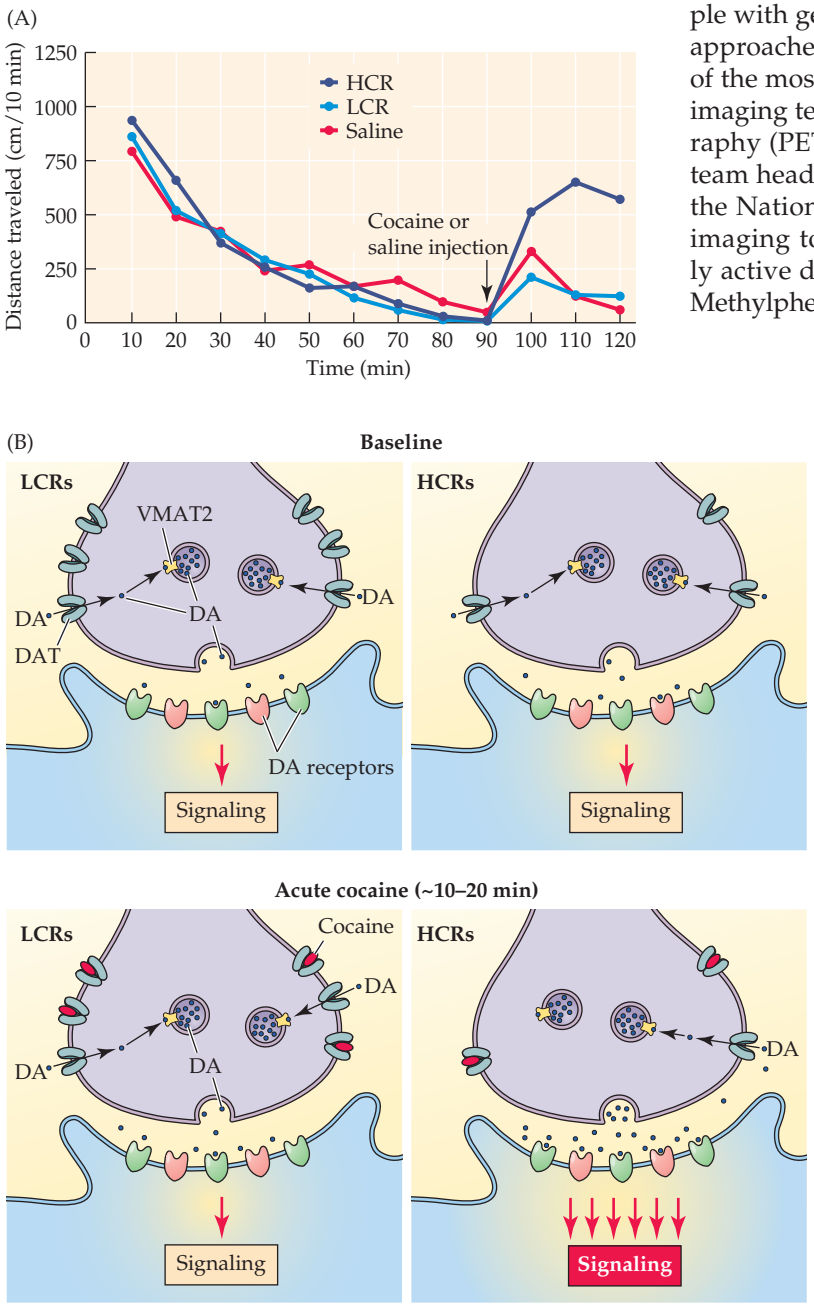


FIGURE 12.10 Behavioral and neurochemical differences between low cocaine responder and high cocaine responder rats (A) When administered 10 mg/kg cocaine IP, high cocaine responders (HCR) showed a strong locomotor response in the open field, whereas low cocaine responders (LCR) barely responded more than the saline-treated control group. Note that the steady decline in locomotor activity during the first 90 minutes prior to injection represents habituation to the novel test environment. The modest locomotor response in the control rats beginning at the 90-minute mark is due to the activating effects of handling and injection. (B) Neurochemical studies aimed at determining the potential mechanism underlying the differential behavioral responses to cocaine challenge showed greater baseline DA transporter (DAT) binding in the LCR than the HCR rats (top). This difference did not influence extracellular DA levels, dopaminergic signaling, or locomotor behavior under drug-free conditions. However, the lower availability of reuptake sites in the HCR animals causes an intermediate dose of cocaine to increase extracellular DA more strongly in those rats than in the LCR animals, thereby enhancing dopaminergic signaling and stimulating locomotor activity (bottom). VMAT2, vesicular monoamine transporter 2. (After Yamamoto et al., 2013.)

treat attention deficit hyperactivity disorder (ADHD), also binds to DAT and blocks DA reuptake (ADHD is discussed further in Box 12.2, later in this chapter). This compound is preferred over cocaine for some studies because as a medication, it can be administered ethically to non-drug-abusing study participants, whereas cocaine cannot.

The studies of Volkow and colleagues (1999a) indicate that once a certain minimum level of DAT occupancy (about 40% to 60%) is attained following either cocaine or methylphenidate administration, the individual *may* experience a drug-induced "high." However, the intensity of the high and even the likelihood that a high will occur depend not only on the amount of DAT occupancy but also on at least two other factors. One factor is the rate at which transporter occupancy occurs after the drug has been taken. Thus routes of administration like smoking or IV injection that lead to quick drug entry into the brain and rapid DAT occupancy are more likely to produce an intense high than oral or intranasal administration, which are associated with delayed drug entry into the brain and slower DAT occupancy (Volkow et al., 1998; 2000). Although we don't yet know how the rate of DA reuptake blockade influences the subjective effects of psychostimulants, this information does help us understand why smoking and IV injection of psychostimulants have greater addiction potential than other routes of administration.

A second factor influencing the psychostimulant "high" is believed to be the baseline level of DA activity in the mesolimbic pathway. Volkow's group found that even with IV administration of cocaine or methylphenidate, some individuals failed to experience a high, even with 60% or greater DAT occupancy, as indicated by PET imaging (Volkow et al., 1997; 1999b). However, when a different imaging procedure was used to assess the effects of IV methylphenidate on the occupancy of D_2 receptors by DA (not by the drug), there was a high correlation between this measure and the subjective high (Volkow et al., 1999c). What does this result mean? Imagine two people, A and B. Because of individual differences in dopaminergic system activity, person A starts with a relatively low level of baseline DA release, whereas person B starts with a relatively high level of release. Both people are now given sufficient methylphenidate or cocaine to produce 60% DAT occupancy. Even with equivalent amounts of reuptake blockade, the effect of this blockade on stimulating postsynaptic DA receptors will be greater in person B than in person A because of the higher initial concentration of DA molecules in the synaptic cleft. Combining these findings with those described above for low and high cocaine responder rats suggests that variation in sensitivity to psychostimulant drugs can be produced by at least two different mechanisms: (1) differing DA transporter levels or (2) differing levels of baseline DA release.

Several DA receptor subtypes mediate the functional effects of psychostimulants

Given that the functional effects of psychostimulants are dependent primarily on inhibition of DA uptake and the resulting increase in synaptic DA levels, it is necessary to consider which postsynaptic DA receptor subtypes mediate these effects. You will recall from Chapter 5 that there are five DA receptor subtypes: D_1 to D_5. D_1 and D_5 constitute the D_1-like family, whereas the D_2-like family comprises the D_2, D_3, and D_4 receptors. Numerous pharmacological studies have shown that relatively nonselective antagonists at either the D_1-like or the D_2-like family of receptors can reduce both the behaviorally activating and the reinforcing effects of psychostimulants (Gold et al., 1989; Bergman et al., 1990; Fibiger et al., 1992; Self et al., 1996). The obvious limitation of these studies is their inability to distinguish which member(s) of each receptor family are critical for the response being measured.

Fortunately, some progress in this area has come from the use of genetic knockouts lacking a particular DA receptor. With respect to the locomotor-stimulating effects of cocaine, the knockout studies have shown that D_1 receptors are absolutely required for such effects (Xu et al., 1994), whereas neither D_2 nor D_3 receptors are needed (Chausmer et al., 2002; Karasinska et al., 2005). Likewise, mutant mice lacking D_2 or D_3 receptors were reported to self-administer cocaine, although with some differences in dose–response function compared with wild-type mice (Caine et al., 2002; 2012). In contrast, D_1 receptor knockout mice do not self-administer cocaine, which suggests a critical role for this receptor subtype in cocaine reinforcement (Caine et al., 2007). Recent studies using sophisticated cellular imaging methods have confirmed that the rewarding effects of cocaine in mice and the drug's ability to promote conditioned associations with environmental cues are dependent on activation of D_1 receptor–expressing medium spiny neurons in the NAcc (Calipari et al., 2016). Furthermore, chronic cocaine exposure dysregulates these cells, a process that likely contributes to relapse in cocaine-dependent users who are attempting to become abstinent.

Section Summary

- Cocaine exerts powerful effects on mood and behavior. The cocaine "high" is characterized by feelings of exhilaration, euphoria, well-being, heightened energy, and enhanced self-confidence. Smoked or intravenously injected cocaine also causes a "rush" in the user.
- At high doses and/or with prolonged use, cocaine can give rise to a number of negative effects such as irritability, anxiety, exhaustion, total insomnia, and even psychotic symptomatology.

- In animal studies, cocaine elicits locomotor stimulation and, at higher doses, stereotyped behaviors.
- Cocaine can function as a discriminative stimulus, and it exhibits powerful reinforcing effects in standard self-administration paradigms.
- Physiologically, cocaine produces sympathomimetic effects such as increased heart rate, vasoconstriction, hypertension, and hyperthermia. High doses can be toxic or even fatal as the result of seizures, heart failure, stroke, or intracranial hemorrhage.
- Microinjection, lesion, and gene knockout studies have demonstrated that most of the behavioral effects of cocaine are attributable to DAT inhibition and the resulting activation of dopaminergic transmission, particularly in the NAcc and striatum.
- Other research performed in outbred rats found that naturally occurring differences in sensitivity to cocaine's locomotor activating effects were related to differences in DAT levels in the NAcc and dorsal striatum.
- Brain imaging studies in humans have found that the subjective effects (e.g., the "high") of psychostimulants are related to the amount of DAT occupancy, the rate at which occupancy occurs, and baseline DA activity.
- Of the various DA receptor subtypes, the D_1 receptor plays the most critical role in mediating cocaine's locomotor activating and reinforcing effects in animals.

Cocaine Abuse and the Effects of Chronic Cocaine Exposure

Most individuals who try cocaine do not progress to a pattern of abuse or dependence. Those who develop cocaine dependence experience tolerance and/or sensitization and many adverse health effects, including brain abnormalities and cognitive deficits. Treatments for cocaine dependence have yielded mixed results, with pharmacotherapies being especially disappointing thus far.

Experimental cocaine use may escalate over time to a pattern of cocaine abuse and dependence

Cocaine users give many different reasons for their initial decision to use the drug. Some of these reasons are to satisfy their curiosity; to facilitate social interactions; to relieve feelings of depression, anxiety, or guilt; to have fun and celebrate; or simply to get "high." Cocaine users typically describe early experimentation with both legal (e.g., alcohol) and illegal drugs, often beginning by 13 or 14 years of age. Early use of other substances may therefore be an important risk factor for the initiation of cocaine use.

People usually begin taking cocaine via the intranasal route, that is, by snorting it. As mentioned above, initial cocaine use most frequently does not lead to subsequent abuse or dependence. There are various reasons why the majority of people who try cocaine either do not continue their use or, if they do continue for some time, they do not subsequently undergo a transition to cocaine abuse or dependence. Some individuals report a strong anxiety response as their initial reaction to cocaine and are thereby dissuaded from further experimentation. Other factors may likewise mitigate against the development of a long-term abuse pattern, including unavailability of the drug, the cost of maintaining a steady supply, the social and legal consequences of illicit drug use, and the very real fear of losing control over one's drug-taking behavior. These factors often lead to a termination of cocaine use, although there are some intranasal users who maintain long-term periodic and controlled cocaine consumption.

The most recent edition of the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-5*; American Psychiatric Association, 2013) no longer uses terms like *cocaine abuse* or *cocaine dependence*. Instead, there is now a new category, "stimulant use disorder," that covers maladaptive use of any stimulant drug. Severity can be rated as mild, moderate, or severe depending on the number of diagnostic criteria (i.e., symptoms) that apply to the individual. Moreover, a particular drug type may be specified, as in "cocaine use disorder." Nevertheless, because of the historical (and in some cases contemporary) use of the older terminology, in this chapter we will continue to refer to cocaine (or methamphetamine, etc.) abuse and dependence except for instances in which researchers themselves have applied *DSM-5* diagnostic criteria and terminology to their study.

Surveys performed by the National Institute on Drug Abuse suggest that approximately 10% to 15% of initial intranasal users eventually become cocaine abusers. A smaller percentage, namely, 5% to 6%, already meet criteria for cocaine dependence within 24 months after their first use (O'Brien and Anthony, 2005). The details of this transition process certainly vary for different individuals, yet a few factors have been identified that may generally be important. The stimulating, euphoric, and confidence-enhancing effects described earlier provide a powerful reinforcing effect during the early stages of cocaine use. Furthermore, these aspects of cocaine reinforcement may be augmented by social responses from friends and acquaintances who

respond positively to the user's newfound energy and enthusiasm. Over time, cocaine use escalates as the individual discovers that higher doses produce a more powerful euphoric effect. Even more importantly, the user may switch from intranasal administration to crack smoking, freebasing, or IV injection. For many, this is a significant event in their drug history because of the greater abuse potential of these latter routes of administration. Moreover, some individuals develop a pattern of **cocaine binges**, which are episodic bouts of repeated use lasting from hours to days with little or no sleep. During these periods, nothing is important to the user except maintaining the "high," and all available supplies of cocaine are consumed in this pursuit. A 3-day freebasing binge may involve the consumption of as much as 150 g of cocaine, which is an enormous amount. More than 25 years ago, Gawin and Kleber (1986; 1988) reported the presence of an abstinence syndrome that they observed following a cocaine binge. They proposed that this abstinence syndrome occurred in three phases, which they called *crash*, *withdrawal*, and *extinction*. During the crash, the user feels exhausted and suffers from a depressed mood. Later, during the withdrawal phase, some of the important symptoms include anhedonia (inability to experience normal pleasures), anergia (a lack of energy), anxiety, and a growing craving for cocaine that increases the risk of relapse. Symptoms subside during the extinction phase.

Other factors may also contribute to the transition from recreational cocaine use to abuse and dependence, to the maintenance of excessive cocaine use, and/or to relapse in individuals who are attempting to achieve abstinence from the drug. As described in Chapter 9 for drug addiction generally, such factors may include comorbidity with other psychiatric disorders, such as depression, anxiety disorders, or personality disorders (Quello et al., 2005), stress (Mantsch et al., 2014), exposure to environmental stimuli previously associated with cocaine use (Crombag et al., 2008), or cocaine priming (i.e., exposure to a small amount of the drug that elicits craving for more) (Mahoney et al., 2007). Interestingly, animal studies indicate that cocaine craving and relapse to cocaine use actually increase over time (although not permanently) following withdrawal from drug use (Lu et al., 2004). This phenomenon, which has been termed **incubation** of cocaine craving, is consistent with the high rate of relapse in cocaine-dependent individuals, even those enrolled in treatment programs (see later in this chapter).

The neurobiology of cocaine craving has been investigated using PET imaging studies of people with cocaine dependence as well as neurochemical studies involving relevant animal models of cocaine-directed motivation. PET imaging studies have found that DA release is increased in the dorsal and ventral striatum, amygdala, hippocampus, and PFC when cocaine users (diagnosed as having cocaine dependence, in most of the cited studies) are presented with cocaine-related stimuli such as videotapes of people smoking cocaine (Volkow et al., 2006; Wong et al., 2006; Fotros et al., 2013; Milella et al., 2016). These studies make use of radiolabeled D_2 receptor ligands that can be displaced from the receptor by DA released from the dopaminergic nerve terminals. Consequently, reductions in drug binding as measured by PET imaging are interpreted as increased local DA release. An example of this approach using radiolabeled raclopride is shown in **FIGURE 12.11**. The data illustrate a significant correlation between the craving elicited by the cocaine cues and the neuroimaging-derived index of DA release in the dorsal striatum (caudate and putamen) (Volkow et al., 2006). DA release in the NAcc (ventral striatum) has also been implicated in cocaine "craving" using an experimental rat model (Saunders et al., 2013). Other rat models have been developed to reproduce the features of escalating cocaine use and to discover its underlying neurobiological mechanisms.

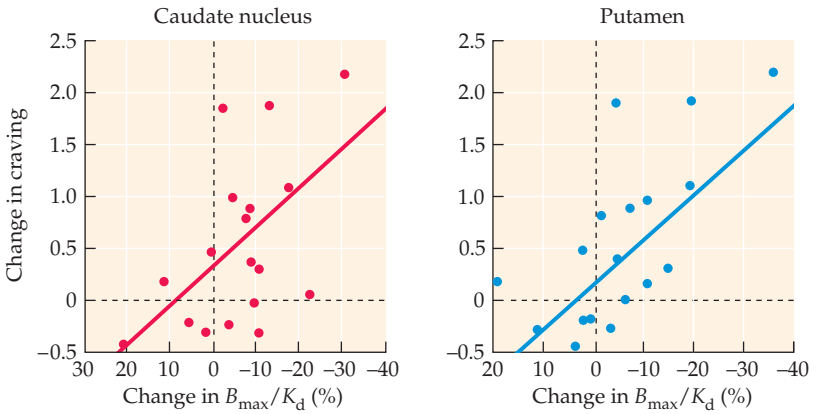


FIGURE 12.11 Relationship between striatal DA release and craving elicited by exposure to cocaine-related visual stimuli
Cocaine-addicted individuals were exposed to a video of cocaine-related cues including purchase, preparation, and smoking of the drug while undergoing PET scanning of striatal DA release measured by DA displacement of radiolabeled raclopride from D_2 receptors. The *y*-axis of the graph plots changes in subjective cocaine craving from before to the end of the video. The *x*-axis plots changes in DA release measured by alterations in raclopride binding. Significant positive correlations were found for both the caudate nucleus and the putamen, indicating that cue-induced cocaine craving is associated with heightened striatal DA release. (After Volkow et al., 2006.)

Several of these models are described in **Web Box 12.2**. Finally, we should mention that research using functional magnetic resonance imaging (fMRI), which measures regional neural activity independently of specific neurotransmitters, has implicated many of the same brain areas mentioned above in the process of cocaine craving (e.g., Risinger et al., 2005). Thus, we can conclude that the midbrain–striatal DA pathway is part of a larger circuit comprising various cortical and limbic structures that are activated when cocaine users experience craving for the drug.

Chronic cocaine exposure leads to significant behavioral and neurobiological changes

TOLERANCE AND SENSITIZATION As with many abused substances, chronic exposure to cocaine or other psychostimulants causes either **tolerance** (reduced drug responsiveness) or **sensitization** (increased responsiveness), sometimes called **reverse tolerance**. One of the amazing aspects of sensitization is that just a few exposures to cocaine or amphetamine can produce an increased responsiveness that lasts for weeks, months, or even longer (Paulson et al., 1991). Although this kind of long-lasting sensitization has usually been studied in experimental animals, a laboratory study by Boileau and colleagues (2006) demonstrated sensitized behavioral and neurochemical responses to amphetamine at 1 year after a mere four doses of the drug given orally to human participants over a 2-week period.

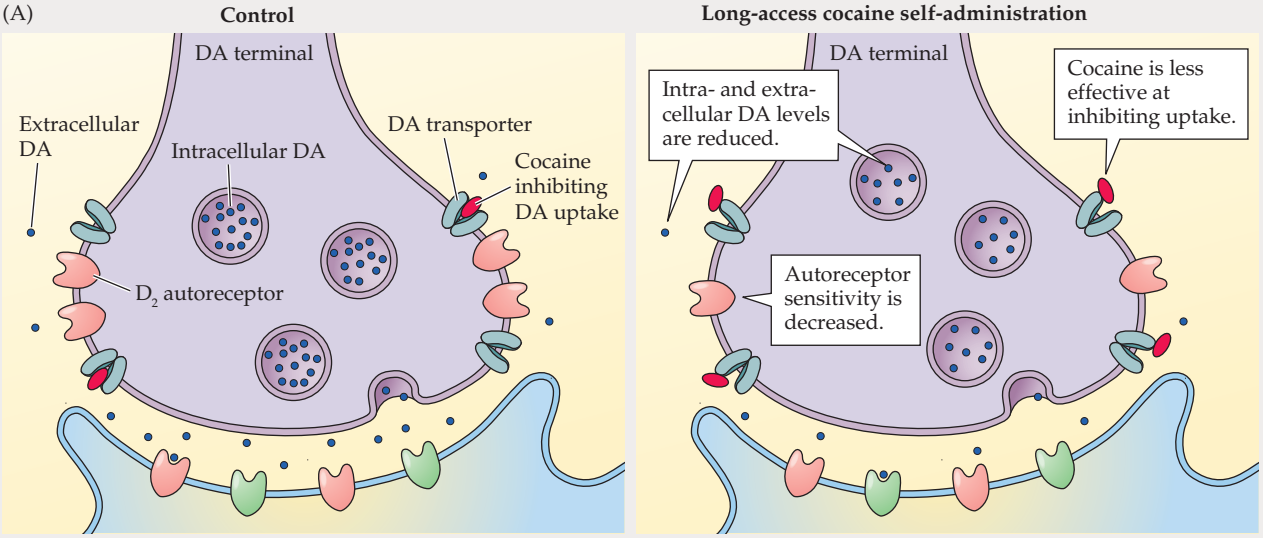
How can psychostimulants produce both tolerance and sensitization? Various studies have shown that the kind of change one observes depends on the pattern of drug exposure, the response that's being measured, and the time interval that has elapsed since the last dose. For example, continuous cocaine infusion into rats causes tolerance to the drug's locomotor-stimulating effect (Inada et al., 1992), whereas once-daily cocaine injections lead to behavioral sensitization, as shown by enhanced stereotyped behaviors (Post and Contel, 1983). Recent studies have examined tolerance or sensitization to self-administered cocaine rather than cocaine given by the experimenter. Once again, different results were obtained depending on the pattern of drug exposure. Cocaine self-administered by IV daily for 6 hours per day (long-access model; see Web Box 12.1 for details) caused a reduction in the stimulatory effects (both locomotor activity and stereotypies) of a 5 mg/kg IP cocaine challenge given on the day after the last self-administration session (Calipari et al., 2014a). In contrast, intermittent access to self-administered cocaine led to a sensitization of the drug's behavioral effects (Calipari et al., 2014b; 2015). Earlier studies of psychostimulant sensitization showed that this phenomenon can be divided into two phases: **induction**, which means the process by which sensitization is established, and **expression**, which refers to the process by which the sensitized response is manifested. During the period between these two phases (i.e., after the last drug dose has been administered), sensitization can actually increase in strength as a result of ongoing neurochemical changes in the brain.

In human cocaine users, evidence for both tolerance and sensitization has been observed, depending on the specific outcome measure (Narendran and Martinez, 2008; Small et al., 2009). Importantly, researchers have generally reported that cocaine's euphoric effects tend to show tolerance over time, which could contribute to increased drug-taking behavior in an attempt to recapture the level of pleasure experienced during earlier episodes of use. The neurochemical mechanisms underlying both psychostimulant tolerance and sensitization have been studied extensively in animal models and to a lesser extent in humans, using brain imaging methods. These mechanisms are described in **BOX 12.1**.

OTHER PROCESSES THAT MAY CONTRIBUTE TO COCAINE DEPENDENCE Individuals diagnosed as being cocaine-dependent have lost control over their drug-seeking and drug-using behaviors. Numerous studies have demonstrated an association between cocaine abuse/dependence and deficits in numerous cognitive domains. Among the most severely affected cognitive functions are sustained attention, impulse control, working memory, verbal learning and memory, performance on psychomotor tasks, and decision making in reward-based learning tasks (Spronk et al., 2013; Potvin et al., 2014). Structural and functional brain imaging studies have found numerous differences between cocaine-using participants and nonusers in the PFC (including medial PFC, dorsolateral PFC, and orbitofrontal cortex), anterior cingulate cortex, insula, dorsal striatum, amygdala, and thalamus (Crunelle et al., 2012; Hanlon and Canterberry, 2012; Taylor, S. B., et al., 2013). Depending on the brain area, reported abnormalities include reduced gray matter volume or cortical thickness, increased volume (caudate nucleus), reduced functional connectivity within a neural circuit, impaired integrity of white matter tracts, and/or altered functional activation during cognitive task performance. Impairments in the PFC, striatum, and their associated networks are thought to be particularly important for the loss of inhibitory control in cocaine-dependent individuals and their inability to monitor and evaluate the consequences of their behavior. A study by Ersche and coworkers (2011) provides a good example of research relating gray matter volume to cocaine use and relevant psychological measures. In this study, structural MRI was used to compare brain region volumes in cocaine-dependent individuals and healthy controls. Some of

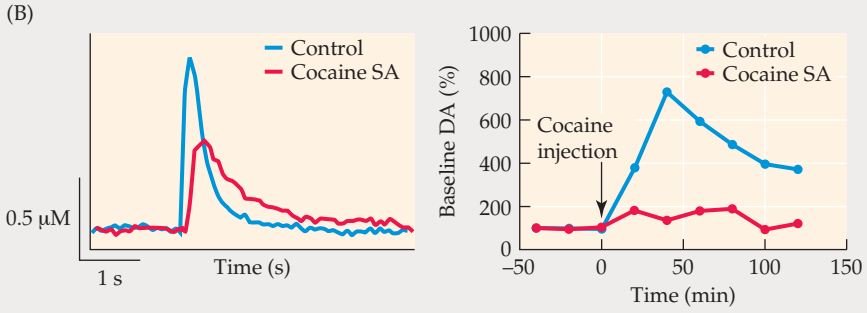
BOX 12.1 The Cutting Edge

Neurochemical Mechanisms of Cocaine Tolerance and Sensitization



(A) Molecular adaptations in the nucleus accumbens underlying tolerance due to long-access cocaine self-administration (After Siciliano et al., 2015.)

Several molecular adaptations occur in NAcc dopaminergic nerve terminals in rats that have been made cocaine tolerant using the long-access self-administration paradigm. These adaptations are illustrated in **Figure A** and can be summarized as follows: (1) reduced intracellular DA levels and reduced DA release, (2) reduced baseline rate of DA uptake, (3) reduced ability of cocaine to block DAT and to inhibit DA reuptake, and (4) reduced sensitivity of terminal D2 DA autoreceptors (Siciliano et al., 2015). Evidence for the first three mechanisms is shown in **Figure B** (Calipari et al., 2014a). The left side of the figure depicts representative measurements of stimulation-evoked DA release and clearance in brain slices containing the NAcc obtained from a control rat and from a rat previously. allowed long-access cocaine self-administration. The upward slope of each trace shows the amount of DA released because of the electrical stimulation, and the downward part of the trace is related to DA clearance due, in part, to uptake by DAT. No cocaine was present when the measurements were taken. Note that the peak height is lower in the tissue from the cocaine-exposed rat, indicating reduced baseline DA release. This effect is interpreted to mean lower amounts of DA available to be released. Moreover, the flatter slope of the downward trace indicates a slower rate of DA clearance. This change may be due to a reduced number of transporters or a reduction in the efficiency of DA uptake. The right side of the figure presents group data from a microdialysis experiment to determine the ability of an acute cocaine challenge (15 mg/kg IP) to increase DA overflow in the NAcc in rats subjected to long-access



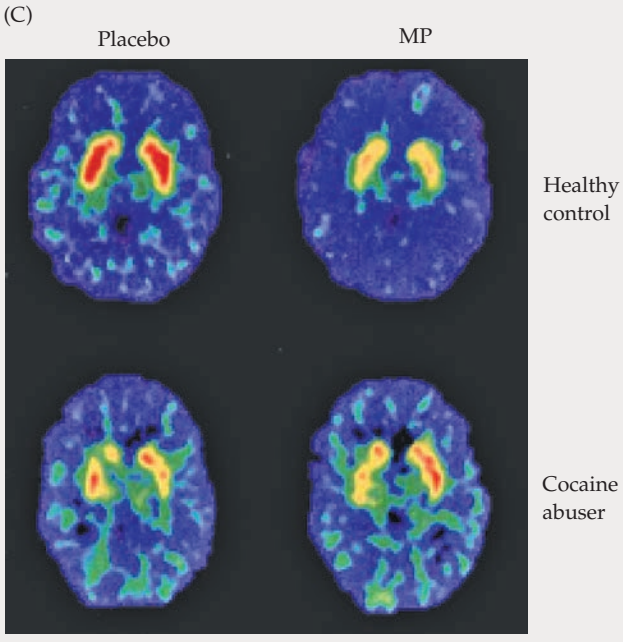
(B) Effects of long-access cocaine self-administration on stimulation-evoked DA release, rate of DA clearance, and changes in DA overflow following cocaine challenge Male rats self-administered cocaine (1.5 mg/kg/infusion IV; FR-1 schedule) for 6 hours per day, 5 consecutive days before neurochemical measurements were obtained. Controls were drug-naive animals maintained in their home cages. The left side depicts in vitro voltammetry measurements of electrical stimulation–evoked DA release and subsequent clearance from a brain slice containing the nucleus accumbens. The right side depicts in vivo microdialysis measurements of DA overflow in the nucleus accumbens following a cocaine challenge (15 mg/kg IP). SA, cocaine self-administration. (After Calipari et al., 2014a.)

BOX 12.1 The Cutting Edge (*continued*)

cocaine self-administration. The unexposed control rats showed the expected large increase in extracellular DA (DA overflow) due to the effect of the cocaine challenge blocking DA reuptake. In contrast, data from the cocaine self-administering rats showed very little increase in extracellular DA. This lack of effect is due to a combination of reduced DA release and a reduced ability of cocaine to inhibit DA reuptake. Given this profound tolerance of the dopaminergic system, it is not surprising that long-access self-administering rats also exhibit behavioral tolerance to cocaine.

We mentioned in the text that *intermittent* access to self-administered cocaine causes behavioral sensitization instead of tolerance. Indeed, researchers have shown that this procedure also leads to *increased* rather than *decreased* potency of cocaine to inhibit DA uptake (Siciliano et al., 2015). It is likely that the production of tolerance versus sensitization depends on differences in the pattern of cocaine concentrations in the brain. The intermittent access procedure has been estimated to produce repeated spikes in brain cocaine, as opposed to the high and relatively constant brain cocaine concentration produced by long-access self-administration. These differing patterns of neural exposure to cocaine are believed to play a critical role in producing the different dopaminergic changes seen in the tolerant compared with the sensitized animals.

The clinical relevance of the results from the rat self-administration studies has been tested by examining dopaminergic sensitivity to psychostimulant challenge in people who are either abusing or dependent on cocaine. Several such studies have used brain imaging to assess DA displacement of radiolabeled drug binding to D_2 receptors, as described earlier in Figure 12.11. One important difference, however, is the addition of a challenge with a psychostimulant such as methylphenidate. Increased sensitivity to methylphenidate would result in greater reuptake blockade and, therefore, greater displacement of the radiolabel by endogenous DA. On the other hand, decreased sensitivity would result in less reuptake blockade and, therefore, less displacement of the radiolabel. The results of this research are consistent with reduced dopaminergic sensitivity to the psychostimulant, which is in line with the rodent findings of cocaine tolerance (Narendran and Martinez, 2008). **Figure C** from a study by Volkow and colleagues (1997) presents PET images of the dorsal striatum (caudate and putamen) from a typical healthy control participant compared with those from a representative cocaine-dependent participant both under baseline conditions (placebo) and following the methylphenidate challenge (MP). Two things are evident from these images. First, the baseline level of D_2 receptor binding is reduced in the cocaine-dependent participant. This difference, which either may be a result of the chronic cocaine use or may have existed before the onset of such use, could be a factor in the lower (i.e., depressive-like) mood reported in cocaine abusers in the absence of the drug. Second, it is clear that the methylphenidate had a much greater effect in reducing raclopride binding in the control participant than in the cocaine-dependent participant. These results, which were statistically significant across the range of participants examined in the study, suggest that the dopaminergic system in cocaine-dependent individuals is less responsive to DA reuptake blockade (as would be produced by cocaine itself if given instead of methylphenidate), which likely contributes to the previously mentioned tolerance to cocaine's euphoric effects. A more recent study of cocaine-abusing participants showed a similar blunting of dopaminergic responses in the ventral striatum and PFC, indicating that cocaine-induced tolerance in this neurotransmitter system encompasses multiple forebrain target areas (Volkow et al., 2014).



(C) PET images showing striatal binding of radiolabeled raclopride A cocaine abuser and a healthy control were compared after each received an IV treatment of either a placebo or 0.5 mg/kg methylphenidate (MP). Compared with the control, the cocaine-abusing individual had lower baseline raclopride binding (placebo) and showed little reduction in binding following MP administration (compare the placebo versus MP image for each individual). Highest levels of binding are shown in red. (From Volkow et al., 1997.)

the resulting data are shown in **FIGURE 12.12**. The brain sections in Figure 12.12A depict areas (shown in yellow) that not only exhibited reduced volume in the cocaine-dependent group but also showed a significant correlation with duration of cocaine abuse (longer period of abuse associated with smaller volumes). Figure 12.12B depicts the orbitofrontal cortex (shown in green), a region for which the reduction in volume was correlated with scores on a test of compulsive cocaine taking (greater compulsivity associated with smaller volume). Figure 12.12C depicts areas with altered volume in relation to inattention-related impulsivity. Red symbols represent the left caudate nucleus, for which volume was positively correlated with inattention (poorer attention associated with larger volume), and blue symbols represent the insula and medial temporal gyrus, for which volumes were negatively correlated with inattention (poorer attention associated with smaller volumes). Overall, all brain areas mentioned except for the left caudate nucleus (part of the dorsal striatum) were found to have reduced gray matter volumes in the cocaine-dependent group. Determining the cellular changes responsible for reduced gray matter volume requires a microscopic analysis of postmortem brain tissues, and unfortunately such information is not yet available. Potential causes of gray matter loss include cell death, retraction of dendritic processes, and synapse elimination (Rasakham et al., 2014; Guha et al., 2016).

Researchers have understandably been concerned that the cognitive deficits and neurobiological abnormalities observed in people diagnosed with cocaine abuse or dependence might *precede* rather than follow from their repeated drug exposure. According to this hypothesis, differences in brain structure/function and the resulting cognitive deficits are produced by factors other than substance use; but once in place, these deficits increase vulnerability to substance use and abuse (including abuse of cocaine). Although this hypothesis may partially be correct, there are also several arguments in favor of the alternative hypothesis of long-term cocaine use *causing* many of the abovementioned

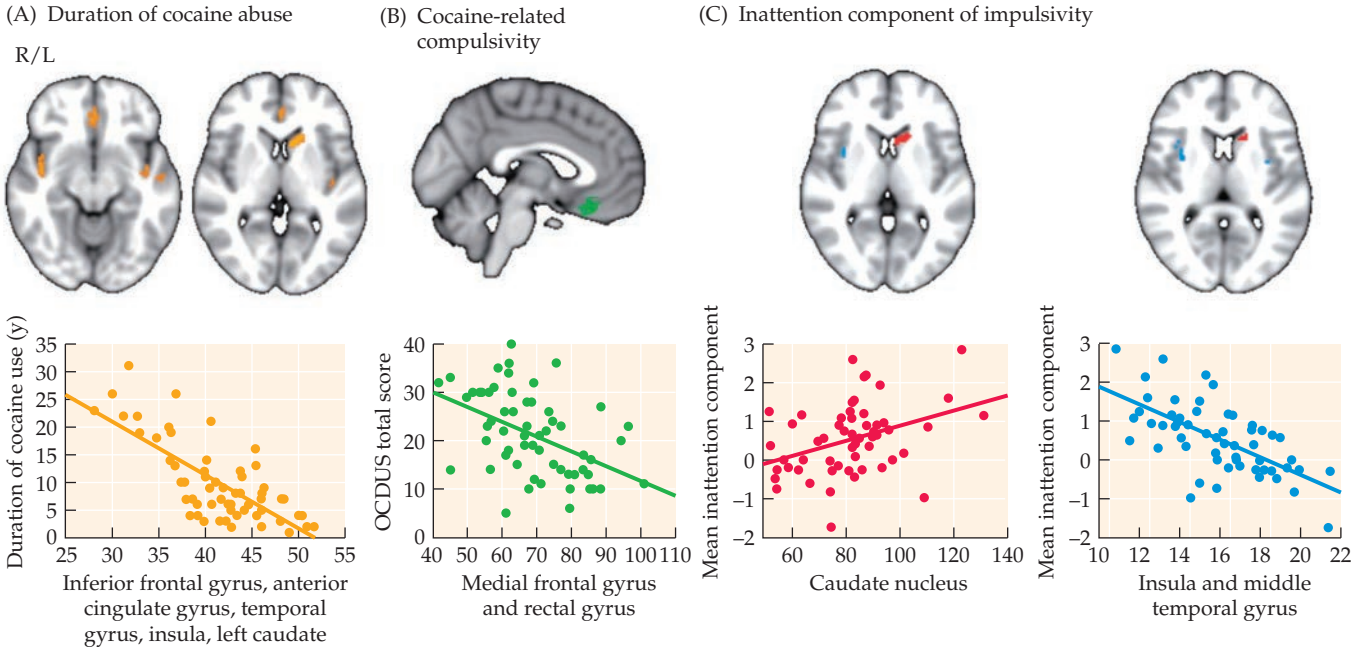


FIGURE 12.12 Regional associations of gray matter volume and cocaine-related measures Structural magnetic resonance imaging (MRI) was performed on cocaine-dependent individuals and healthy controls to measure the volume of gray matter in various cortical areas and in the caudate nucleus (part of the dorsal striatum). In the cocaine-dependent individuals, (A) gray matter volumes were significantly reduced in the inferior frontal gyrus, anterior cingulate gyrus, temporal gyrus, insula, and left caudate (yellow areas in the brain sections), and the combined volume of these areas was negatively correlated with the duration of cocaine abuse. (B) Reduced gray matter volumes of the medial frontal gyrus and rectal gyrus were negatively correlated with an index of cocaine compulsivity (based on the obsessive-compulsive drug use scale, OCDUS) (green areas in the brain section). (C) Reduced gray matter volume of the caudate nucleus was positively correlated with an inattention component of impulsivity (based on the Barratt Impulsiveness Scale) (red area in the brain section), whereas reduced gray matter volumes of the insula and medial temporal gyrus were negatively correlated with the inattention component of impulsivity (blue areas in the brain section). Brain sections in (A) and (C) are shown in the horizontal plane, whereas the brain section in (B) is shown in the parasagittal plane. R/L shows right and left sides of the brain in the horizontal sections. (From Ersche et al., 2011; CC BY-NC 2.5, with permission of the authors.)

findings from cognitive and neuroimaging studies. First, Figure 12.12 showed that at least for several brain areas, reduced volume was significantly correlated with duration of cocaine abuse. This is an unlikely finding if the observed volume differences were already in place before the onset of cocaine use. Second, neuroimaging studies in monkeys have demonstrated that repeated cocaine exposure leads to increased effects on the PFC compared with the effects observed at the initial dosing (Beveridge et al., 2008). The third piece of evidence comes from research on a subgroup of rats engaging in compulsive cocaine-seeking behavior (i.e., these rats endured a highly aversive stimulus in order to obtain cocaine) following long-access cocaine self-administration. Compared with noncompulsive cocaine-exposed rats, this subgroup developed significantly reduced activity of pyramidal neurons in the medial PFC. Such hypoactivity is in line with PFC abnormalities observed in cocaine-dependent users. Most importantly, stimulation of these pyramidal neurons suppressed the compulsive cocaine-seeking behavior, thereby suggesting a causal link between PFC dysfunction and uncontrollable cocaine use in humans and offering a novel target for treating people suffering from a cocaine use disorder (Chen et al., 2013).

Repeated or high-dose cocaine use can produce serious health consequences

The previous section summarized the effects of long-term cocaine use/abuse at typical recreational doses on brain structure and function. Higher doses taken over a long period or acute overdosing on cocaine can exert other adverse physiological and behavioral consequences. Such consequences can include a stroke or seizure, as mentioned earlier (Siniscalchi et al., 2015). Behaviorally, high-dose cocaine use can lead to panic attacks or the development of a temporary paranoid psychosis with delusions and hallucinations (Roncero et al., 2014; Tang et al., 2014). Risk factors for cocaine-induced psychotic disorder include being male, using cocaine in relatively greater amounts and for longer durations, and taking the drug by the IV route of administration. One particularly frightening type of hallucination is called "cocaine bugs," which refers to the sensation of tiny creatures crawling under or over the user's skin. More than 100 years ago, some of Freud's colleagues were already seeing patients with these kinds of psychotic reactions. Episodes of cocaine-induced psychotic disorder occur more frequently with repeated use, which is consistent with a growing sensitization to this effect of the drug.

Other organs or organ systems such as the heart, lungs, gastrointestinal system, and kidneys can also be adversely affected by cocaine. Complications associated with the heart range from chest pains to cardiac arrhythmias (irregular heart rate), cardiac myopathy (damaged heart muscle), and even myocardial infarction (heart attack). Frequent snorting of cocaine can lead to perforation of the nasal septum (the tissue that separates the two sides of the nose). Survey data indicate that heavy cocaine users may die from many different causes, including acute toxicity from overdose, traumatic deaths (e.g., homicide, motor vehicle accidents, falls, and fires), heart or liver disease, and pneumonia (Degenhardt et al., 2011). Those who take the drug by IV injection are also at high risk for contracting HIV and dying from AIDS. Finally, ingestion of cocaine by a pregnant woman has variable effects on the unborn child. Many offspring seem to escape without obvious harm; others may show behavioral problems, attention deficits, and/or other cognitive abnormalities; and in a small number of cases, the fetus is killed prior to birth. Although space constraints do not permit a detailed account of this research, interested readers seeking more information are referred to recent reviews by Buckingham-Howes et al. (2013), Cain et al. (2013), Gkioka et al. (2016), Lambert and Bauer (2012), and Martin et al. (2016).

Pharmacological, behavioral, and psychosocial methods are used to treat cocaine abuse and dependence

High rates of cocaine abuse and dependence in our society have spurred a great deal of interest in developing effective therapies for cocaine users. We will describe pharmacotherapeutic approaches, the idea of a cocaine vaccine, and programs that rely on behavioral and psychosocial methods.

PHARMACOTHERAPIES A large variety of compounds targeting several different neurotransmitter systems have been tested as potential medications to help cocaine users stop their current use and then to help maintain abstinence. Indeed, over 100 blinded placebo-controlled clinical trials have been conducted in which 64 different medications have been tested (Czoty et al., 2016). Yet despite this enormous effort, at the present time there is not a single FDA-approved medication for the treatment of cocaine use disorder. There are numerous reasons for this lack of success, including the surprising observation that many medications have been tested without the benefit of prior laboratory studies to at least suggest possible therapeutic efficacy (Czoty et al., 2016). Nevertheless, even in cases where therapeutic efficacy seemed plausible based on preclinical animal studies of cocaine-seeking behavior and/or cocaine relapse, outcomes have usually not been encouraging. This failure is generally attributable to lack of therapeutic benefit, unacceptable side effects, or a combination of both.

For many years, the National Institute on Drug Abuse (NIDA) has been the main driving force in the

effort to identify medications for cocaine dependence (NIDA, 2016). Much of this effort has been directed toward reducing cocaine's euphoric effects and/or the craving that ensues during cocaine withdrawal. Because of the well-known role of DA in cocaine reinforcement in animal models and its presumed involvement in human cocaine addiction, significant attention has been directed to various dopaminergic drugs, including DA releasing agents, receptor agonists, antagonists, and uptake inhibitors, which might compete with cocaine for access to the DA transporter (Platt et al., 2002). Psychostimulants such as amphetamine, methylphenidate, and modafinil, all of which increase dopaminergic transmission, have been tested as potential "replacement" compounds for cocaine. This approach is similar to that involved in the use of the opioid agonist methadone to treat opioid dependence, as previously discussed in Chapter 11. Although psychostimulants have yielded promising results in preclinical animal studies, the outcome of human clinical trials has been mixed at best (Dürsteler et al., 2015; Castells et al., 2016). Furthermore, Negus and Henningfield (2015) discuss various roadblocks ahead before any psychostimulant replacement therapy could be approved by the FDA for the treatment of cocaine use disorder. One key issue is the abuse potential of these compounds themselves, although it's worth noting that the three drugs listed above *are* already approved for treating other psychiatric disorders (see later sections of the chapter). Therefore, continued research on psychostimulant replacement therapy seems to be warranted.

The search for cocaine medications has led researchers to explore many other neurotransmitter systems besides DA (Shorter et al., 2015). These include the noradrenergic, serotonergic, glutamatergic, and GABAergic systems. Note that much of the current evidence in support of drugs targeting these systems comes from experimental animal studies. Hence, we are still far away from having a proven pharmacotherapeutic agent for cocaine users, regardless of which neurotransmitter system is being targeted.

COCAINE VACCINE Through a much different approach, researchers have shown that vaccines against cocaine can be developed. The resulting antibodies may simply bind cocaine molecules, or alternatively, they may have catalytic activity that actually breaks down cocaine in the bloodstream. Both methods cause less cocaine to get into the brain, and both have been shown to reduce (or even completely block) cocaine self-administration and reinstatement in animals. Cocaine-binding antibodies have been tested in several clinical trials. Thus far, the results are mixed, as some people who developed relatively high antibody titers (high concentrations of cocaine-targeted antibodies) in their bloodstream did show reduced cocaine use, but many other people showed a poorer response to the vaccination and continued a high level of cocaine use (Kosten et al., 2014; Orson et al., 2014). An important limitation of cocaine-binding antibodies is that their effectiveness can be overcome by taking larger doses of cocaine. Antibody therapy might, therefore, work best when coupled with behavioral approaches to reduce the patient's motivation to seek out and use the drug. In any case, because of the lack of success of conventional pharmacotherapeutic approaches, anticocaine vaccines continue to be developed and tested in the hope that this approach will eventually prove effective in reducing cocaine dependence.

BEHAVIORAL AND PSYCHOSOCIAL THERAPIES It should be apparent that researchers have not yet discovered any compound that is broadly effective in treating cocaine abusers. Although this situation could change with the development of newer medications, it is necessary to consider the potential role of behavioral and social therapies in dealing with this problem. While pharmacotherapy may aid in patient stabilization (e.g., by reducing craving or other abstinence symptoms), equally important are counseling and support structures that enable the patient to learn new coping responses, avoid triggers for relapse, and function effectively in a drug-free lifestyle. Indeed, psychosocial factors such as feelings of self-efficacy, demonstrating a strong commitment to abstinence, and having good social support predict a greater likelihood of recovery from cocaine dependence (McKay et al., 2013).

A variety of different treatment programs are available for cocaine-dependent individuals (Penberthy et al., 2010). Many are conducted on an outpatient basis, although the most severe cases usually receive the greatest benefit from hospitalization and either short- or long-term inpatient treatment. **Psychosocial treatment programs** involve individual, group, or family counseling designed to educate the user, promote behavioral change, and alleviate some of the problems caused by cocaine abuse. **Cognitive behavioral therapy** (**CBT**) is aimed at restructuring cognitive (thought) processes and training the user either to avoid high-risk situations that might cause relapse or to employ appropriate coping mechanisms to manage such situations when they occur. This approach is sometimes called **relapse prevention therapy**. Also available are 12-step programs such as Narcotics Anonymous or Cocaine Anonymous. The general approach of all 12-step programs is based on that of Alcoholics Anonymous, which is described in Chapter 10.

One of the most interesting approaches to treating cocaine users was developed by Stephen Higgins and

coworkers at the University of Vermont (Higgins et al., 1991). This **contingency management program** is a behavioral treatment approach based on the premise that drug taking is an operant response that persists mainly as a result of the reinforcing properties of the drug. Hence altering reinforcement contingencies to reduce drug-associated reinforcement and to increase the availability of nondrug reinforcers should help promote abstinence and the adoption of a drug-free lifestyle. As part of this program, each negative urine test of the client is reinforced with a voucher. These vouchers cannot be redeemed for money per se, to avoid patients accumulating funds for drug purchases; however, they can be exchanged for retail items available locally. Another aspect of this program involves a community reinforcement approach (Hunt and Azrin, 1973), which is designed to enhance the patient's social (including family) relationships, recreational activities, and job opportunities. Contingency management has been shown to be one of the most effective approaches for treating cocaine dependence (Schierenberg et al., 2012; Farronato et al., 2013). Carroll and coworkers (2016) recently conducted a study comparing CBT alone, CBT plus disulfiram medication, CBT plus contingency management, and CBT plus both disulfiram and contingency management in effectiveness of reducing cocaine use in cocaine-dependent individuals who sought treatment. Cocaine use measured by estimated days of use per month declined in all groups and stabilized at a lower level than during pretreatment. However, the greatest treatment efficacy was observed in the CBT plus contingency management group. It is not clear why the latter group used less cocaine than the group in which disulfiram was added to the treatment regimen. The important point, however, is that the combined nonpharmaceutical treatment approach was highly successful over a long follow-up period for individuals who were motivated to seek help for their cocaine dependence. The main problem associated with contingency management programs is that they are labor-intensive and costly, particularly when paired with community reinforcement methods. Yet, results such as those shown here suggest that wider implementation of such programs may be beneficial in reducing the burden of cocaine dependence on our society.

Section Summary

- Early use of other substances seems to be an important risk factor for the initiation of cocaine use.
- Some users quickly stop taking cocaine for various reasons, some maintain controlled use for long periods, and still others progress to a pattern of uncontrolled use (i.e., abuse). Such a progression may come about through dose escalation and/or switching from intranasal use to smoking or IV injection—routes of administration with greater abuse potential.
- Maladaptive use of psychostimulants generally is categorized as stimulant use disorder in the *DSM-5*. Maladaptive cocaine use can be categorized more specifically as cocaine use disorder.
- Maladaptive cocaine use (i.e., cocaine abuse) may be manifested by daily or near-daily use or by a pattern of bingeing. Many individuals who abuse cocaine also suffer from other psychiatric disorders.
- Cocaine craving and relapse to cocaine use increase over time following withdrawal, which has been called incubation of cocaine craving.
- Neuroimaging studies have found that cocaine-dependent individuals show abnormal prefrontal cortical functioning and that cocaine-related cues elicit DA release in the dorsal striatum. The midbrain–striatal DA pathway is part of a larger circuit comprising various cortical and limbic structures that are activated when cocaine users experience craving for the drug.
- Animal models of cocaine dependence include such features as escalation of drug intake, relapse to cocaine seeking after a period of abstinence, cocaine-seeking behavior despite aversive consequences, and increased motivation to take cocaine, as shown by an elevated breaking point on a progressive-ratio schedule.
- Animal models have supported the hypothesis that both sensation seeking and impulsivity are traits that contribute to the development of compulsive cocaine use.
- Chronic exposure to cocaine or other psychostimulants can lead to tolerance and/or sensitization. Changes in drug responsiveness depend on the pattern of drug exposure, the outcome measure, and the time since the last dose.
- Animal studies have implicated increased dopaminergic activity in the VTA and increased NAcc DA release as being important for locomotor sensitization to psychostimulants.
- Human cocaine-dependent study participants show reduced DA release in the striatum compared with controls. This finding is consistent with evidence for tolerance to the drug's euphoric effects over time, thus leading to increased drug-taking behavior by these individuals.
- Individuals suffering from cocaine abuse or dependence show deficits in many cognitive domains, including sustained attention, impulse control, working memory, verbal learning and memory,

performance on psychomotor tasks, and decision making in reward-based learning tasks. These deficits are additionally associated with structural and functional differences in several cortical and subcortical brain areas, including the PFC, orbitofrontal cortex, and dorsal striatum. Evidence from both human and experimental animal studies suggests that at least some of these cognitive and neurobiological differences are *caused* by repeated cocaine exposure.

- The adverse effects of repeated or high-dose cocaine use include stroke or seizure, abnormalities in both gray and white matter in the cortex, cardiovascular problems including heart attack, damage to other organ systems, and possible abnormalities in the development of offspring exposed prenatally to cocaine. High-dose cocaine use can also lead to panic attacks or the onset of a paranoid psychotic reaction.
- Much effort in the area of treating cocaine abuse has been focused on the development of medications that might reduce craving and promote abstinence among users. Some of these medications act on the dopaminergic system (e.g., DA receptors or the DA transporter), whereas others that are being tested target the noradrenergic, serotonergic, glutamatergic, and GABAergic systems. The psychostimulants amphetamine, methylphenidate, and modafinil have been investigated as potential replacement drugs for cocaine. Despite these efforts, there is currently no FDA-approved medication to treat cocaine dependence.
- Yet another approach still under consideration is to use an anticocaine vaccine that either traps the drug in the bloodstream or breaks the drug down enzymatically.
- Current behavioral and psychosocial treatments include various types of counseling, cognitive behavioral therapies aimed at relapse prevention, 12-step programs like Narcotics Anonymous and Cocaine Anonymous, and contingency management programs based on a combination of vouchers and a community reinforcement approach. Contingency management programs seem to have the greatest success rates in treating cocaine dependence; however, such programs are labor-intensive and costly compared to other approaches, which has limited their application.

The Amphetamines

Background and History

Amphetamine is the parent compound of a family of synthetic psychostimulants. It is available in two chemical forms, L-amphetamine (trade name Benzedrine) and D-amphetamine (also called dextroamphetamine; trade name Dexedrine). Another key member of this family is **methamphetamine**. Two naturally occurring plant compounds have structures similar to those of amphetamine and methamphetamine, namely **cathinone** and **ephedrine**. As can be seen in **FIGURE 12.13**, all of these compounds are structurally related to the neurotransmitter DA.

Cathinone is the primary active ingredient in khat (alternately spelled qat) (*Catha edulis*), an evergreen shrub native to East Africa and the Arabian Peninsula (**FIGURE 12.14**). Ephedrine is a constituent of the herb *Ephedra vulgaris*. Chinese physicians have used *Ephedra* (known to them as ma huang) for more than 5000 years as an herbal remedy. Like other amphetamine-like substances, ephedrine reduces appetite, and it also provides a subjective feeling of heightened energy. For these reasons, a number of companies began to market ephedra-containing dietary supplements as weight loss products sold in health food stores. These supplements became so popular that in 1999, the General Accounting Office estimated that Americans were consuming about 2 billion doses of ephedra-containing products each year! Unfortunately, ephedrine sharply elevates blood pressure and exerts other sympathomimetic effects as well. These effects can increase the risk of heart attack or stroke, particularly with large drug doses or in vulnerable individuals. Because of the many reports of adverse reactions (including several deaths)

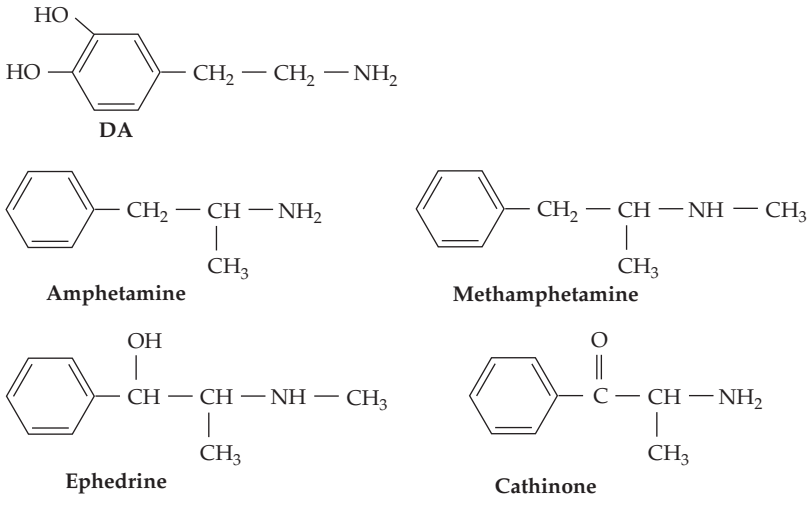


FIGURE 12.13 Amphetamine and related psychostimulants resemble the neurotransmitter DA in their chemical structure This accounts for their potent effects on the dopaminergic system.

FIGURE 12.14 Khat for sale in an Ethiopian marketplace (Toby Adamson/Perspectives/Getty Images.)

associated with the use of ephedra, the U.S. Food and Drug Administration (FDA) became increasingly concerned about this substance. Then in February of 2003, national headlines were made when a young Baltimore Orioles pitcher named Steve Bechler collapsed and died during spring training in Florida. Struggling to control his weight, Bechler had been taking high doses of a popular ephedra-containing supplement, and the Broward County coroner ruled that ephedra was a likely contributory factor in the pitcher's death. As a consequence of this and other reported adverse reactions, the FDA officially banned the sale of ephedra-containing dietary supplements in 2004.

Interestingly, ephedra played a key role in the development of amphetamine. In the 1920s, purified ephedrine was found to be a valuable antiasthmatic agent because of its powerful bronchodilator (widening of the airways) action.[3] However, the medical profession soon became concerned that demand for ephedrine might exceed its supply and therefore began to search for an appropriate synthetic substitute. That substitute turned out to be amphetamine, which had first been synthesized in 1887 by a chemist named Edeleano. The Smith, Kline & French pharmaceutical company introduced an amphetamine-containing inhaler in 1932. The inhaler, which contained 250 mg of Benzedrine in a cotton plug, proved to be effective in temporarily relieving nasal or bronchial congestion. Unfortunately, however, some individuals began to overuse these inhalers, particularly since many varieties could be purchased without a prescription. Other people learned to open the container, remove the amphetamine-containing plug, and chew the cotton, swallow it, or extract it to recover the drug for the purpose of injection. Amphetamine in tablet form was first marketed in 1935 as a treatment for the sleep disorder narcolepsy (see later section on modafinil and its use in treating this disorder). By the 1940s, amphetamine had become so widely embraced by the medical profession that one author documented 39 supposed clinical uses for the drug. In addition, during World War II many American military personnel were given amphetamine to forestall sleep and maintain a heightened level of alertness during prolonged periods on duty (Rasmussen, 2015).

After the war, the United States experienced a surge in the street use of amphetamine. During the 1950s and 1960s, students were casually using amphetamines in the same way that caffeine is presently taken to remain awake during pre-exam all-nighters. The peak of amphetamine use in the United States occurred during the early 1970s, when the legal production of amphetamine exceeded 10 billion (!) tablets, and at least one survey estimated that approximately 25% of young men had used stimulant drugs (mainly amphetamine or related compounds) on one or more occasions. Since that time, cocaine has generally supplanted amphetamine as the major abused psychostimulant. One exception to this trend has been a current upsurge in methamphetamine use in certain parts of the country. In the following discussion, amphetamine and methamphetamine are presented together because of their similar neurochemical and behavioral effects.

Basic Pharmacology of the Amphetamines

Amphetamine is typically taken either orally or by IV or subcutaneous injection (the latter is sometimes called "skin popping"). Street names for amphetamine include "uppers," "bennies," "dexies," "black beauties," and "diet pills." Because absorption from the gastrointestinal tract is relatively slow, it may take up to 30 minutes for behavioral effects to be experienced after a typical oral dose of 5 to 15 mg. In contrast, IV injection provides a much more rapid and intense "high" than oral consumption and has much greater abuse potential.

Methamphetamine is more potent than amphetamine in its effects on the central nervous system and is therefore favored by substance abusers when it is available. Typical street names for methamphetamine are "meth," "speed," "crank," "zip," and "go." The drug can be taken orally, snorted, injected intravenously, or smoked. Smoking methamphetamine can be accomplished either by using a glass pipe or by heating the compound on a piece of aluminum foil (a practice sometimes called "chasing the dragon"). Smoking is a very effective route of methamphetamine consumption because the drug vaporizes at a low temperature and

[3]Some decongestants still contain pseudoephedrine as their active ingredient, but availability of these drugs (i.e., only from the pharmacist rather than the open shelves) is closely regulated because pseudoephedrine is used illegally to synthesize methamphetamine for street use.

is not readily broken down by heat. In the late 1970s, methamphetamine for recreational use was primarily being manufactured by various motorcycle gangs on the West Coast, and the practice of hiding the drug in motorcycle crankcases is what led to the street name "crank." Subsequently, methamphetamine hydrochloride in a crystalline form particularly suitable for smoking (called "ice" or "crystal" on the street) began showing up in Hawaii in the 1980s. This material has since spread to many parts of the country, particularly the West, South, and Midwest. Because "ice" is inexpensive to make and is highly addictive, it poses a serious risk for society's attempts to control and reduce the incidence of stimulant abuse (Maxwell and Rutkowski, 2008; Gonzales et al., 2010). Methamphetamine is usually synthesized from pseudoephedrine, which accounts for the government's tight control over this precursor. If you have ever needed to obtain pseudoephedrine from a pharmacy, you know that only small quantities of pills are sold at one time even though no prescription is needed from a physician.

Some amphetamine or methamphetamine users (called "speed freaks") go on binges, or "runs," of repeated IV injections to experience recurrent highs. During a run, the drug is typically injected approximately every 2 hours for a period as long as 3 to 6 days or more. Little sleep or eating occurs during a run. The user finally becomes exhausted, ends the run, and goes to sleep for many hours. Barbiturates or other depressant drugs are sometimes used either to "take the edge off" during a run or to assist in sleeping following the run. Yet another approach is to moderate the extreme stimulatory effect of IV amphetamine or methamphetamine by combining it with heroin to yield a so-called "speedball."

Amphetamine and methamphetamine are metabolized by the liver, although at a slow rate. Metabolites, as well as some unmetabolized drug molecules, are mainly excreted in the urine. The elimination half-life of amphetamine ranges from 7 to more than 30 hours depending on the pH of the urine. Methamphetamine has a similar half-life of approximately 10 hours. Because of these long half-lives, users obtain a much longer-lasting high from a single dose of amphetamine or methamphetamine than they can get from a dose of cocaine.

Mechanisms of Amphetamine and Methamphetamine Action

Amphetamine and methamphetamine are indirect agonists of the catecholaminergic systems. Unlike cocaine, which only blocks catecholamine reuptake, amphetamine and methamphetamine also release catecholamines from nerve terminals. At very high doses, these compounds can even inhibit catecholamine metabolism by monoamine oxidase.

Studies on the mechanism of catecholamine release by amphetamine have particularly focused on DA. The results of this research suggest that two related drug actions are involved. One action is to cause DA molecules to be released from inside the vesicles into the cytoplasm of the nerve terminal. These DA molecules are subsequently transported outside of the terminal by a reversal of the DAT (**FIGURE 12.15**). The result is a massive increase in synaptic DA concentrations and an associated stimulation of dopaminergic transmission. Amphetamine-mediated reversal of DAT function is facilitated by an activation of protein kinase C and calcium/calmodulin kinase II, resulting in phosphorylation of the transporter (Robertson et al., 2009). Importantly, the dopaminergic nerve terminals cannot resynthesize and store DA quickly enough to keep up with the massive release provoked by amphetamine. Hence, the acute release of DA is followed by a period of reduced DA concentrations until neuronal stores can be replenished.

In rodents, amphetamine- or methamphetamine-stimulated DA release has been demonstrated by using techniques such as in vivo microdialysis. Brain imaging studies have likewise provided evidence for DA release in humans (Slifstein et al., 2010; Schrantee et al., 2015) and non-human primates (Gallezot et al., 2014; Ota et al., 2015) following amphetamine administration either orally or by IV injection. It is important to recognize that the NE-releasing effects of amphetamines occur not only in the brain but also in the sympathetic nervous system. Consequently, these compounds exert potent sympathomimetic actions similar to those seen with cocaine.

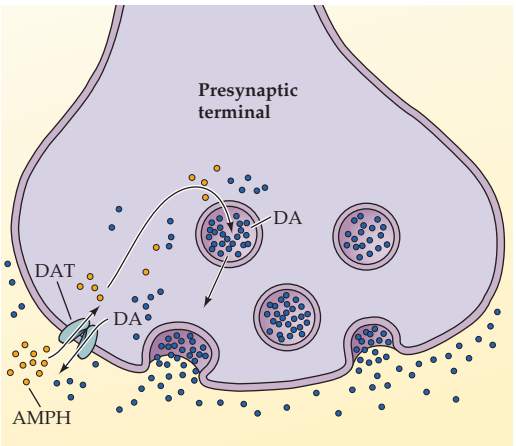


FIGURE 12.15 Mechanisms of amphetamine-stimulated DA release Amphetamine (AMPH) molecules enter DA nerve terminals in part through uptake by dopamine transporter (DAT). Once inside the terminal, the drug provokes DA release from the synaptic vesicles into the cytoplasm. In addition, DAT functions in a reverse direction to release DA from the cytoplasm into the extracellular fluid. The combined effect of these processes is a massive increase in synaptic DA levels.

Behavioral and Neural Effects of Amphetamines

Like cocaine, amphetamine causes heightened alertness, increased confidence, feelings of exhilaration, reduced fatigue, and a generalized sense of well-being in human users. A number of other effects have also been observed, including improved performance on simple, repetitive psychomotor tasks; a delay in sleep onset; and a reduction in sleep time, particularly with respect to REM (rapid-eye-movement) sleep. Indeed, amphetamine permits sustained physical effort without rest or sleep, which accounts for its distribution to military personnel during World War II, as well as its occasional use by truck drivers and other workers desirous of foregoing sleep for extended periods. The drug can also enhance athletic performance and is therefore one of the many banned substances in athletic competitions. In rodents and other animals, amphetamine elicits behavioral activation (locomotor stimulation and stereotypy) similar to that seen with cocaine. It is also highly reinforcing, as shown by numerous studies involving drug self-administration or place conditioning.

Amphetamine and methamphetamine have therapeutic applications but are also subject to abuse and dependence when used recreationally. Heavy use of these compounds can lead to psychotic reactions, neurotoxicity, and other adverse consequences.

Amphetamine and methamphetamine have therapeutic uses

As mentioned earlier, one medical use for amphetamine is in the treatment of narcolepsy; however, this drug is more recently being replaced by other stimulants such as modafinil (Didato and Nobili, 2009). Amphetamine and particularly methylphenidate are even more widely used in treating children with ADHD. The pharmacology and therapeutic applications of modafinil and methylphenidate are discussed later in the chapter. Earlier in the chapter, Table 12.1 mentioned that cocaine has an anorexic effect (i.e., production of weight loss). Suppression of appetite and weight reduction are common to all psychostimulants, including not only cocaine but also amphetamine and methamphetamine. For many years, methamphetamine tablets (trade name Desoxyn) have been available as an antiobesity treatment. One of the mechanisms thought to underlie the appetite-suppressing effect of psychostimulants is the induction of an interesting neuropeptide called CART (cocaine- and amphetamine-regulated transcript) (Rogge et al., 2008). Needless to say, because of methamphetamine's high abuse potential, this treatment is now only rarely used to treat obese patients who have failed to respond to more conventional therapeutic approaches.

Narcolepsy typically involves recurring and irresistible attacks of sleepiness during the daytime hours, often along with other symptoms such as cataplexy (sudden and transient loss of muscle tone that can be severe enough to cause bodily collapse, often brought about by strong emotional stimuli), hypnagogic hallucinations (vivid sensations that occur at the onset of sleep, upon awakening, or sometimes during a cataplectic attack), and sleep paralysis (muscle paralysis that persists after waking from a rapid-eye-movement sleep episode). Narcolepsy is caused by loss (perhaps autoimmune related) of hypothalamic neurons that secrete the neuropeptide hypocretin (also called orexin). Refer back to Box 3.1 for more information on this disorder.

High doses or chronic use of amphetamines can cause a variety of adverse effects

Illicit use and abuse of amphetamines is a significant worldwide problem, with high rates of usage in the United States, Mexico, Japan, China, and countries of South Asia and the Middle East (Chomchai and Chomchai, 2015). The 2016 World Drug Report of the United Nations Office on Drugs and Crime reveals that 170 tons(!) of amphetamine-type stimulants (mostly methamphetamine and amphetamine) were seized by authorities worldwide during 2014 (United Nations Office on Drugs and Crime, 2016). **FIGURE 12.16** shows that worldwide seizures of these substances has been skyrocketing over the past several years compared with other classes of illicit drugs.

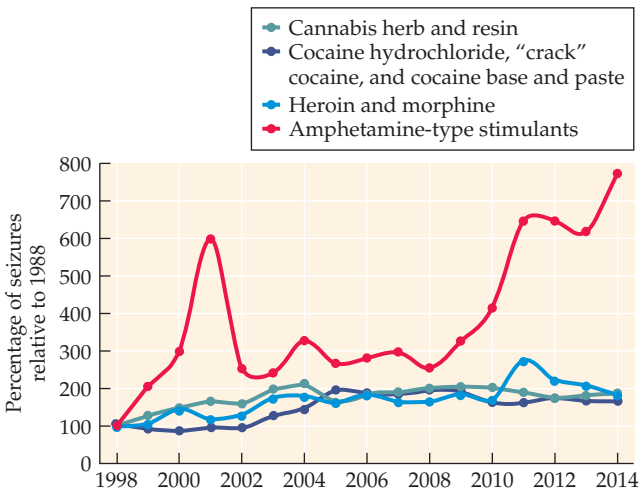


FIGURE 12.16 Yearly trends in worldwide drug seizures from 1998 to 2014 For each class of abused drug, changes in quantities seized worldwide are shown relative to a baseline of seizures in 1998. For example, the data for amphetamine-type stimulants (ATS) in 2014 reached a value of almost 800 on the graph, which indicates that ATS seizures were nearly eight times (800%) as high as the seizures recorded in 1998. Note that the ATS category includes amphetamine, methamphetamine, methcathinone, ephedrine, pseudoephedrine, methylphenidate, and MDMA. (After United Nations Office on Drugs and Crime, 2016.)

Because methamphetamine is currently abused more heavily than amphetamine in the United States, this section of the chapter will focus primarily on the adverse behavioral, physiological, and neurobiological effects of chronic methamphetamine abuse. Nevertheless, it is important to note that at least some of these same effects can occur in cases of heavy amphetamine rather than methamphetamine use.

DEPENDENCE, WITHDRAWAL, AND COGNITIVE FUNCTION Chronic methamphetamine use commonly leads to dependence on the drug and a withdrawal syndrome when the individual undergoes abstinence (Cruickshank and Dyer, 2009). There is some evidence that females are more likely than males to use methamphetamine and are more likely to become dependent on the drug (Dluzen and Liu, 2008). Principal symptoms of methamphetamine withdrawal include depressed mood, increased anxiety, sleep disturbances, cognitive deficits, and craving for the drug. Users may also experience agitated behavior, reduced energy, and increased appetite. Withdrawal symptoms may take up to several weeks to subside, during which time the user is at particularly high risk for relapse.

Numerous studies have reported cognitive deficits in individuals with methamphetamine abuse or dependence compared with controls. Because such studies are almost always cross-sectional in design, they cannot readily distinguish between cognitive differences that preceded the onset of drug use and differences *caused* by the drug. Nevertheless, a careful examination of the literature led to the conclusion that methamphetamine abuse may lead to cognitive decline in some individuals (Dean et al., 2013).

PSYCHOTIC REACTIONS More than 30 years ago, several research groups first described in some high-dose amphetamine users a psychotic reaction consisting of visual and/or auditory hallucinations, behavioral disorganization, and the development of a paranoid state with delusions of persecution. Users may experience the same hallucination of a parasitic skin infestation described earlier for cocaine. These reactions to amphetamine usually do not occur upon first exposure to the drug, but only after a chronic abuse pattern has developed. Furthermore, in at least one study, the paranoia and hallucinations did not typically begin until the second or third day of a "speed run."

As methamphetamine use has increased, the incidence of psychotic reactions to this substance has grown. Risk factors for the development of methamphetamine-induced psychosis include a long history of use, use of high doses, becoming dependent on the drug, and (not surprisingly) having a preexisting history of psychotic symptoms (Darke et al., 2008). Various studies have found that between 26% and 46% of methamphetamine-dependent individuals have experienced psychotic episodes (Glasner-Edwards and Mooney, 2014). Continued methamphetamine use can cause a transition to a psychotic state that persists long after abstinence from the drug and has a symptomatology that is difficult to distinguish from paranoid schizophrenia. Two theories have been proposed to account for such a transition: first, that the individual has an underlying schizophrenic disorder that is unmasked by methamphetamine use, and second, that methamphetamine-induced psychosis and schizophrenia have similar neurochemical bases. Regardless of which theory is correct, methamphetamine users who present with psychotic symptoms are typically administered the same kinds of antipsychotic medications as are given to patients with schizophrenia.

NEUROTOXICITY There is a special danger to methamphetamine users that is due to the neurotoxic properties of this substance. Investigators have known for many years that administration of multiple doses of methamphetamine to animals causes long-lasting reductions in the levels of DA, tyrosine hydroxylase (the key enzyme in DA synthesis), and DAT in the dorsal striatum (Moratalla et al., 2017). These changes are indicative of damage to DA axons and terminals, which has been confirmed by histological experiments showing the presence of degenerating fibers. Additional studies have demonstrated significant death of dopaminergic neurons in the substantia nigra, the source of the nigrostriatal DA system. For reasons that aren't completely understood, the mesolimbic DA system is relatively spared in methamphetamine-treated animals. This difference can be seen in **FIGURE 12.17**, which presents tissue sections from a methamphetamine-treated mouse compared with a saline vehicle–treated mouse in which the sections were stained for tyrosine hydroxylase immunoreactivity. Note the striking reduction in staining in the dorsal striatum (caudate–putamen, or CPu) compared with the more ventral NAcc subregions (AcbC and AcbSh) and nearby areas. A number of factors contribute to methamphetamine-induced neurotoxicity. Binge-treated animals experience severe hyperthermia, and blocking this effect has a substantial ameliorative effect on the drug's toxic effects. Activation of both D_1 and D_2 receptors also appears to play an important role. At a molecular level, damage to the dopaminergic system has been linked to multiple processes, including oxidative stress (cellular stress due to increased production of oxygen-containing free radicals), excitotoxicity, neuroinflammation, and mitochondrial dysfunction (Halpin et al., 2014; Moratalla et al., 2017).

The experimental animal studies just described raise a critical health question of whether repeated methamphetamine exposure causes damage to the dopaminergic system in humans. There is ample

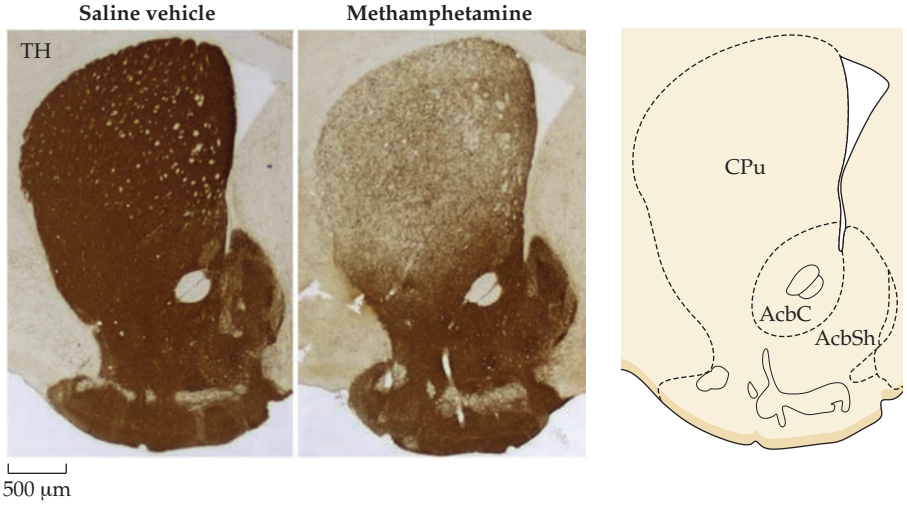


FIGURE 12.17 Methamphetamine-induced loss of tyrosine hydroxylase immunoreactivity in the nigrostriatal compared with the mesolimbic DA pathways in mice Female mice were given IP injections of either 4 mg/kg methamphetamine or saline vehicle, three times on a single day, with a 3-hour interdose interval. The animals were killed 7 days later, and coronal brain sections through the caudate–putamen (CPu) and nucleus accumbens core and shell (AcbC and AcbSh) were stained using an antibody against tyrosine hydroxylase (TH) as a marker of dopaminergic fibers and nerve terminals. The two photomicrographs on the left show that methamphetamine-induced DA neurotoxicity was more pronounced in the nigrostriatal pathway (CPu) than in the mesolimbic pathway (AcbC and AcbSh). The figure on the right is a plate from a mouse brain atlas used to identify the key anatomical areas shown in the photomicrographs. (From Moratalla et al., 2017.)

evidence that this system is, at the very least, highly dysfunctional in chronic methamphetamine users. Brain imaging studies combined with one biochemical study of postmortem brain tissues have shown reduced striatal DA levels, reduced DAT binding, and reduced binding to D_2/D_3 receptors in the drug users compared with nonusers (Volkow et al., 2015; Kish et al., 2017). Yet, the available evidence is not consistent with loss of substantia nigra dopaminergic neurons, suggesting that the dopaminergic dysfunction may be a neurochemical response to the recurring drug insult rather than physical damage to the system. This may seem to be relatively good news to hard-core methamphetamine users; however, epidemiological studies show an elevated risk for developing Parkinson's disease in the methamphetamine-using population (Curtin et al., 2015; Kish et al., 2017). We can speculate either that a subset of heavy users does incur dopaminergic cell loss that contributes to later Parkinson's disease onset, or that repeated drug-induced stress on that cell system accelerates the overall process of age-related dopaminergic cell death (see Chapter 20), thereby causing a greater incidence of the disease over time.

GENERAL PHYSICAL HEALTH In addition to the neurotoxic and cognitive effects mentioned above, heavy methamphetamine use has been associated with a variety of adverse health outcomes. These include cardiovascular problems such as elevated heart and blood pressure, atherosclerosis, myocardial infarction, and increased risk of stroke; oral diseases such as "meth mouth" (De-Carolis et al., 2015); and an increased mortality rate (Darke et al., 2008; Marshall and Werb, 2010). There is also anecdotal evidence for premature aging among chronic methamphetamine users. This can be seen from a website called Faces of Meth (www.facesofmeth.us/main.htm), which was developed by the Sheriff's Office of Multnomah County in Oregon and which posts "mug shots" of the same people taken at different times during the course of their drug use (and arrest). Two of these photographs are shown in **FIGURE 12.18**, which illustrates the physical toll inflicted on

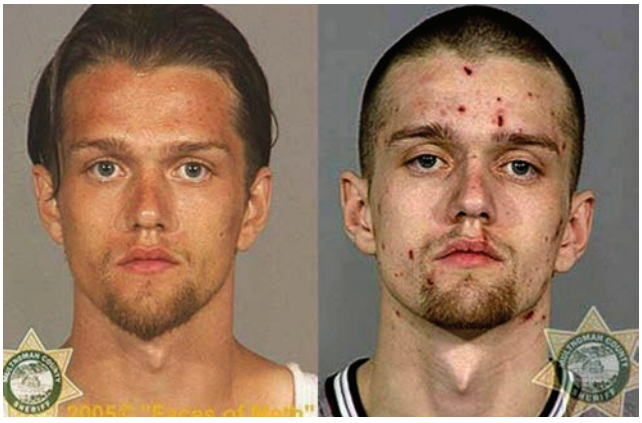


FIGURE 12.18 An example of the physical deterioration produced by chronic methamphetamine use (Multnomah County Sheriff/Splash News/Alamy Stock Photo.)

heavy methamphetamine users over the course of just a few years. It is hoped that the message of these pictures will deter at least some people from becoming trapped in the scourge of methamphetamine use.

Section Summary

- Amphetamine and methamphetamine are synthetic psychomotor stimulants that are closely related structurally to two similarly acting plant compounds, cathinone and ephedrine.
- Amphetamine was first introduced in the United States in 1932 in the form of a nasal inhaler. People soon realized that they could achieve powerful stimulatory and euphoric effects by consuming the drug orally or by injecting it. The incidence of amphetamine use and abuse grew until a peak was attained in the 1970s. Since that time, the drug has been largely supplanted by cocaine, except for a recent upsurge in methamphetamine use in certain parts of the country.
- Amphetamine is typically taken orally or by IV or subcutaneous injection. Crystalline methamphetamine, which is more potent than amphetamine, can also be taken by snorting or smoking. Some amphetamine or methamphetamine users take the drug repeatedly in binges called speed runs. Both drugs are metabolized slowly by the liver, thus causing a longer duration of action than cocaine.
- Amphetamine and methamphetamine are indirect catecholamine agonists. They stimulate release of DA and NE from nerve terminals and block the reuptake of these neurotransmitters. At high doses, there is also an inhibition of the catecholamine-degrading enzyme monoamine oxidase. Central DA release has been demonstrated in both animals and humans. Acute release of DA is followed by a period of DA depletion due to an inability of the dopaminergic nerve terminals to resynthesize the transmitter at a sufficiently fast rate.
- Amphetamine and methamphetamine also have sympathomimetic effects that are due to their effects on NE in the sympathetic nervous system.
- Acute administration of amphetamine to humans leads to a well-known constellation of behavioral reactions, including increased arousal, reduced fatigue, and feelings of exhilaration. Sleep is delayed, and performance of simple, repetitive tasks is improved.
- Amphetamine and another stimulant, methylphenidate, are widely prescribed for children with attention deficit hyperactivity disorder (ADHD). At relatively low doses, these stimulants produce calming and attention-enhancing effects that differ from the typical responses seen in adults taking higher drug doses.
- In experimental animals, amphetamine acts much like cocaine. It elicits dose-dependent stimulation of locomotion and stereotyped behaviors, and it is highly reinforcing in self- administration and place-conditioning paradigms.
- Heavy use of amphetamine or particularly methamphetamine can result in a number of adverse consequences, including the development of dependence, cognitive deficits, psychotic reactions that closely resemble paranoid schizophrenia, and dysfunction neurotoxicity of the DA system. Chronic methamphetamine users may be at elevated risk for developing Parkinson's disease.
- Other health consequences of repeated methamphetamine exposure include cardiovascular problems, increased risk of stroke, oral diseases, premature aging, and increased mortality rate.

Methylphenidate, Modafinil, and Synthetic Cathinones

Methylphenidate

Methylphenidate (trade name **Ritalin**) has been mentioned a number of times previously in this chapter. Its chemical structure is distinct from that of either cocaine or amphetamine-type drugs (**FIGURE 12.19**). Methylphenidate was first synthesized in 1944 by the CIBA pharmaceutical company. By the late 1950s and early 1960s, the drug was being marketed clinically for several disorders, including "hyperkinetic syndrome" (now expanded and reconceptualized as ADHD), narcolepsy, depression, and extreme sedation/coma due to overdose with drugs such as barbiturates (Challman and Lipsky, 2000; CESAR, 2013). As discussed below, by far the major continued medical use of methylphenidate is in the treatment of ADHD.

The neurochemical actions of methylphenidate are well established. Once inside the brain, the drug binds to DAT on the membrane of dopaminergic neurons and to the norepinephrine transporter (NET) on the membrane

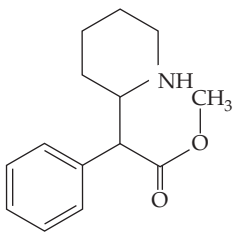


FIGURE 12.19 Chemical structure of methylphenidate

of the noradrenergic cells. The effect of this binding is to block reuptake of both DA and NE in their respective target areas, thereby elevating extracellular levels of both transmitters and stimulating overall catecholaminergic neurotransmission (Heal et al., 2009). Initial studies of methylphenidate binding to catecholamine transporters were performed using experimental animals; however, in vivo occupancy of both DAT (Volkow et al., 1998) and NET (Hannestad et al., 2010) has been demonstrated in the human brain using PET imaging.

Taken orally, methylphenidate has the subjective and behavioral profile of a typical psychostimulant. Laboratory studies of healthy, non-ADHD participants found dose-dependent increases in subjective arousal and alertness, perceived ability to concentrate, positive mood/drug liking, and (at higher doses) anxiety (Chait, 1994; Kollins et al., 2009). Linssen and colleagues (2014) reviewed the results of studies that specifically focused on methylphenidate's influence on cognitive function. The authors reported strong evidence for positive drug effects on working memory and cognitive processing speed. Some evidence was also found for beneficial effects on verbal learning and memory, and on vigilance and attention. Dose-response functions varied across domains, with some cognitive processes most strongly enhanced by low methylphenidate doses (defined as ≤10 mg) but other processes most strongly enhanced by medium doses (defined as >10 mg and ≤20 mg). Overall, no reliably measurable cognitive benefits for healthy participants were observed at high methylphenidate doses (defined as >20 mg). A much different approach was taken by German researchers who recently published a randomized, double-blind, placebo-controlled study on the ability of methylphenidate or modafinil to improve competitive performance by highly skilled chess players (Franke et al., 2017). Both compounds enhanced chess performance compared with placebo treatment. A subsequent commentary in the *Journal of the American Medical Association* cited evidence for use of such cognitive enhancers by several types of professionals, including physicians, business executives, and academicians (Lyon, 2017).

Probably the most widespread diversion of prescription stimulants for nonmedical use is by young people. One longitudinal survey of students enrolled at a large public university in the mid-Atlantic region found that over 60% of respondents had been offered a prescription stimulant at least once over a 4-year period (Garnier-Dykstra et al., 2012). Over 30% had used a prescription stimulant for nonmedical purposes. Most often, studying was offered as the motive for stimulant use. Other common motives were staying awake to party, getting "high," and, in the first year of school, simple curiosity. Furthermore, prescription stimulant use is not restricted to college students. A large national survey of high school seniors found that almost 10% reported having used prescription stimulants for nonmedical purposes at least once (McCabe and West, 2013). When healthy, non-ADHD students use stimulants, they feel more alert and can probably study for a longer time before becoming fatigued. But do these substances actually improve academic performance? The latest data answer "no." One recently published study found no significant influence of nonmedical prescription stimulant use on grade point average (GPA) among a subsample of the students who contributed to an earlier-cited 4-year longitudinal study (Arria et al., 2017). Another study published at the same time sampled students enrolled at six different public universities while focusing on individuals who had relatively poor academic performance related to low executive functioning. Even within this group, which might be expected to benefit most from stimulant use, no beneficial effect on GPA was observed (Munro et al., 2017). These findings do not support a conclusion that prescription stimulants like methylphenidate can help students improve their academic performance.

Methylphenidate has clear abuse potential due to its pharmacological profile of elevating brain dopaminergic transmission; however, the degree of risk depends greatly on the route of administration. There is little risk of abuse when the drug is taken orally at recommended doses. On the other hand, methylphenidate tablets that are formulated for immediate release can be crushed and taken either by intranasal insufflation (i.e., "snorting") or by IV injection. As we have seen previously for other compounds, these routes of administration produce much more rapid drug entry into the brain (especially IV injection), thereby causing a methylphenidate "high" similar to that produced by cocaine or amphetamine (Kollins et al., 2001; Bogle and Smith, 2009). When taken repeatedly by these methods, methylphenidate use can lead to dependence, just like other psychostimulants. Nevertheless, the drug's abuse liability appears to be lower than that of cocaine, amphetamine, or methamphetamine.

Finally, it is important to discuss the therapeutic applications of methylphenidate. The main features of ADHD and its treatment by methylphenidate and amphetamine are discussed in **BOX 12.2**. We also mentioned that methylphenidate is sometimes prescribed for patients with the sleep disorder narcolepsy; however, narcolepsy is more commonly treated with a different stimulant, modafinil, which is discussed in the next part of the chapter. Yet another possible therapeutic application of methylphenidate concerns using the drug to accelerate emergence from general anesthesia. Animal studies have demonstrated that emergence from anesthesia occurs more quickly after IV administration of methylphenidate (Solt et al., 2011; Chemali et al., 2012). Subsequent research found that

BOX 12.2 Clinical Applications

Psychostimulants and ADHD

The most important clinical use for psychostimulants is in the treatment of a developmental disorder known as attention deficit hyperactivity disorder (ADHD). Children with ADHD exhibit extreme degrees of inattentiveness, impulsivity, and hyperkinesis (excessive motor activity). They have a very short attention span and impulsively turn their attention to almost anything in the environment. The *Diagnostic and Statistical Manual of Mental Disorders* identifies three different subtypes of ADHD: a predominantly inattentive subtype, a predominantly hyperactive–impulsive subtype, and a combined subtype. In severe cases of the hyperactive–impulsive or combined subtype, there may be destructiveness, stealing, lying, fire setting, and sexual "acting out." The child is frequently unruly in the classroom and disruptive of family interactions within the home.

Based on recent statistics, ADHD occurs in roughly 5% to 7% of school age children around the world (Leung and Hon, 2016). The disorder is more prevalent in males than in females, but with differences in manifestation. Boys are more likely to exhibit hyperactivity and impulsivity, whereas girls are more likely to exhibit inattention. Although ADHD most often resolves with age, approximately 40% of children with this disorder will still be affected in adulthood. In general, adult patients with ADHD show signs of distractibility, impulsivity, restlessness, hyperemotionality, and problems both at work and in interpersonal relationships. Moreover, such individuals are at heightened risk for developing conduct disorder, antisocial personality disorder, and/or substance abuse problems.

The relevance of psychostimulants for the treatment of ADHD is that low doses of these drugs benefit about 75% to 80% of affected children. Most strikingly, psychostimulant administration has a seemingly paradoxical calming effect in hyperactive children. This phenomenon was first reported by Bradley in 1937 and has since been observed in many other studies. Many different stimulant medications are now available for the treatment of ADHD that differ not only in the active ingredient but also in the length of action (i.e., with short-acting, intermediate-acting, and long-acting effects). The active ingredient in most of these medications is D-amphetamine (e.g., Dexedrine), mixed amphetamine salts (e.g., Adderall), lisdexamfetamine (Vyvanse, which is a prodrug for D-amphetamine), or methylphenidate (Ritalin and other trade names) (Briars and Todd, 2016). The most important benefit of intermediate- and long-acting preparations is that a single dose given in the early morning will continue to benefit the child throughout the day without the need for additional dosing. However, a secondary benefit is that the delayed-release aspect of intermediate- and long-acting ADHD drugs makes them unsuitable for snorting or IV injection of crushed tablets. A recently developed methylphenidate skin patch (Daytrana) serves the same function as the long-acting oral medications and is particularly useful for children who find it difficult or may be unwilling to swallow pills.

A major advance in ADHD treatment occurred in 2002 with the introduction of atomoxetine (Strattera), the first nonstimulant medication for ADHD. Atomoxetine is a selective norepinephrine transporter inhibitor that is thought to work, at least in part, by enhancing the action of NE on α_{2A}-adrenergic receptors in the PFC (see below). More recently, extended-release formulations of the α_{2A}-receptor agonists clonidine (Kapvay) and guanfacine (Intuniv) have also been approved for the treatment of ADHD. These latter drugs may be used alone or in combination with a stimulant to achieve maximum reduction in symptomatology. It is important to note that although many children are treated with drugs alone, concomitant psychotherapy and parental counseling are often required for best results. Moreover, there is evidence supporting the efficacy of cognitive behavioral therapy, either with or without medication, in treating adults with ADHD (Knouse and Safren, 2010).

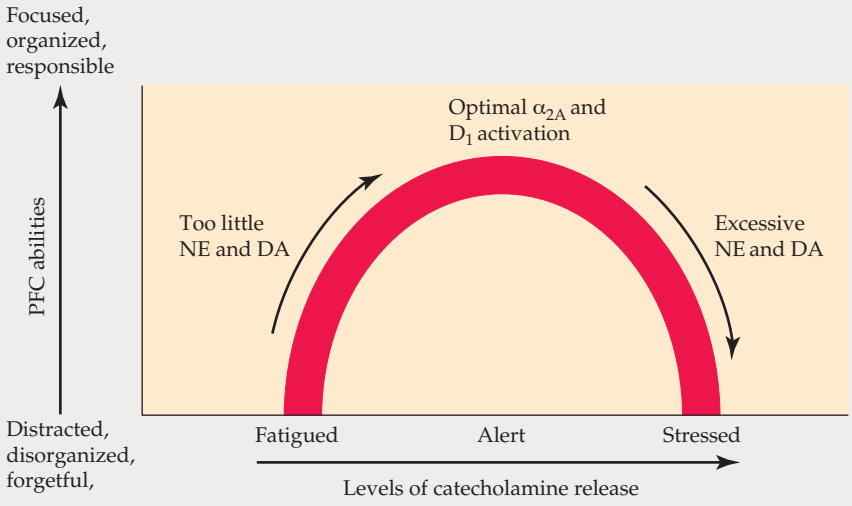
Certain safety concerns have been raised regarding the long-term use of stimulants to treat ADHD. One of the first concerns is the possibility that these compounds may result in reduced growth. Large-scale studies looking at average effects have found either small or no effects of long-term stimulant use on growth, although the possibility remains that some individual children could be adversely affected in this manner (Kaplan and Newcorn, 2011). Second, because of their sympathomimetic effects, stimulants cause small but measurable increases in heart rate and blood pressure. These compounds should, therefore, be avoided by any children or adults who have preexisting cardiovascular disease or are at increased risk of developing cardiovascular problems. Similarly, there is some concern (although not without controversy) that stimulants may exacerbate the occurrence of tics in patients with tic disorders. Clinicians concerned about this possibility have the option of prescribing the nonstimulant noradrenergic drugs (e.g., atomoxetine) for those patients. Finally, there is the issue of abuse potential of psychostimulants, as discussed in the text. Parents of medicated ADHD children need to be vigilant for signs of either

BOX 12.2 Clinical Applications (*continued*)

abuse by the child or distribution of the medication to siblings or friends for recreational use. No other significant adverse consequences have consistently been identified in humans undergoing long-term psychostimulant treatment for ADHD. On the other hand, animal model studies examining both behavioral and neurochemical effects of psychostimulants given repeatedly during the relevant developmental periods remind us that these compounds can exert powerful and widespread effects on the brain (Bock et al., 2010; Marco et al., 2011; Jaboinski et al., 2015), and therefore, they should not be administered unless there has been a thorough evaluation of the child to ascertain the risk–benefit ratio of starting drug treatment.

Understanding the symptomatology of ADHD and the mechanisms by which psychostimulants and noradrenergic agents can reduce this symptomatology requires a brief review of the neurobiology of ADHD. A large number of neuroimaging studies have identified multiple neural circuits that appear to be dysfunctional in patients with ADHD. Among these are (1) frontoparietal intracortical circuits important for attention and orienting responses; (2) dorsal frontostriatal circuits encompassing the dorsolateral PFC, dorsal striatum, and thalamus, which play a key role in inhibitory control; and (3) mesocorticolimbic circuits containing the orbitofrontal cortex, ventral striatum (including NAcc), and anterior hippocampus, which help mediate reward anticipation and motivation (Gallo and Posner, 2016). Given that all of these areas receive dopaminergic and/or noradrenergic innervation, the drugs used to treat ADHD undoubtedly have manifold effects across these circuits. Nevertheless, many researchers have focused on the PFC as potentially playing a critical role in ADHD pharmacotherapy. The pyramidal (i.e., output) neurons of the PFC are innervated by both the noradrenergic and dopaminergic systems. The key receptors involved are α_{2A} in the case of NE and D_1 in the case of DA. Experimental animal studies have suggested that the role of NE activation of α_{2A} receptors is to enhance the strength of relevant sensory input to the PFC (i.e., increase the "signal"), whereas the role of DA activation of D_1 receptors is to weaken irrelevant sensory input (i.e., decrease the "noise") (Berridge and Arnsten, 2015). These effects work together to increase the signal-to-noise ratio of sensory input to the PFC, thus helping the individual attend to the appropriate stimuli. Bringing this information to bear on the issue of ADHD, Arnsten and colleagues have argued (1) that PFC functioning is an inverted U-shaped function of the activity of both catecholaminergic systems, (2) that individuals with ADHD may have deficient catecholaminergic activity within the PFC, and (3) that a critical mode of action of compounds used to treat ADHD is to restore optimal catecholaminergic activity within this brain area (Berridge and Arnsten, 2013; 2015) (**Figure**). This hypothesis accounts for the therapeutic efficacy of catecholamine-releasing agents like amphetamine as well as dual DA and NE uptake inhibitors such as methylphenidate. Less clear is how the hypothesis explains the efficacy of the purely noradrenergic agents atomoxetine, guanfacine, and clonidine, assuming that elevating PFC dopaminergic activity is important for reversing ADHD-related cognitive deficits. At least for atomoxetine, there is additional information that helps us understand its therapeutic benefit in patients with ADHD. Unlike the dopaminergic fibers innervating the striatum, those innervating the PFC express very low levels of DAT (Sesack, 2014). In contrast, the noradrenergic fibers innervating the PFC express ample levels of NET. Because DA is a very effective substrate for NET, it turns

(*Continued*)



Hypothesized role of prefrontal cortical α_{2A} and D1 receptors in cognitive performance and the treatment of ADHD Activation of α_{2A} and D_1 receptors in the PFC is thought to depend on the organism's state of arousal. A moderate level of arousal leads to intermediate levels of receptor activation that are optimal for PFC functioning, whereas either too little or too much activation has detrimental effects on PFC functioning and cognitive performance. (After Arnsten, 2009 and Berridge et al., 2012.)

BOX 12.2 Clinical Applications (*continued*)

out that NET, not DAT, is the major source of DA uptake and clearance in the PFC. Thus, a selective NE uptake inhibitor like atomoxetine acts like methylphenidate in the PFC by increasing extracellular levels of *both* NE and DA in this brain area.

In summary, ADHD is characterized by several kinds of deficits in cognition and executive function, particularly an inability to concentrate, lack of impulse control, and hyperkinesis. These deficits are thought to be related to dysfunction in several complex neural circuits, especially circuits that include the PFC. Most patients with ADHD respond positively to psychostimulants (amphetamine and methylphenidate formulations) or to other agents that enhance catecholaminergic activity (atomoxetine, guanfacine, and clonidine). The therapeutic efficacy of these compounds is hypothesized to rely on activation of α_{2A} and D_1 receptors in the PFC, thereby alleviating a state of suboptimal catecholaminergic activity in this brain area.

this effect depends on an arousal-promoting effect of DA, since similar results were obtained by selective optogenetic activation of VTA dopaminergic neurons (N. E. Taylor et al., 2016) or by administration of a D_1 receptor agonist (N. E. Taylor et al., 2013). To our knowledge, methylphenidate has not yet been clinically tested for this purpose in patients undergoing a medical procedure requiring general anesthesia. But if such testing eventually occurs and no adverse side effects are observed, then this novel therapeutic application of methylphenidate could make its way into routine medical practice.

Modafinil

Modafinil (trade name **Provigil**) is an unusual stimulant, with respect to both its history and its mechanism of action. Like many of the compounds that were first developed to treat depression, anxiety, or psychotic disorders, modafinil was discovered serendipitously in the course of pharmaceutical development for other purposes (Rambert et al., 2006). In the 1970s, a small French pharmaceutical company named Laboratoire Louis Lafon was searching for novel nonsteroidal anti-inflammatory drugs. While screening a group of newly synthesized test compounds, one of the company's experimenters noticed that mice treated with one of these compounds became hyperactive. This compound, which was given the name adrafinil, not only caused increased locomotor activity but also antagonized barbiturate-induced sedation. Interestingly, despite its stimulant-like characteristics in some behavioral and physiological tests, adrafinil had a unique pharmacological profile that differed substantially from amphetamine (a classic psychostimulant) in many other such tests. The lead compound adrafinil was subsequently modified chemically to produce modafinil, which was more potent and longer acting than the parent drug.

The structure of modafinil is shown in **FIGURE 12.20**. Some similarity can be seen to the structure of methylphenidate, but with several key differences including a change in one of the two rings, a sulfur atom in the side chain, and a terminal amino group. The mechanism underlying modafinil's stimulant activity seems to be more complex than the mechanisms underlying the effects of cocaine, amphetamine, or methylphenidate. In vitro studies have shown that modafinil binds to DAT with relatively low affinity and acts as a weak DA uptake inhibitor (Loland et al., 2012). Brain imaging studies have further confirmed modafinil occupancy of DAT in the human brain and an ability of the drug to elevate extracellular DA (Volkow et al., 2009). The importance of these effects was further demonstrated by the observation that modafinil's stimulant properties are blocked in mice genetically engineered to lack DAT and in wild-type mice pretreated with either a D_1 or D_2 receptor antagonist (Wisor, 2013). Yet, even though DAT may be the most important direct molecular target of modafinil, some of the downstream effects of the drug's dopaminergic activity also seem to be important. Specifically, modafinil administration stimulates release of NE and orexin, both of which are integral components of the brain's arousal system (see Chapters 3 and 4), and it additionally inhibits GABA release (Ishizuka et al., 2012). The combined effects on orexin and GABA lead to increased release of histamine, another component of the waking/arousal neural circuitry (Chapter 3).

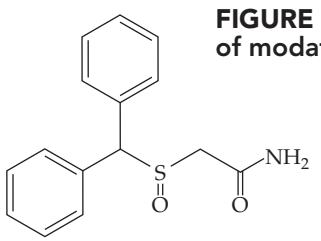


FIGURE 12.20 Chemical structure of modafinil

Together, these actions of modafinil are hypothesized to cause increased wakefulness as well as enhanced alertness and vigilance.

Because of its ability to increase wakefulness, modafinil is currently approved by the FDA for the treatment of excessive daytime sleepiness in patients with narcolepsy (see Box 3.1), people with obstructive sleep apnea,[4] and individuals with disordered sleep due to shift work (Valentino and Foldvary-Schaefer, 2007; Kumar, 2008). Like other psychostimulants, modafinil has also shown the ability to enhance cognitive functioning in healthy, non-sleep-deprived individuals (Minzenberg and Carter, 2008; Battleday and Brem, 2015). Numerous studies have found improvement in executive function, attention, learning, and episodic memory following administration of modafinil. As described for methylphenidate, these effects have been attributed to stimulation of DA and NE systems, including within the PFC. Despite its ability to increase dopaminergic activity, modafinil is generally thought to have relatively low abuse potential. Thus, modafinil has potential for helping to ameliorate cognitive deficits in patients with schizophrenia or other disorders involving cognitive dysfunction (Scoriels et al., 2013).

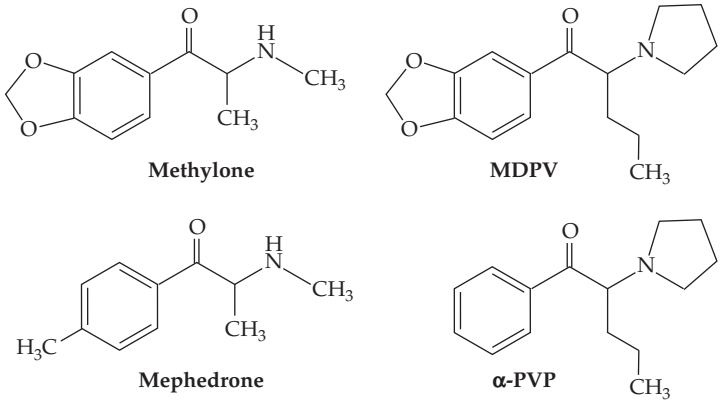


FIGURE 12.21 Chemical structures of some abused synthetic cathinones

Synthetic Cathinones

At the beginning of the section on amphetamines, we briefly mentioned and showed the structure of cathinone, a naturally occurring psychostimulant found in the khat plant of eastern Africa and Arabia (see Figure 12.13). Over the past 10 to 15 years, there has been a surge in Europe, the United States, and Asia in availability and recreational use of synthetic cathinone derivatives. Although over 30 such compounds have appeared on the street, we will focus here on the following four drugs that have received the most attention in the popular media and have been studied most extensively by researchers: **mephedrone** (4-methylmethcathinone), **methylone** (3,4-methylenedioxy-*N*-methylcathinone), **MDPV** (3,4-methylenedioxypyrovalerone), and **α-PVP** (α-pyrrolidinovalerophenone) (**FIGURE 12.21**). Mephedrone and methylone were the first synthetic cathinones to be sold widely for recreational use (Valente et al., 2014; Karila et al., 2015). Mephedrone came to the attention of European authorities in 2007, after which its dissemination and use grew rapidly. Common street names for mephedrone include "meow meow," "M-Cat," "Meph," "bounce," and "bubbles." Mephedrone was also sold in Israel under the name "Neodove." Methylone originated in the mid-2000s in the Netherlands and Japan under the name "Explosion." Other methylone-containing products include "Neocor" and "Room Odorizer." MDPV began to appear in 2009 under various product names such as "Vanilla Sky," "Ivory Wave," and "Energy-1." Most recently, use of α-PVP has surged in Europe and the United States, particularly in southern Florida (Katselou et al., 2016). Common street names for this compound are "flakka" and "gravel," the latter name arising from the similarity in appearance of α-PVP crystals to aquarium gravel (**FIGURE 12.22**).

Besides the brand or street names associated with individual synthetic cathinones, the general terms "bath salts," "plant food," "pond water cleaner," and "legal

FIGURE 12.22 Crystals of "flakka" (© iStock.com/bert_phantana.)

[4]Obstructive sleep apnea is a condition in which the airway closes partially or fully upon going to sleep. This causes the person to wake up, often without realizing it. In severe cases, several hundred brief awakenings may occur in the course of the night, thereby leading to sleep deprivation and extreme sleepiness during the daytime hours.

highs" have been used to sell this group of compounds in head shops and over the internet. As an additional protective measure, websites typically state that the materials for sale are "not for human consumption." Of course, neither buyers nor government authorities are fooled by these tactics. Interestingly, when synthetic cathinones first began to be taken recreationally, they *were* legal. But this changed rather quickly once the abuse and addiction liability of these compounds were recognized. Accordingly, mephedrone and related compounds were banned by the U.S. Drug Enforcement Administration (DEA) in September 2011, and they were banned even earlier by Britain, where the "legal high" problem has been particularly severe. In the remainder of this section, we will discuss the pharmacology and toxicology of the four major synthetic cathinones, while keeping in mind that new compounds continue to be synthesized in an ongoing race between underground chemists and the DEA.

Synthetic cathinones are taken by several routes of administration, the most common of which are nasal insufflation (snorting) and oral ingestion. Snorting within the synthetic cathinone user community is often done by "keying," which involves using a key that has been dipped into the powdered drug. Oral ingestion may involve consuming capsules of the drug or an alternative called "bombing," which entails wrapping drug powder or crystals inside a cigarette wrapper and then swallowing the wrapper. These drugs can also be taken by IV injection or by sublingual or rectal administration. Acute subjective and physiological effects of synthetic cathinones are similar to those of other psychostimulants discussed earlier. The subjective effects consist of euphoria, increased energy, alertness, disinhibition/impulsivity, talkativeness, and sexual stimulation (Dybdal-Hargreaves et al., 2013; Karila et al., 2016b). Acute physiological effects include tachycardia, hypertension, and hyperthermia. At relatively low doses, adverse reactions to synthetic cathinones are not usually problematic. However, high doses can lead to severe somatic toxic reactions including liver failure, kidney damage, rhabdomyolysis (skeletal muscle breakdown), and abnormal blood clotting within the systemic vasculature (German et al., 2014; Karila et al., 2015). Synthetic cathinone overdose has even been associated with a number of fatalities in countries where use is common (Busardò et al., 2015; deRoux and Dunn, 2016). Adverse psychiatric reactions range from acute anxiety to a state of excited delirium, with the most extreme cases involving psychotic reactions characterized by delusions, hallucinations, violent aggression, and self-injury in some cases. Mephedrone, MDPV, and α-PVP are more likely to produce severe psychiatric reactions than methylone.

In terms of their neurochemical mechanisms of action, synthetic cathinones fall into two categories that parallel the mechanisms of amphetamine and methamphetamine versus cocaine and methylphenidate (Baumann et al., 2014; German et al., 2014). Mephedrone and methylone are substrates for DAT, NET, and SERT. Consequently, these drugs act like amphetamine in their ability to release DA, NE, and 5-HT from their respective nerve terminals and to block the reuptake of these neurotransmitters. In contrast, MDPV and α-PVP are more like cocaine and methylphenidate in that they block monoamine transporters without leading to release. But unlike cocaine, MDPV and α-PVP are selective for DAT and NET and have relatively little influence on SERT. Moreover, these compounds are much more potent than cocaine, which contributes to the risk of toxic overdose. To summarize, all four synthetic cathinones elevate extracellular levels of DA and NE, with mephedrone and methylone additionally stimulating 5-HT release. The ability of these compounds to enhance dopaminergic activity and to produce a euphoric state all point to a risk for users to develop a condition of dependence. Not surprisingly, experimental animal studies examining various synthetic cathinones in tests of drug self-administration, place conditioning, and effects on brain electrical self-stimulation have demonstrated potent rewarding and reinforcing properties of this drug class (Baumann et al., 2014; Watterson and Olive, 2014). In accordance with these results, synthetic cathinone users report experiencing symptoms of dependence and withdrawal; however, empirical studies of cathinone dependence are still in their infancy.

High doses of methamphetamine or MDMA, especially when administered in a binge-like dosing regimen, can cause long-lasting reductions in biochemical markers for DA (methamphetamine; see earlier in this chapter) or 5-HT (MDMA; see Chapter 6) in rodents. Due to the structural similarities of synthetic cathinones with one or the other of these other compounds, researchers have begun to investigate whether the cathinones likewise exert toxic actions on the monoamine systems. As recently summarized by Angoa-Pérez and coworkers (2017), the results to date are variable and depend on the specific drug, the dosing regimen, and the species (rats versus mice). A few studies have reported regional decreases in dopaminergic and serotonergic markers in mephedrone-treated rats and mice, but other studies found no drug-related changes. Moreover, methylone administration to rats was found to cause widespread 5-HT depletion in one study but not another. No in vivo studies have found any evidence for monoamine neurotoxicity produced by MDPV; however, both MDPV and methylone were recently reported to cause a concentration-dependent apoptotic cell death

in an in vitro study using a human dopaminergic cell line (Valente et al., 2017). Taken together with the in vivo rodent studies, these findings indicate that certain synthetic cathinones have potential to cause neurotoxicity in either the dopaminergic or serotonergic system. Much more research in this area is clearly warranted, particularly as more and more young people become exposed to these substances over time.

Section Summary

- Methylphenidate is a prescription psychostimulant that activates catecholamine transmission by blocking DAT and NET, thereby increasing extracellular levels of DA and NE.
- Methylphenidate has typical psychostimulant subjective and behavioral effects, including increased arousal and alertness, perceived ability to concentrate, elevated mood, and (at higher doses) anxiety. Low to medium doses of methylphenidate exert a positive influence on various cognitive functions such as working memory, cognitive processing speed, verbal learning and memory, and vigilance and attention.
- Methylphenidate is frequently diverted for nonprescription use, particularly by students. Although users feel more alert and less fatigued, current evidence does not indicate that use of this drug enhances academic performance.
- Recreational use of methylphenidate can lead to abuse and dependence, particularly when the drug is taken by snorting or by IV injection.
- The primary clinical application of methylphenidate is for the treatment of ADHD. This disorder is characterized by extreme inattentiveness, impulsivity, and hyperkinesis. Other pharmacological treatments for ADHD include various amphetamine formulations, the NE reuptake inhibitor atomoxetine, and two different α_{2A}-receptor agonists.
- Individuals with ADHD are thought to have deficient catecholaminergic activity in multiple neural circuits that subserve cognitive functioning. One of the principal sites of action of ADHD medications may be the PFC, which is a key brain area participating in several of the abovementioned circuits. In this brain area, DA is taken up primarily by NET instead of DAT, which helps explain why catecholamine releasing agents (i.e., amphetamines), catecholamine reuptake inhibitors (methylphenidate), and a selective NE reuptake inhibitor (atomoxetine) share the ability to reduce ADHD symptomatology.
- Modafinil is an unusual psychostimulant that is often prescribed to treat daytime sleepiness associated with the sleep disorder narcolepsy, obstructive sleep apnea, and being employed as a shift worker.
- Modafinil is a weak DA reuptake inhibitor, which helps account for its stimulant properties. However, there are several downstream effects of the drug that are also thought to be important, including increased release of NE, orexin, and histamine, and an inhibition of GABA release.
- In recent years, a variety of synthetic cathinone derivatives have become available for recreational use under names such as "bath salts," "plant food," "meow meow," and "flakka." Four such compounds are mephedrone, methylone, MDPV, and α-PVP.
- Synthetic cathinones are most commonly taken by snorting or oral ingestion, although other routes of administration such as IV injection have been reported.
- Acute subjective and physiological effects of low to moderate doses are like those of other psychostimulants. However, higher doses can produce severe adverse reactions all the way up to organ failure, psychotic episodes, and death.
- Mechanistically, mephedrone and methylone are similar to amphetamine in that they are substrates for DAT, NET, and SERT, thereby causing acute release of DA, NE, and 5-HT. In contrast, MDPV and α-PVP are selective reuptake inhibitors of DA and NE, which is similar to the neurochemical action of methylphenidate.
- Animal studies have shown that synthetic cathinones are highly rewarding and reinforcing, which suggests that these compounds have the potential to produce dependence in regular users. Other studies indicate that certain synthetic cathinones could produce neurotoxic deficits in the dopaminergic or serotonergic systems, depending on the conditions of exposure. More research is needed to define the conditions for such neurotoxic effects in animal and in cell culture models and to determine whether these effects may be occurring in human cathinone users.

STUDY QUESTIONS

1. What are the defining characteristics of psychomotor stimulant drugs in terms of the behavioral and psychological effects they exert?
2. Describe the typical routes of administration of cocaine, the time course of effects produced by these different routes, and the metabolism of cocaine.
3. What are the primary molecular targets of cocaine in the brain? What are the actions of cocaine on these targets, and what are the resulting effects on synaptic transmission?
4. Acute administration of cocaine causes an increased frequency of transient DA release events. Describe the hypothesized mechanism for this effect.
5. What is the mechanism of action of cocaine-based local anesthetic drugs such as Novocaine?
6. Compare and contrast the effects of cocaine on mood and behavior when taken occasionally at low to moderate doses versus when taken chronically and/or at high doses.
7. Discuss the role of the sympathetic nervous system in mediating cocaine's physiological effects.
8. Discuss the varying lines of evidence (in animal studies) for a role of DA, especially in the nucleus accumbens and dorsal striatum, in the behaviorally activating and rewarding/reinforcing effects of cocaine and other psychostimulants such as amphetamine.
9. Describe results from brain imaging studies that have implicated DA in the mechanism of psychostimulant action in humans.
10. Discuss evidence from pharmacological and genetic engineering studies aimed at determining the involvement of different dopaminergic receptor subtypes in the behavioral effects of cocaine.
11. (a) Describe the observed patterns of cocaine use that most commonly lead to a progression from occasional use to cocaine abuse and dependence. (b) Describe the phenomenon of a cocaine binge and the phases of the abstinence syndrome that have been observed following a typical binge.
12. What is the role of craving in the development of cocaine dependence, and what is known about the neurobiology of cocaine craving?
13. Discuss the processes of cocaine tolerance and sensitization, including the relevant changes in drug sensitivity, patterns of drug administration that can produce tolerance versus sensitization, and current information on the neurochemical mechanisms that contribute to these processes.
14. Describe the results of structural brain MRI studies of cocaine-dependent individuals. Describe the possible relationship between changes in the brain discovered by these studies and the cognitive differences between cocaine-dependent and non-cocaine-using individuals.
15. What are the major health consequences (other than neurobiological) of chronic or high-dose cocaine use.
16. Discuss the current treatment approaches for cocaine dependence. Include a consideration of pharmacological approaches that have been used, thus far unsuccessfully, to deal with this disorder.
17. Web Box 12.1 asks the question "Is a sweetened water solution more reinforcing than cocaine?" How has this question been addressed experimentally, and what is the answer? How do the results of this line of research bear on the broader questions of how cocaine dependence occurs and how it should be treated?
18. The structures of amphetamine, methamphetamine, cathinone, and ephedrine are related to a particular neurotransmitter. What is that transmitter?
19. Describe the typical routes of administration of amphetamine and methamphetamine, and describe the characteristics of a "speed run."
20. How do the mechanisms of action of amphetamine and methamphetamine differ from that of cocaine?
21. Discuss the adverse consequences of chronic and/or high-dose use of amphetamines, including dependence and withdrawal, effects on cognitive function, psychotic reactions, potential neurotoxic effects, and consequences for general physical health.
22. Describe the neurochemical mechanisms of action of methylphenidate.
23. Discuss the characteristics of ADHD, the various kinds of drugs used to treat this disorder,

STUDY QUESTIONS (*continued*)

and the proposed mechanism of action of these drugs in alleviating ADHD symptomatology.

24. What is modafinil? Describe the neurochemical actions of this compound that are thought to be involved in its therapeutic efficacy in treating narcolepsy.

25. (a) List the names of the four synthetic abused cathinones mentioned in the text. (b) Discuss how these four compounds can be placed into two different categories based on their neurochemical mechanisms of action. (c) Discuss evidence for synthetic cathinone–related neurotoxicity.

Go to the *Psychopharmacology* Companion Website at oup-arc.com/access/meyer-3e for animations, web boxes, flashcards, and other study aids.

CHAPTER 13

Vape shops specialize in offering a variety of e-cigarette brands and flavored nicotine-containing solutions. (Matt Mawson/Corbis Documentary/Getty Images.)

Nicotine and Caffeine

V2, VAPORFI, MIGVAPOR, SOUTH BEACH SMOKE, JUUL VAPOR, HALO CIGS, EPUFFER, AND NJOY. What do all these terms have in common? As you may already know or have guessed, they are all names of popular brands of e-cigarettes. Use of e-cigarettes has skyrocketed in recent years. At the same time, a huge controversy has arisen among both researchers and public health officials about whether e-cigarette use should be encouraged as a safer alternative to cigarette smoking (and a novel approach to smoking cessation) or discouraged because of its potential as a path to nicotine addiction among young people.

Nicotine, the psychoactive ingredient in tobacco, e-cigarettes, and other electronic nicotine delivery systems (ENDS) such as vape pens and e-hookahs, is the third most widely consumed recreational drug in the world after caffeine and alcohol. Every day, millions of men and women around the world consume this drug, usually by smoking tobacco in cigarettes, cigars, or hookahs or by inhaling nicotine through various ENDS. What is the lure of nicotine? Why do so many people smoke, despite the known dangers of lung cancer and other respiratory diseases? The first part of this chapter will address these and other questions about nicotine and smoking or vaping. We will discuss the controversy over the benefits versus the harms of e-cigarette use. The second part of the chapter is concerned with the properties and mechanisms of action of caffeine, the other widely used and legal stimulant drug. ■

Nicotine

Background and History

Nicotine is an alkaloid found in tobacco leaves (**FIGURE 13.1**). There are two major species of tobacco plant: a large-leaf form and a small-leaf form. The large-leaf variety (*Nicotiana tabacum*), which is the principal source of present-day tobacco, originated in South America, where it was domesticated by native peoples more than 5000 years ago (**FIGURE 13.2**). The small-leaf variety (*Nicotiana rustica*) is native to eastern North America and the islands of the West Indies. Tobacco and nicotine were unknown to Europeans until Columbus's expedition to the New World in 1492. There his sailors discovered tobacco smoking by the native peoples and brought samples of *Nicotiana rustica* back on their return voyage (Burns, 2007). An early proponent of tobacco was Jean Nicot de Villemain, the French ambassador to Portugal who was instrumental in introducing the plant to his native country from Portugal. Indeed, the botanical name of the more popular tobacco plant, *Nicotiana tabacum*, is derived from both Nicot's surname and *tabaco*, the term for "tobacco" in both Spanish and Portuguese.

In Britain, tobacco was initially scarce and thus costly. Early pipes, called "fairy pipes" in England and "elfin pipes" in Scotland, were extremely small to conserve the dried leaves. But the popularity of smoking grew so rapidly that by the early 1600s, it is estimated, there were thousands of shops in London alone where tobacco could be purchased. At first, most tobacco use took place through pipe smoking, cigar smoking, and chewing, but this was later supplanted to a large extent by the snorting of finely powdered tobacco leaves called snuff.

In 1610, England attempted to commercialize tobacco growing in the Virginia colony using the native *Nicotiana rustica*. However, this venture failed because the *N. rustica* species had a disagreeable flavor to Europeans compared with *N. tabacum*, which had been a Spanish monopoly up to that time. Luckily for the British, John Rolfe, the local leader of this effort, managed to obtain some *N. tabacum*, which grew just as well in Virginia as it had for thousands of years in South America. Thus was the American tobacco industry born.

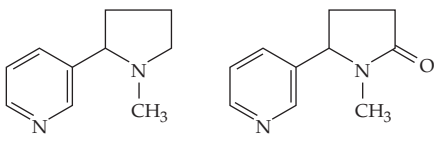


FIGURE 13.1 Chemical structure of nicotine and the principal metabolite cotinine

FIGURE 13.2 Leaves of the *Nicotiana tabacum* plant from which tobacco is derived (Courtesy of David McIntyre.)

Cigarettes began to be used in Europe in the mid-nineteenth century, and their popularity in the United States exploded over the next 30 years. This change was fostered by two separate developments: new methods of curing tobacco leaves that improved their flavor, and the invention of the cigarette machine. When cigarettes were rolled manually, a skilled worker could make about 2500 to 3000 cigarettes per day, which may seem like a large number. In 1884, however, a cigarette machine was built that could make 120,000 cigarettes in one day, and modern machines can produce 4000 to 8000 per minute!

A Chinese pharmacist named Hon Lik is considered to be the inventor of the modern e-cigarette (Demick, 2009). Hon was motivated by the death of his father, a heavy cigarette smoker, from lung cancer. His invention, which was patented in 2003, heated and vaporized a solution of nicotine, thereby resulting in an aerosol that is inhaled by the user. This vaporization step, which is common to all ENDS, is the source of the street term "vaping." From its start as a tiny industry, the e-cigarette has grown enormously in popularity, with global sales recently exceeding $6 billion (Davidson, 2015) and estimated to reach $50 billion by 2025 (PRN Newswire, 2015). Based on the annual National Youth Tobacco Survey, much of this rise in e-cigarette popularity is attributable to young people, as e-cigarette use by high school students surpassed the use of tobacco cigarettes in 2014 (**FIGURE 13.3**; Jamal et al., 2017). Early e-cigarettes delivered relatively small amounts of nicotine, but this has changed with the advent of newer designs that allow much greater drug delivery. In response to the growing use of e-cigarettes, the U.S. Food and Drug Administration (FDA) in 2016 extended its regulatory authority over tobacco products to include e-cigarettes and other ENDS (Food and Drug Administration, 2016).

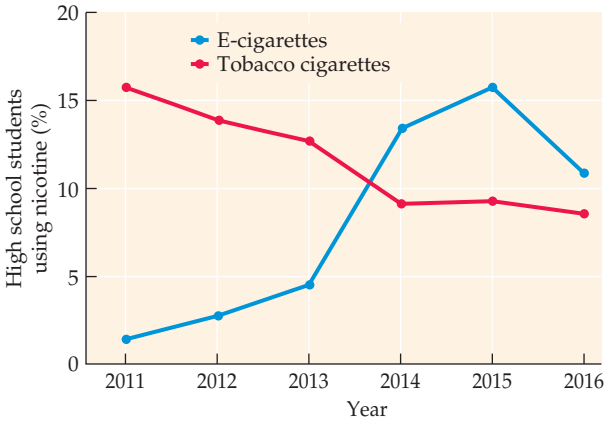


FIGURE 13.3 Yearly changes from 2011 to 2016 in percentage of high school students using e-cigarettes compared with tobacco cigarettes
Current usage is defined as one or more uses within the past 30 days. Data are from the National Youth Tobacco Survey. (After Jamal et al., 2017.)

Basic Pharmacology of Nicotine and Its Relationship to Smoking

Features of tobacco smoking and nicotine pharmacokinetics

When nicotine was first isolated in 1828 by Wilhelm Posselt and Karl Reimann, it was found to constitute about 5% of the weight of dry tobacco leaves. However, this relatively minor fraction imbues tobacco with many physiological and psychological effects when the leaves are smoked, chewed, or snorted and the nicotine is absorbed into the human bloodstream. Without nicotine, it is quite likely that tobacco would be regarded as a useless weed.

The typical tobacco cigarette contains between 6 and 11 mg of nicotine, although no more than 1 to 3 mg actually reaches the bloodstream of the smoker. The amount of available nicotine depends mainly on features of the smoker's behavior such as the number of puffs and the length of each puff. Nicotine in the tobacco is vaporized by the 800°C temperature at the burning tip of the cigarette. It enters the smoker's lungs mainly on tiny particles called **tar**, a complex mixture of hydrocarbons of which some are known to be carcinogenic. Tar is an important contributor to the taste and smell of cigarette smoke, and along with nicotine, these sensory qualities contribute significantly to the reinforcing effects of smoking. Once the smoke has been inhaled, the nicotine readily passes through the absorbent surface of the lungs, whose total area has been estimated as about equal to the surface of a tennis court. Nicotine is absorbed to a lesser extent through the membranes of the mouth and nostrils when tobacco is chewed or snorted as snuff.

A typical smoker takes about 10 to 15 total puffs on a cigarette at intervals of approximately 30 to 60 seconds. Researchers have shown that each puff delivers nicotine to the brain beginning within about 7 seconds, which is roughly twice as fast as when the drug is administered intravenously. Thus smoking a cigarette is the quickest and most efficient method of delivering nicotine to the brain, where the drug produces its reinforcing effects (see the section on behavioral and physiological effects later in the chapter). For many years, the accepted lore was that each individual puff produced a large "spike" of nicotine in the brain; however, brain imaging using positron emission tomography (PET) suggests that nicotine rapidly accumulates in the brain starting with the first puff (Rose et al., 2010). Hence, each subsequent puff causes just a small increase in brain nicotine levels that is superimposed on the previously existing level.

Features of e-cigarette vaping and nicotine pharmacokinetics

Recent studies have determined the amount of nicotine available in various e-cigarette brands, the parameters of vaping[1] behavior in e-cigarette users, and the levels of circulating nicotine produced in these users. This research enables us to compare and contrast vaping e-cigarettes versus smoking conventional tobacco cigarettes.

A typical e-cigarette consists of a plastic cartridge containing a solution of nicotine dissolved in propylene glycol or glycerin, a battery-powered heating element/atomizer that vaporizes the nicotine so it can be inhaled, a microprocessor control system with a sensor that activates the heating element when the unit is puffed, and a light-emitting diode (LED) that simulates the glow of a burning cigarette tip (**FIGURE 13.4**). A survey performed in early 2014 recorded over 400 available brands of e-cigarettes and over 7000 unique flavors (Zhu et al., 2014). Available flavorings fall into many categories such as tobacco flavors, menthol flavors, fruit flavors, dessert flavors, coffee flavors, and other beverage flavors. Nicotine content varies across a wide range. For example, you can vape an e-cigarette with no nicotine just for the purpose of experiencing the taste and other sensory qualities of using the device. The stated nicotine concentrations in nicotine-containing e-cigarettes vary from low (typically 3–6 mg/ml), to medium (9–12 mg/ml), to high (16–24 mg/ml) levels. However, laboratory measurements have demonstrated that the actual nicotine content is frequently lower than the content stated by the manufacturer (Goniewicz et al., 2013; Lisko et al., 2015). As in the case of tobacco cigarettes, only a portion of the nicotine in the

[1]We will use the term *vaping* instead of *smoking* when referring to inhalation of vapor from e-cigarettes or other ENDS.

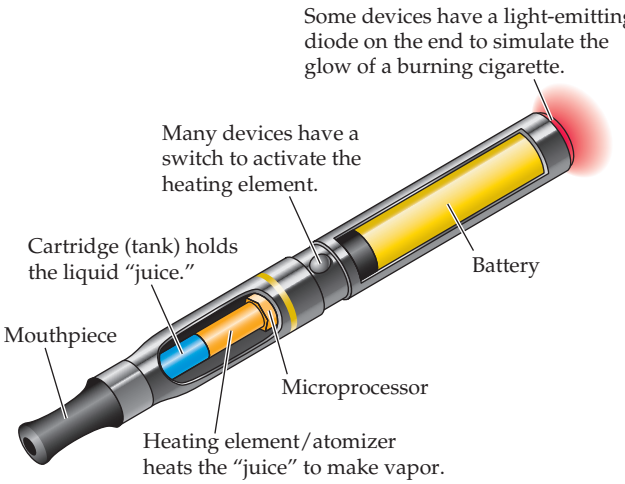


FIGURE 13.4 Components of a typical e-cigarette (After U.S. Federal Emergency Management Agency, 2017.)

e-cigarette is inhaled by the user, and the amount of nicotine ingested and its subjective effects are self-titrated by experienced users to obtain the optimum subjective effects. Indeed, the peak plasma nicotine levels produced by vaping vary substantially as a function not only of the nicotine concentration in the cartridge, but also of the rate of puffing and puff duration (Dawkins and Corcoran, 2014; Dawkins et al., 2016). Importantly, the rapidity of e-cigarette nicotine delivery through the lungs is similar to that from tobacco cigarettes, thereby providing the same opportunity for rapid nicotine-mediated reinforcement (St. Helen et al., 2016).

Nicotine metabolism

About 70% to 80% of nicotine in the body is transformed into the principal metabolite, **cotinine** (see Figure 13.1), by a specific liver enzyme known as **cytochrome P450 2A6** (**CYP2A6**). Cotinine and other nicotine metabolites such as nicotine-*N*-oxide, nornicotine, and norcotinine are excreted mainly in the urine. Because of genetic variation, some individuals have low CYP2A6 activity and thus reduced nicotine metabolism. Slow metabolizers of nicotine smoke fewer cigarettes, are less likely to become dependent on smoking, and have greater success quitting smoking than people with moderate or high rates of nicotine metabolism (Tanner et al., 2015; Zdanowicz and Adams, 2014). Thus, a slow rate of nicotine breakdown exerts a somewhat protective effect against cigarette smoking.

The elimination half-life of nicotine is typically about 2 hours, although this value can vary with individual differences in metabolism rate. Continuous removal of circulating nicotine means that people who have become dependent must smoke or vape repeatedly over the day to avoid withdrawal symptoms due to falling blood nicotine levels. Such withdrawal symptoms are discussed later in the chapter. Frequent smoking leads to ever-increasing peak levels of nicotine across the day, since each dose builds on the residual nicotine left over from that day's previous cigarettes. However, this does not cause greater and greater effects, because tolerance also develops over the same time period. Mild nicotine withdrawal emerges during the overnight period while the smoker is sleeping, yet at the same time the nicotine tolerance built up over the previous day partially dissipates. Because of these two processes, the dependent individual awakens the next morning with a substantial craving for a cigarette but also may experience the strongest or best response that she will have all day.

Mechanisms of Action

Nicotine works mainly by activating **nicotinic acetylcholine receptors** (**nAChRs**), one of the two basic subtypes of acetylcholine (ACh) receptor (see Chapter 7). You will recall that nAChRs are ionotropic receptors that conduct Na^+ ions across the cell membrane and produce a rapid excitatory response by the postsynaptic cell. Each nAChR is composed of five separate protein subunits, the composition of which differs between neurons and muscle cells and also varies across different brain areas. One of the functional consequences of this receptor diversity is differential sensitivity to receptor agonists and antagonists. For example, neuronal receptors containing two $\alpha 4$ or $\alpha 3$ subunits along with three β subunits (typically $\beta 2$) are much more sensitive to nicotine than those composed of five $\alpha 7$ subunits because of their higher affinity for the compound (Albuquerque et al., 2009). These high-affinity nAChRs are found in many parts of the brain, including the cerebral cortex, thalamus, striatum, hippocampus, and monoamine-containing nuclei such as the substantia nigra, ventral tegmental area (VTA), locus coeruleus, and raphe nuclei. Peripherally, such receptors are found in the ganglia of the autonomic (parasympathetic and sympathetic) nervous system. Some nAChRs, particularly those containing $\alpha 7$ subunits, are located presynaptically on nerve terminals where they enhance neurotransmitter release. Thus, a secondary consequence of nicotine exposure is glutamate-mediated neuronal excitation due to increased release from glutamatergic neurons (Dani, 2015).

High-affinity nAChRs rapidly desensitize in response to agonist exposure. Because of this effect, nicotine can paradoxically decrease the effectiveness of normal cholinergic transmission (Dani, 2015). Very high doses of nicotine lead to a persistent activation of nAChRs and a continuous depolarization of the postsynaptic cell. As we saw in Chapter 7, this causes a depolarization block, and the cell cannot fire again until

the nicotine is removed. In this way, a high dose of nicotine exerts a biphasic effect that begins with stimulation of nicotinic cholinergic functions but then turns to a nicotinic receptor blockade. This biphasic action accounts for the features of nicotine poisoning discussed later.

Section Summary

- Nicotine is an alkaloid found in tobacco leaves. Tobacco plants are native to North and South America, and these plants were domesticated several thousand years ago by Native Americans.
- When tobacco was first brought back to Europe from the New World, use of this substance was primarily by means of pipe smoking, cigar smoking, and chewing. Snorting finely powdered tobacco leaves (snuff) later became popular.
- Tobacco cigarettes were first introduced in the mid-nineteenth century, and cigarette smoking subsequently increased as the result of improved methods of curing the tobacco leaves, as well as the advent of modern cigarette manufacturing machines.
- The contemporary e-cigarette was introduced in 2003 by a Chinese pharmacist. Since that time, e-cigarettes and other electronic nicotine delivery systems (ENDS) have greatly increased in popularity.
- A typical tobacco cigarette contains 6 to 11 mg of nicotine, of which only about 1 to 3 mg actually reaches the smoker's bloodstream. Nicotine is vaporized by the high temperature at the tip of the burning cigarette and enters the smoker's lungs on tiny particles called tar.
- Once in the lungs, the nicotine is readily absorbed into the blood and quickly reaches the brain. The rapid delivery of nicotine to the brain is believed to be a powerful reinforcer of smoking behavior.
- A typical e-cigarette consists of a plastic cartridge containing nicotine dissolved in propylene glycol or glycerin, a battery-powered heating element, a microprocessor control system, and an LED that simulates the glow of a burning cigarette tip. Flavorings are often added to the nicotine solution to enhance its taste. Manufacturers' stated nicotine concentrations vary from low (3–6 mg/ml), to medium (9–12 mg/ml), to high (16–24 mg/ml) levels, although the actual nicotine concentration is often lower than the stated concentration. The rapidity of nicotine delivery from an e-cigarette is similar to that from tobacco cigarettes.
- Nicotine is metabolized primarily to cotinine by the liver enzyme CYP2A6. The cotinine and other nicotine metabolites are then excreted mainly in the urine. People who metabolize nicotine inefficiently because of genetically determined low CYP2A6 activity seem to be less vulnerable to cigarette smoking than efficient metabolizers.
- The elimination half-life of nicotine is typically about 2 hours. Nicotine clearance from the body is an important reason why most smokers and vapers smoke or vape throughout the day. Tolerance to at least some of nicotine's effects occurs during this period, but during sleep this tolerance partially dissipates and the smoker awakens in a state of mild withdrawal.
- The principal mechanism of nicotine action is to stimulate nAChRs in the brain and the autonomic nervous system. In particular, there are high-affinity nAChRs that most commonly are composed of two $\alpha 4$ or $\alpha 3$ subunits, along with three $\beta 2$ subunits. Some nicotinic receptors, typically composed of $\alpha 7$ subunits, are located presynaptically on nerve terminals where they function to enhance the release of neurotransmitters such as glutamate.
- The opening of nAChR channels permits Na^+ to flow across the cell membrane, thereby causing membrane depolarization and a fast excitatory response. High-affinity nAChRs desensitize rapidly in the presence of nicotine, thereby leading to reduced transmission by ACh. Very high doses of nicotine can cause persistent activation of nAChRs, leading to a temporary depolarization block of the postsynaptic cell.

Behavioral and Physiological Effects

If one wishes to determine the pharmacological effects of nicotine itself, separated from the complex behavioral aspects of smoking or vaping, it is necessary to give study participants the pure drug. This is routinely accomplished through the use of nicotine injections, nicotine patches, or nicotine-containing gum. Nevertheless, many early studies suffer from a significant methodological problem due to the use of current smokers, who are required to refrain from smoking for a specified period of time, typically ranging from 8 to 24 hours. Because nicotine abstinence produces withdrawal symptoms in dependent individuals, it is often difficult to determine whether nicotine-induced changes represent true differences from "normal," or simply reversal of withdrawal symptoms. Fortunately, researchers subsequently began to study participants who had never smoked, thereby permitting us to compare the findings of those studies with the results from abstinent smokers.

Nicotine elicits different mood changes in smokers compared with nonsmokers

With respect to mood states, nicotine is usually found to increase calmness and relaxation in recently abstinent smokers. This fits well with numerous self-reports of smokers indicating that smoking a cigarette has a relaxing, tension-reducing effect. However, it seems likely that these mood changes are related at least partly to relief from nicotine withdrawal symptoms, because nicotine administration to nonsmokers tends to elicit feelings of heightened tension or arousal, along with lightheadedness, dizziness, and even nausea (Kalman, 2002). If you either smoke now or have ever smoked in the past, you may recall having experienced some of these same effects when you tried your first cigarette.

Not all the mood-altering effects of smoking are related to nicotine intake. For example, Perkins and coworkers (2010) examined the influence of smoking either nicotine-containing or denicotinized cigarettes on negative affect induced by several different mood induction procedures, namely, (1) overnight abstinence from tobacco, (2) performance on a difficult computer task, (3) viewing of highly arousing negative mood slides, and (4) preparation of a speech for public presentation. The investigators found that smoking strongly reduced negative affect in the abstinence condition, with additional but smaller effects seen in the computer challenge and negative mood slide conditions. Interestingly, there was little difference between nicotine-containing and denicotinized cigarettes in any of these conditions (**FIGURE 13.5**). These findings suggest that in addition to nicotine, conditioned stimuli associated with smoking play a significant role in the calming effects of this behavior in regular smokers.

Nicotine enhances cognitive function

We saw in Chapter 7 that ACh plays an important role in certain aspects of cognitive functioning, including memory and attention. In this section we focus on the specific involvement of nicotinic receptors in cognition and studies demonstrating an ability of nicotine to enhance cognitive performance.

Abstinent smokers given nicotine show enhanced performance on many kinds of cognitive and motor tasks, particularly those involving attentional demands. However, as in the case of mood effects, much of this enhancement appears to be due to alleviation of withdrawal-related deficits (Sherwood, 1993). On the other hand, a meta-analysis[2] by Heishman and coworkers (2010) covering a wide range of studies supported the conclusion that nicotine has a positive influence on cognitive and motor functioning even in nonsmokers. Statistically significant effects of nicotine were observed for fine motor performance, and on accuracy and response latency in certain types of attentional and memory tasks. A recent review by Hahn (2015) likewise concluded that nicotine and other nicotinic agonists facilitate performance on attentional tasks.

Tobacco smoking is common in people with cognitive disorders such as attention deficit hyperactivity disorder (ADHD) (McClernon and Kollins, 2008) and

[2]Meta-analysis is a statistical tool that enables investigators to combine the results of many studies concerning the same experimental treatment, thereby increasing statistical power and determining with greater confidence whether the effect of the treatment in question is statistically reliable.

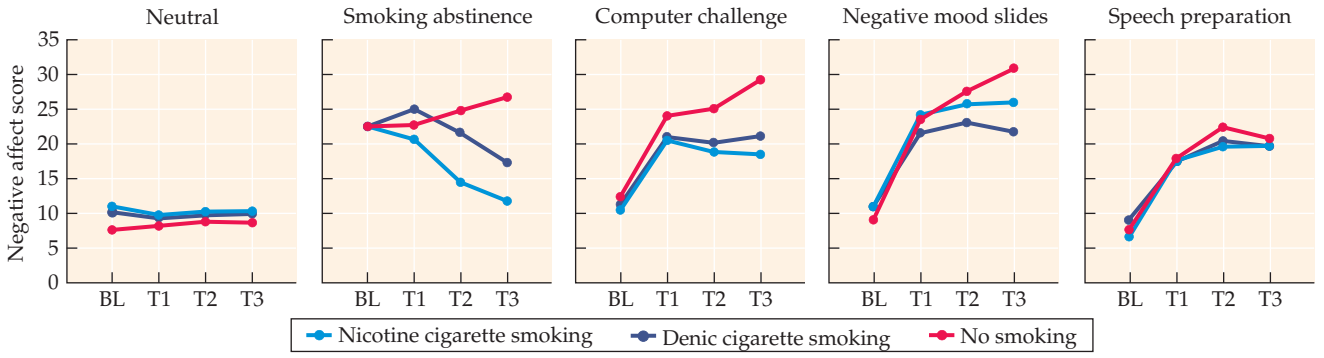


FIGURE 13.5 Influence of mood induction procedure and smoking condition on negative affect The study participants were regular adult smokers (10 cigarettes or more per day) tested at four time points on the negative affect scale of the Diener and Emmons mood form: baseline (BL), before mood induction; T1, after 5 minutes of the mood induction procedure and before cigarette smoking; T2, after four cigarette puffs and 3 more minutes of mood induction; and T3, after another 10 minutes of mood induction during which ad lib smoking was allowed in the smoking groups. The smoking abstinence mood induction procedure involved overnight abstinence (more than 12 hours) from smoking. The three cigarette conditions included smoking a standardized nicotine-containing cigarette, smoking a denicotinized (Denic) cigarette, and no smoking at all during the session. The no-smoking group showed significantly greater negative affect than either smoking group at T3 in the abstinence, computer challenge, and negative mood slide conditions. (After Perkins et al., 2010.)

schizophrenia (D'Souza and Markou, 2012). This finding has given rise to the theory that patients may, to some extent, be attempting to medicate their cognitive impairment with nicotine (Evans and Drobes, 2008). Poltavski and Petros (2006) tested the hypothesis that nicotine would have a greater influence on attention in people with poor baseline levels of attention than in people who had higher baseline attentional performance. In this study, nonsmoking college students were prescreened for baseline levels of attention, after which they were divided into a low-attention and a high-attention group. Both groups were then subjected to the Conners' Continuous Performance Test (CPT), a well-known test of sustained attention that is sometimes used to diagnose ADHD. In this test, the participants were instructed to respond as rapidly as possible by pressing a specific key on a computer keyboard when a target stimulus (the letter *X*) was briefly displayed on a computer monitor, but they were to withhold their response when any other letter was displayed. Before CPT testing, approximately half of the participants in each group were given a transdermal nicotine patch containing 7 mg of the drug; the other members of the group received a placebo patch. The results supported the authors' hypothesis by showing that nicotine treatment significantly increased the d' score (a measure of stimulus detectability) in the low-attention but not the high-attention group (**FIGURE 13.6**). Indeed, stimulus detectability in the nicotine-treated low-attention group was just as high as detectability in both of the high-attention groups.

Animal studies have also been useful in determining the cognitive effects of nicotine, since such studies obviously do not suffer from the interfering influence of prior smoking. Rats and mice given nicotine show improvement on a variety of different tasks, including tasks requiring sustained attention as well as working memory (Newhouse et al., 2004; Hahn, 2015). Both high- and low-affinity nAChRs are expressed in the hippocampus, and hippocampal-dependent tasks such as contextual fear conditioning and spatial learning are sensitive to nicotine and other nAChR agonists (Kutlu and Gould, 2015). Of note, whereas acute or chronic nicotine administration may enhance performance on many tasks, animals show performance deficits when withdrawing from chronic nicotine treatment.

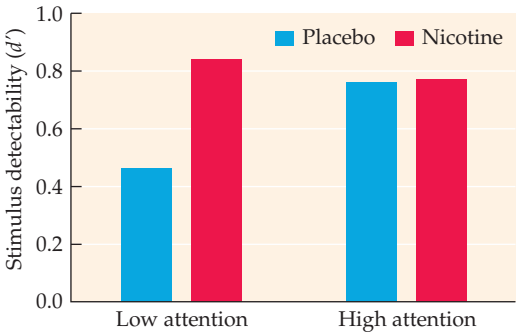


FIGURE 13.6 Nicotine enhancement of stimulus detectability in low-attention study participants
After screening for baseline attentional performance, participants were tested for their ability to detect and correctly identify a brief visual stimulus displayed on a computer monitor. Those who received a transdermal nicotine patch (7 mg) prior to testing showed enhanced stimulus detectability if they were in the low-baseline-attention but not in the high-baseline-attention group. (After Poltavski and Petros, 2006.)

The diversity of nAChR subunit combinations and their differential expression across the brain has challenged investigators trying to determine which subunit combinations mediate nicotine-induced cognitive enhancement. Experiments addressing this issue have relied on administering nAChR agonists or antagonists that show selectivity for particular receptor subunit-containing receptors and/or have used genetic engineering to knock out or otherwise mutate a specific subunit. For example, a typical pharmacological approach would involve administering nicotine to animals pretreated either with **dihydro-beta-erythroidine** (**DhβE**), an antagonist of high-affinity nAChRs (e.g, receptors containing α4 and β2 subunits), or **methyllycaconitine** (**MLA**), which blocks low-affinity nAChRs consisting only of α7 subunits. Taken together, the pharmacological and genetic studies generally suggest that high-affinity nAChRs play a more important (though not exclusive) role than low-affinity nAChRs in mediating nicotine's enhancement of hippocampal-dependent tasks (Kutlu and Gould, 2015).

Finally, it's important to recognize that nicotine affects cognition by acting through a neural circuit that includes cells using neurotransmitters other than ACh. Identification of at least some of these additional transmitters has been achieved by determining whether nicotine enhancement of task performance can be reversed by blocking noncholinergic receptors. An example of this approach can be seen in a rat study using the five-choice serial reaction time task (5-CSRTT) of attention. A typical 5-CSRTT apparatus consists of an operant chamber equipped with a food pellet dispenser, a food magazine where the pellets can be obtained, and a curved front containing five apertures, each of which can be illuminated with a signal light (**FIGURE 13.7**). During training and testing, one of the signal lights is illuminated for a brief period such as 1 second or less. The rat must sustain its attention sufficiently to detect the stimulus, which it signifies by poking its nose into the correct aperture. This permits the animal to receive a reinforcement (food pellet) in the food magazine. Computer software enables researchers to obtain data on variables such as accuracy (percentage of trials with a correct response), errors of omission (failure to respond

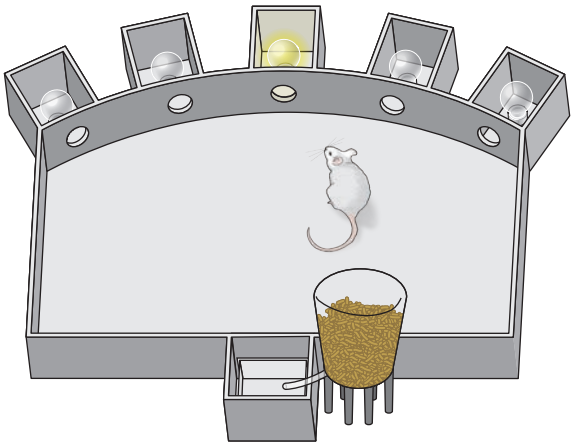


FIGURE 13.7 Diagram of the type of apparatus used in the five-choice serial reaction time task (After Dailey et al., 2004 and Carli et al., 1983.)

to the signal, suggesting a lapse in attention), premature responses (responding before the signal was displayed), and response latency (time to respond after the signal was presented). In the present study, rats were tested in the presence or absence of nicotine, and some animals were additionally pretreated with the β-adrenergic antagonist propranolol or the α-adrenergic antagonist prazosin. The results showed that nicotine enhancement of the percent correct responses on the attention task was blocked by propranolol but not by prazosin (Hahn and Stolerman, 2005). From these results we can conclude that the positive effect of nicotine on sustained attention in this task required a stimulation of norepinephrine release and subsequent activation of β-adrenergic receptors somewhere within the relevant neural circuit. Other research of this type has further suggested a role for glutamate activation of *N*-methyl-D-aspartate (NMDA) receptors but not for dopamine activation of either D_1 or D_2 receptors in the circuitry underlying nicotine's effects on attention (Hahn, 2015).

Nicotine exerts both reinforcing and aversive effects

By definition, smoking tobacco cigarettes or vaping e-cigarettes is reinforcing, because the behavior is learned by the cigarette user and continues to be maintained unless the smoker quits. As the principal reinforcing component within tobacco is nicotine, the main part of this section is devoted to the mechanisms of nicotine reinforcement. However, nicotine can also be aversive, and we will summarize information on the neural pathways mediating nicotine aversion versus nicotine reinforcement. In a later section we will consider evidence for reinforcing properties of other components of cigarette smoke.

NICOTINE REINFORCEMENT Under the right experimental conditions, experienced cigarette smokers as well as laboratory animals will self-administer pure nicotine by intravenous (IV) injection (Le Foll and Goldberg, 2009; Caille et al., 2012; Goodwin et al., 2015). This shows that nicotine by itself can be reinforcing even in the absence of the behavioral components of cigarette smoking. On the other hand, it is clear that the reinforcement provided by smoking is much more complex than simply the delivery of nicotine to the individual. In a later section, we will discuss the relative contributions of nicotine versus other aspects of smoking in the reinforcing properties of this behavior.

Nicotine reinforcement can be influenced by a number of factors, including sex and age. For example, female rats show greater nicotine reinforcement than males, based on IV self-administration, and they also show greater nicotine reward, using the place conditioning paradigm (O'Dell and Torres, 2014). These preclinical findings are consistent with human studies demonstrating that compared with men, women are more sensitive to nicotine reward/reinforcement, report greater positive mood effects of smoking, and have a more difficult time quitting smoking (O'Dell and Torres, 2014). Rodent studies have additionally found greater nicotine reward and reinforcement in adolescent animals than in adults (Yuan et al., 2015). This age difference is hypothesized to stem from the incomplete maturation of key neurochemical systems, such as the dopaminergic system, in the adolescent subjects. Of course, we have known for many years that tobacco use almost always begins at a young age, well before adult brain development has been attained.

Animal studies have played a key role in assessing the mechanisms underlying nicotine reinforcement. As in the case of cocaine and amphetamine, the mesolimbic dopamine (DA) pathway from the VTA to the nucleus accumbens (NAcc) plays a key role in nicotine's reinforcing effects. The reader will recall from Chapter 5 that midbrain dopaminergic neurons exhibit two different modes of firing: a slow regular rate of firing, termed single-spiking firing mode, and periods of rapid bursts of firing, termed phasic or burst firing. Burst firing of the cells depends on excitatory synaptic inputs, which include glutamatergic projections from several brain areas (e.g., medial prefrontal cortex, or mPFC) as well as cholinergic projections from neurons in the pedunculopontine tegmental nucleus (PPTg) and the laterodorsal tegmental nucleus (LDTg) (Faure et al., 2014). Acetylcholine release from these neurons onto the VTA dopaminergic cells activates high-affinity nicotinic receptors located in the VTA, thereby stimulating burst firing of the dopaminergic neurons and increasing DA release in the NAcc. Most importantly, administration of nicotine to animals has been shown to produce the same effects as shown in **FIGURE 13.8** (Subramaniyan and Dani, 2015). The importance of accumbens DA for nicotine reinforcement was demonstrated by Corrigall and coworkers (1992),

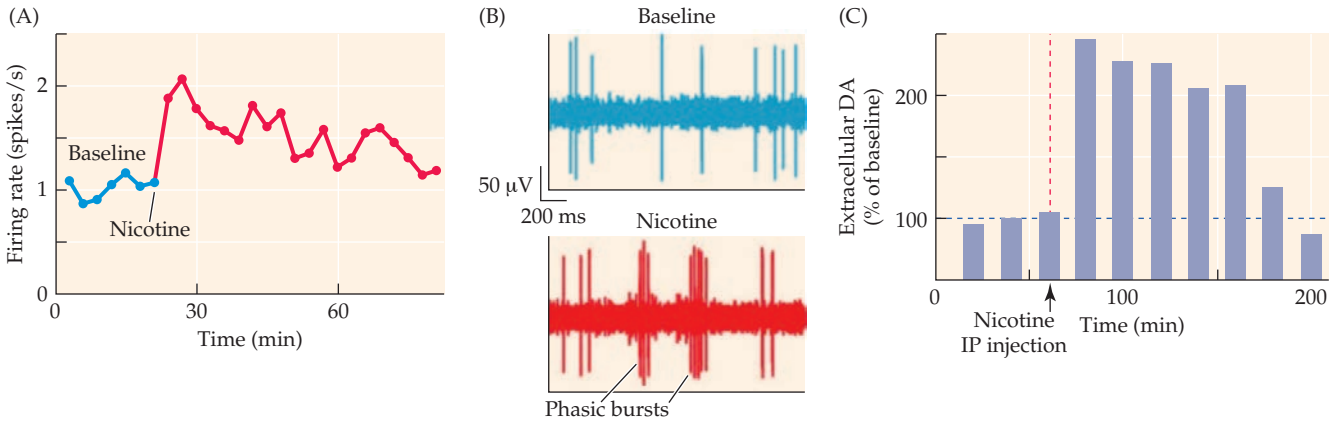


FIGURE 13.8 Nicotine stimulation of VTA dopaminergic neuron firing and DA release in the NAcc
(A) Nicotine administration (0.4–0.5 mg/kg IP) to awake, freely moving rats caused a doubling of the average firing rate of VTA dopaminergic neurons compared with the baseline control condition. (B) This effect was mediated by increased burst firing by the cells. (C) Microdialysis performed in the shell of the NAcc of a separate group of rats showed a large increase in DA release following nicotine administration (0.6 mg/kg IP). (After Subramaniyan and Dani, 2015; and Zhang et al., 2009; original data in panel C from Pidoplichko et al., 2004).

who showed that lesioning the dopaminergic innervation of this area with 6-hydroxydopamine (6-OHDA) significantly attenuated nicotine self-administration.

Nicotine self-administration studies using knockout mice with a genetic deletion of specific nicotinic receptor subunits have provided information regarding the involvement of these subunits in the activation of DA neurons and the elicitation of behavioral reinforcement (Faure et al., 2014; Morel et al., 2014). Such studies have shown that nicotine-mediated dopaminergic neuronal activation and IV self-administration were both lost in mice lacking the β2 subunit that is usually present in high-affinity nAChRs. Both functions were restored when the missing subunit was reexpressed specifically in the VTA. Additional evidence has shown that the VTA receptors necessary for nicotine reinforcement also contain primarily α4 and/or α6 subunits. These findings from mutant mice are consistent with human genetic studies showing that polymorphisms (variations in DNA sequence) in the genes for the α4, α6, and β2 subunits have been linked to the subjective effects of smoking, smoking rates, and the risk for developing nicotine dependence (Brunzell et al., 2015).Brain imaging studies of smokers have revealed important information on nicotine occupancy of nAChRs, particularly high-affinity α4β2 subunit–containing nAChRs, as well as the drug's effects on DA release. In one study, tobacco-dependent smokers underwent an initial period of abstinence to clear the brain of nicotine. Participants were then subjected to PET imaging under one of three conditions: no smoking, smoking a denicotinized cigarette (containing 0.05 mg nicotine), or smoking a low-nicotine cigarette (containing 0.6 mg nicotine). The images shown in **FIGURE 13.9** reveal that nicotine from the low-nicotine cigarette was sufficient to cause substantial occupancy (mean of 79%) of high-affinity nAChRs in all of the brain areas examined (Brody et al., 2009). Even smoking denicotinized cigarettes, which are sometimes used in research to simulate the sensory and motoric experiences of smoking in the relative absence of nicotine consumption (see Figure 13.5), produced a noticeable effect on high-affinity nAChRs. An earlier PET imaging study showed that even a single puff on a standard cigarette led to measurable occupancy of high-affinity nAChRs measured 3 hours later! As would be predicted, receptor occupancy was dose-dependent, with greater occupancy following three puffs and nearly complete receptor saturation following the smoking of three entire cigarettes (Brody et al., 2006). These findings emphasize that neurons expressing high-affinity nAChRs are exquisitely sensitive to nicotine.

AVERSIVE EFFECTS OF NICOTINE Depending on the dose and other circumstances, nicotine can produce aversive instead of reinforcing effects. In nonsmokers, subcutaneous injection of a high dose of nicotine elicited various unpleasant reactions including nausea, dizziness, sweating, headache, palpitations, stomachache, and clammy hands (Foulds et al., 1997). This reaction did not occur in smokers, which demonstrates the presence of chronic nicotine tolerance in smokers (see section below on nicotine tolerance and dependence). The results additionally raise the possibility that tolerance to these aversive effects must occur before individuals can fully experience nicotine reinforcement.

Genetic studies have helped elucidate which nAChRs determine the balance between nicotine reinforcement and aversion. We have already noted a role for α4, α6, and β2 subunits in nicotine reinforcement. In contrast, there is converging evidence that nicotine

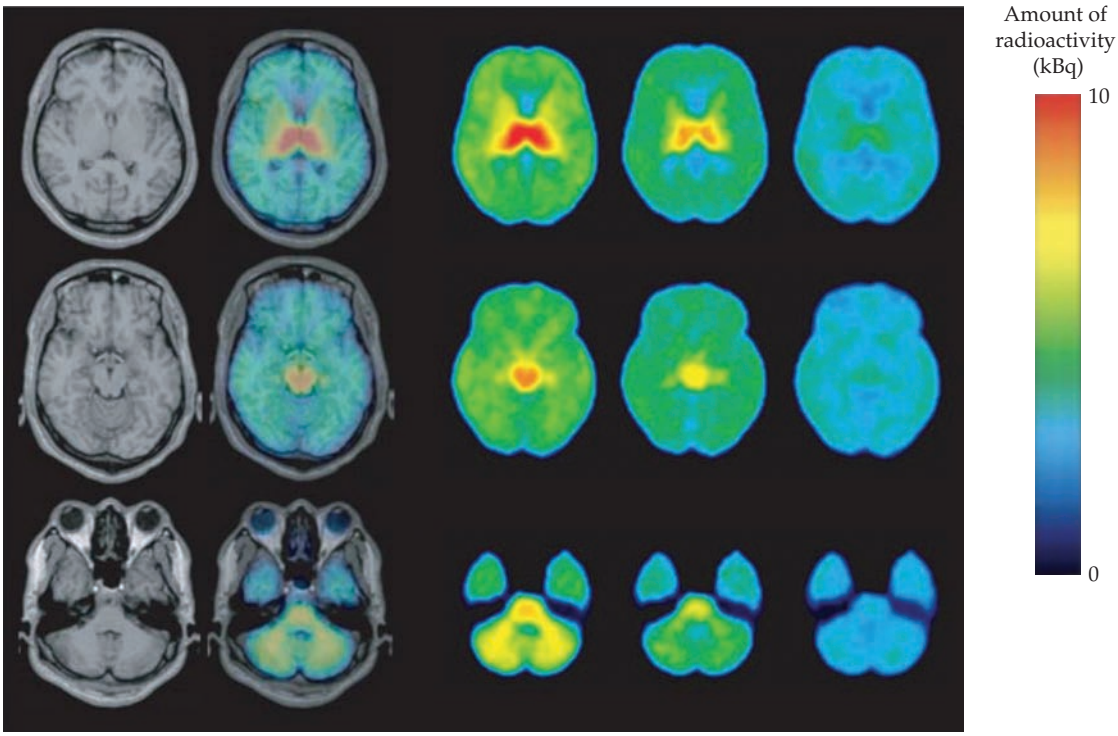


FIGURE 13.9 Dose-dependent occupancy of brain α4β2 subunit–containing nAChRs by cigarette-derived nicotine Tobacco-dependent cigarette smokers were subjected to PET imaging using a radiolabeled drug that binds selectively to high-affinity α4β2 subunit–containing nAChRs in the brain. Following 43 hours of abstinence from smoking, participants were imaged under control (no smoking) conditions or after smoking a denicotinized (Denic) cigarette or a low-nicotine (Low-nic) cigarette. The images in column 1 (far left) show magnetic resonance imaging (MRI) scans obtained for the purpose of anatomical localization of drug binding. Columns 3 to 5 show PET scans obtained under the various smoking conditions. The second column (Fusion) shows the MRI images laid over the PET images. The three rows represent different planes of imaging that capture receptor binding in three brain areas of interest. The top row shows binding at the level of the thalamus; the middle row shows binding at the level of the brain stem; and the bottom row shows binding at the level of the cerebellum. The highest amount of receptor binding is depicted in red, followed in descending order by yellow, green, and blue. The concentration values beneath the labels under columns 3, 4, and 5 are the mean plasma levels of nicotine produced by each experimental condition. The reductions in radiolabeled drug binding (i.e., displacement of the radiolabel by nicotine) show that even low levels of nicotine cause significant occupancy of high-affinity nAChRs in multiple brain areas. (From Brody et al., 2009.)

aversion is related to a gene cluster on human chromosome 15 (Fowler and Kenny, 2014). This chromosomal region, which is called *CHRNA5-CHRNA3-CHRNB4*, encodes the genes for the nAChR α5, α3, and β4 subunits respectively. Animal studies have revealed that just as β2 subunits tend to assemble together with α4 or α6 subunits, α5, α3, and β4 subunits tend to be found together in the same nAChRs (note that in this case, the receptors in question contain two different kinds of α subunits mixed together). Activation of these α5-, α3-, and β4-containing receptors appears to enhance nicotine's aversive properties, thereby reducing the propensity to consume or self-administer the drug (Fowler and Kenny, 2014; Antolin-Fontes et al., 2015). On the other hand, reducing receptor expression by selectively knocking out the α5 subunit enhances nicotine self-administration at high doses where the aversive effects usually limit the animal's consumption (Fowler et al., 2011; **FIGURE 13.10**). The nAChRs that mediate nicotine aversion are heavily concentrated in the neural pathway from the medial habenula to the interpeduncular nucleus. The significance of this localization was demonstrated by the finding that nicotine's aversive effects can be modulated by altering the expression of these receptors *just* in the medial habenula alone (Fowler et al., 2011; Frahm et al., 2011).

Nicotine produces a wide range of physiological effects

We mentioned earlier that nAChRs are abundantly expressed in autonomic ganglia. Consequently, nicotine can activate elements of both the sympathetic

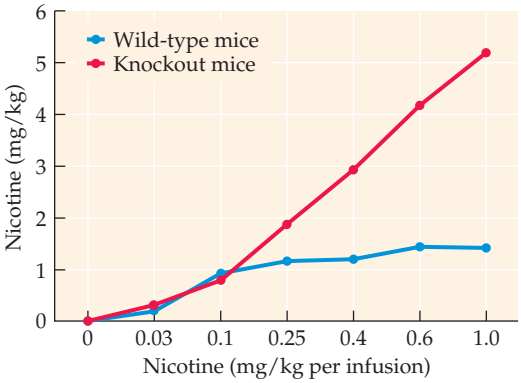


FIGURE 13.10 Role of the nAChR α5 subunit in nicotine aversion Results of dose-dependent changes in nicotine self-administration in α5 subunit knockout mice compared with wild-type mice. The *y*-axis shows total nicotine intake at each dose. The knockout mice administer successively greater amounts of nicotine as the dose per infusion increases, whereas the wild-type mice plateau at a relatively low level of intake because of the aversive effects of nicotine at higher doses. (After Fowler et al., 2011.)

and parasympathetic systems to cause a wide spectrum of physiological manifestations. For example, smoking a cigarette stimulates the adrenal glands to release epinephrine (adrenaline) and norepinephrine (noradrenaline). These hormones, along with direct nicotine-induced activation of sympathetic ganglia, lead to symptoms of physiological arousal such as tachycardia (increased heart rate) and elevated blood pressure. This mild physiological arousal is thought to contribute to the reinforcing features of smoking. On the other hand, repeated exposure to nicotine and other components of cigarette smoke alters the balance of autonomic nervous system activity, resulting in chronically elevated sympathetic activity (Middlekauff et al., 2014). This effect helps explain why smoking increases the risk for cardiovascular disease and cerebrovascular accidents (strokes), particularly if the smoker has high blood pressure to begin with.

The action of nicotine on parasympathetic ganglia increases hydrochloric acid secretion in the stomach, which exacerbates or contributes to the formation of stomach ulcers. There is also increased muscle contraction in the bowel, which sometimes leads to chronic diarrhea that is especially harmful to individuals vulnerable to colitis, a condition of chronic irritability of the colon. Together, these autonomic nervous system effects contribute to the deleterious consequences of heavy and prolonged use of tobacco products (see the section on smoking-related illness).

One consequence that many cigarette smokers find desirable is the constraining effect of nicotine on body weight. Adult cigarette smokers weigh an average of 8 to 11 pounds less than gender- and age-matched nonsmokers, and quitting smoking usually results in weight gain (Audrain-McGovern and Benowitz, 2011). This effect of nicotine has been attributed to an increase in metabolic rate combined with appetite suppression. Indeed, new findings suggest that a group of cholinergic neurons within the basal forebrain strongly inhibit feeding behavior and that the effect of these cells is mediated at least partly through activation of nAChRs (Herman et al., 2016). Nevertheless, no one would recommend smoking for weight control, because the terrible health consequences of smoking far outweigh the modest benefit derived from losing a few pounds.

Nicotine is a toxic substance that can be fatal at high doses

Nicotine is quite toxic, primarily because of its effects on the autonomic nervous system. As small a dose as 60 mg can be fatal to an adult. If you do the math based on the nicotine content of a typical cigarette, you will see that a single pack contains several lethal doses of the drug. Of course, cigarettes are only smoked one at a time, and most of the nicotine is not taken in because of burning of the tobacco and the loss of sidestream smoke (smoke not inhaled by the smoker). Cases of nicotine poisoning sometimes occur through swallowing (usually by children) of tobacco or of e-cigarette refill cartridges containing a concentrated nicotine solution. Although such episodes can be fatal, nicotine exposure by this route is generally less toxic than would be expected, because of slow absorption of the drug from the stomach, first-pass metabolism in the liver, and possible regurgitation of the ingested material remaining in the stomach due to nicotine activation of the chemical trigger zone (vomiting center) in the medulla. Toxic effects may also result from absorption of excessive nicotine through the skin when field workers are harvesting wet tobacco leaves or from exposure to pure nicotine used in certain insecticides. Indeed, the first reported case of nicotine poisoning was that of a florist who accidentally came into contact with a nicotine-containing insecticide that he had spilled onto a chair (Faulkner, 1933). Nicotine toxicity to insects is believed to have been an important driving force in the evolution of nicotine synthesis by tobacco plants (see **Web Box 13.1**).

The symptoms of nicotine poisoning include nausea, excessive salivation, abdominal pain, vomiting, diarrhea, cold sweat, headache, dizziness, disturbed hearing and vision, mental confusion, and marked weakness. This is quickly followed by fainting and

prostration; falling blood pressure; difficulty in breathing; weakening of the pulse, which becomes rapid and irregular; and collapse. Left untreated, a fatal dose ends with convulsions followed shortly by respiratory failure due to depolarization block of the muscles of breathing. The treatment of nicotine poisoning involves inducing vomiting if the poison has been swallowed, placing adsorptive charcoal in the stomach, giving artificial respiration, and treating for shock.

Chronic exposure to nicotine induces tolerance and dependence

NICOTINE TOLERANCE Repeated exposure to nicotine leads to a complex pattern of tolerance and, in some instances, sensitization. It is useful to distinguish between acute and chronic nicotine tolerance. For example, acute tolerance can be studied by pretreating subjects (e.g., by injection or by nasal spray) with either nicotine or vehicle and then testing their responses to a subsequent nicotine challenge. In both smokers and nonsmokers, many behavioral and physiological responses are attenuated by nicotine pretreatment, indicating the occurrence of tolerance. In much the same way, cigarette smokers undergo a significant degree of nicotine tolerance during the course of the day. Acute tolerance is short-lived; after an overnight period of abstinence, smokers awaken the next morning more sensitive to nicotine than at the end of the previous day. This neurobiological mechanism helps explain why smokers often report that the first cigarette of the day is the most pleasurable one.

Acute tolerance is related to a desensitization (i.e., temporary inactivation) of central nAChRs, including those that mediate nicotine reinforcement. The nicotine from even a single cigarette is able to induce some degree of receptor desensitization. However, the properties of desensitization, including the concentration of nicotine required for desensitization, latencies to desensitize and to recover, and the proportion of receptors that are inactivated, vary significantly with subunit composition. Receptors composed of both α and β subunits (e.g., α4β2) are desensitized at lower nicotine concentrations than α7-containing receptors, because the former have a higher affinity for nicotine (Picciotto et al., 2008). On the other hand, α4β2 nicotinic receptors recover more rapidly than α7 receptors, which may permit the high-affinity receptors to at least partially regain their sensitivity by the time the next cigarette is smoked. For this reason, some investigators theorize that individuals who smoke frequently over the course of a day undergo numerous cycles of nicotinic receptor activation, desensitization, and resensitization.

Long-term exposure to nicotine causes chronic tolerance. This chronic tolerance is superimposed on the acute within-a-day tolerance, and of course it is present only in smokers and others who use tobacco frequently. An early clue to the existence of chronic nicotine tolerance was the observation that nicotine-related toxicity occurred much more frequently among tobacco harvesters who didn't smoke than among those who were smokers (Gehlbach et al., 1974). The subjective effects of nicotine, both positive (rewarding) and negative (aversive), are most susceptible to chronic tolerance in regular smokers (Perkins, 2002). There is also some tolerance to the cardiovascular effects of the drug. Chronic tolerance to at least some of these effects is surprisingly slow to dissipate, even after prolonged abstinence from smoking. Nicotinic receptor desensitization is generally thought to be the primary mechanism underlying chronic, as well as acute, tolerance, although other factors are probably also involved. Interestingly, chronic exposure to nicotine elicits a compensatory response manifested by an up-regulation of high-affinity nAChR expression in many parts of the brain (Melroy-Greif et al., 2016). In humans, this effect has been demonstrated using both neuroimaging and postmortem binding studies. For example, **FIGURE 13.11** shows a large increase in high-affinity nicotinic receptor binding in layer 6 of the prefrontal cortex of a smoker compared with a nonsmoker (Perry et al., 1999). Such receptor up-regulation may play a significant role in the development of nicotine dependence and withdrawal (see next section).

NICOTINE DEPENDENCE AND WITHDRAWAL Nicotine is a highly addictive drug. Indeed, the National Epidemiologic Survey on Alcohol and Related Conditions, a survey conducted between 2001 and 2005, found that over 67% of nicotine users eventually became dependent, whereas the percentages for alcohol, cocaine, and cannabis were only 22.7%, 20.9%, and 8.9%, respectively (Lopez-Quintero et al., 2011). The *Diagnostic and Statistical Manual of Mental Disorders*, fifth edition, (*DSM-5*) lists "tobacco use disorder" as a subcategory within the broader category of substance abuse disorders (American Psychiatric Association, 2013). To be diagnosed with tobacco use disorder, a person must have used tobacco (i.e., nicotine-containing) products for over 1 year and have met at least two of the following three criteria: (1) use of more tobacco products for a longer time than planned, (2) signs of nicotine tolerance, and (3) appearance of withdrawal symptoms when the person stops using tobacco. For habitual smokers who meet these criteria, even a brief abstinence of a few hours leads to a growing urge to smoke. These feelings correlate with a drop in nicotine levels in the individual's bloodstream. A series of case histories of adolescent-to-young-adult smokers performed by DiFranza and colleagues (2010) led the investigators to propose three temporal stages of compulsion to use tobacco: "wanting," "craving," and "needing," each successive stage being more powerful than the one before. These stages are more fully

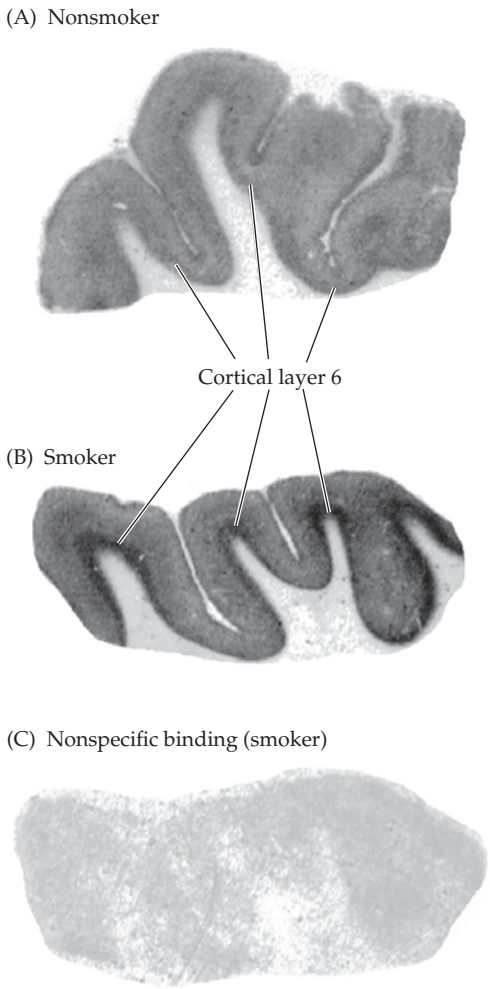


FIGURE 13.11 Up-regulation of nicotinic receptor binding in the prefrontal cortex of smokers Postmortem slices of prefrontal cortex were incubated with radiolabeled epibatidine, a compound that binds to high-affinity nicotinic binding sites. (A and B) Total binding in a nonsmoker and a smoker, respectively, illustrating the particularly large amount of receptor up-regulation in cortical layer 6. (C) Nonspecific epibatidine binding (i.e., binding to tissue elements other than nicotinic receptors), which was very low. (From Perry et al., 1999.)

characterized as follows: "Wanting is mild, short-lived and easily ignored. Craving is a desire to smoke that is more intense and compelling than wanting. Wanting will go away if ignored, but craving is more persistent. Smokers say they are needing to smoke when withdrawal symptoms are so troublesome that they cannot function normally" (DiFranza, 2015, p. 44).

The much longer withdrawal that occurs when people try to quit smoking leads to a more complex abstinence syndrome characterized by three kinds of symptoms: affective (mood related), somatic, and cognitive (McLaughlin et al., 2015). Affective symptoms include anxiety, irritability, depressed mood, anhedonia (decreased ability to experience pleasure), insomnia, restlessness, hyperalgesia (abnormal sensitivity to pain), and of course a powerful desire to smoke. Somatic symptoms consist of tremors, bradycardia (slowed heart rate), nausea and gastrointestinal distress, and hunger and weight gain. Finally, the cognitive features of nicotine withdrawal include difficulty concentrating (i.e., deficient attention) and memory impairment (McClernon et al., 2015). An early study examined the time course of withdrawal symptoms as well as the ability of nicotine gum to prevent such symptoms (Hughes et al., 1991). The results showed that the abstinence syndrome was still present at 1 week postcessation, but most symptoms except for hunger and weight gain had subsided by the 4–week time point. These data suggest that the abstinence syndrome from tobacco is relatively short-lived. Use of nicotine gum prevented almost all of the withdrawal symptoms, supporting the conclusion that these symptoms are largely due to nicotine dependence. It is important to note, however, that even with amelioration of withdrawal symptoms by nicotine replacement, more than two-thirds of the individuals were back smoking at a 6-month follow-up test. These and other experimental results indicate that the nicotine abstinence syndrome is not the only reason that most regular smokers find it so difficult to quit their habit.

Although the *DSM-5* criteria may be used clinically to diagnose nicotine dependence, a number of questionnaires have been devised that are often applied to research studies. These questionnaires include the Fagerström Test for Nicotine Dependence (Heatherton et al., 1991), the Heaviness of Smoking Index (Kozlowski et al., 1994), the Nicotine Dependence Syndrome Scale (Shiffman et al., 2004), and the Cigarette Dependence Scale (Etter, 2008). A few questionnaires have been developed for application to specific groups of nicotine users. For example, DiFranza and colleagues (2002) devised the Hooked on Nicotine Checklist, a questionnaire designed to assess emerging nicotine dependence in young people. More recently, combinations of these older instruments have been revised and supplemented to study dependence in e-cigarette users (Etter and Eissenberg, 2015; Foulds et al., 2015). The results indicated that vapers who either are exsmokers or concurrently use both e-cigarettes and tobacco cigarettes generally show less nicotine dependence than long-term users of cigarettes only. More research is needed to confirm these initial findings. But even if confirmation is obtained, public health researchers are concerned that the advent of e-cigarettes may spawn a new generation of young people dependent on nicotine who might never have taken up smoking tobacco cigarettes (Cobb et al., 2015; Fillon, 2015). This issue is discussed again later the chapter.

Animal models of nicotine dependence have been critical for studying the neurochemical mechanisms

underlying this phenomenon and for testing potential pharmacotherapies for smoking cessation (Falcone et al., 2016). Nicotine dependence can be induced in laboratory animals by giving them continuous exposure to the drug, often by implanting a small device known as an **osmotic minipump** under the skin of the animal. The minipump is filled with a nicotine solution and slowly infuses the solution subcutaneously at a constant rate for a predetermined period such as 1 or 2 weeks. Some withdrawal symptoms can be observed when the nicotine clears the animal's system after the pump either runs out of solution or is removed by the experimenter. However, a stronger reaction can be triggered by administering a nicotinic receptor antagonist such as **mecamylamine**, thereby blocking the action of any residual nicotine still present in the animal. In rats, typical nicotine withdrawal symptoms include gasps, shakes or tremors, teeth chatter, ptosis (drooping eyelids), reduced locomotor activity, and increased startle reactivity (Helton et al., 1993; Hildebrand et al., 1997). Brain reward function measured by the threshold for intracranial electrical self-stimulation is also significantly impaired during nicotine withdrawal (Epping-Jordan et al., 1998)—an effect seen during withdrawal from other abused drugs as well (see Chapter 9). Decreased functioning of the brain reward system is a sign of anhedonia, which is present during tobacco withdrawal in smokers and might contribute to the well-known difficulty in stopping smoking. Current evidence suggests that the nicotine abstinence syndrome is mediated by a combination of resensitization of the desensitized and up-regulated nAChRs, reduced activity of the mesolimbic dopaminergic pathway, increased corticotropin-releasing factor (CRF) signaling in the central nucleus of the amygdala, and activation of the same pathway between the medial habenula and the interpeduncular nucleus that's involved in nicotine aversion (Dani and De Biasi, 2013; McLaughlin et al., 2015).

Section Summary

- The mood-altering effects of nicotine depend on whether the individual is an abstinent smoker or a nonsmoker. In temporarily abstinent smokers, administration of pure nicotine usually increases calmness and relaxation. This effect is due, in part, to relief from nicotine withdrawal symptoms, because nicotine given to nonsmokers more often elicits feelings of tension, arousal, lightheadedness or dizziness, and sometimes nausea.
- Administration of nicotine to abstinent smokers also leads to enhanced performance on various cognitive tasks, particularly those involving attentional demands. In this case, however, some nicotine-related functional enhancement has also been reported for nonsmokers. Some research has found that nicotine enhancement is most clearly evident in people with a low baseline level of attention.
- Animal studies have found that nicotine improves performance on tasks requiring sustained attention and working memory. Certain hippocampal-dependent tasks, such as contextual fear conditioning and spatial learning, are also sensitive to nicotine. Research with nAChR subunit knockout mice as well as the antagonists DhβE (selective for α4β2-containing high-affinity nAChRs) and MLA (selective for α7-containing low-affinity nAChRs) revealed that high-affinity nAChRs play a greater role in the nicotine enhancement of hippocampal-dependent tasks. Other neurotransmitter receptors involved in the positive effects of nicotine on sustained attention include β-adrenergic and NMDA receptors, but not D_1 or D_1 receptors for DA.
- Within a certain dose range, pure nicotine is reinforcing to both humans and experimental animals, as shown by IV self-administration. Females are more sensitive than males to nicotine reward/reinforcement in both humans and rodents. Rodent studies have additionally found greater nicotine reward/reinforcement in adolescent than in adult animals.
- The reinforcing properties of nicotine involve activation of high-affinity receptors located in the VTA, which stimulates burst firing of the dopaminergic neurons and increases DA release in the NAcc. This hypothesis is supported by the finding that nicotinic receptors in the VTA containing β2 subunits in combination with α4 and/or α6 subunits are necessary for nicotine self-administration. Polymorphisms in the genes coding for these subunits have been associated with the subjective effects of smoking, smoking rates, and the risk for developing nicotine dependence in humans.
- Nicotine can also exert aversive effects, which seem susceptible to chronic tolerance with continued nicotine exposure. Nonsmokers injected with a high dose of nicotine experienced a number of symptoms such as nausea, dizziness, sweating, headache, palpitations, and stomachache. Nicotine aversion in both humans and experimental animals involves the stimulation of nAChRs containing α5, α3, and β4 subunits. These receptors are expressed at high levels in the medial habenula–interpeduncular nucleus pathway, and the genes for the subunits are clustered together on human chromosome 15.

- Nicotine additionally produces a variety of peripheral physiological effects. These include release of epinephrine and norepinephrine from the adrenal glands, tachycardia, and elevated blood pressure, all of which contribute to the arousing effects of the drug. Nicotine also increases hydrochloric acid secretion in the stomach and muscle contraction in the bowel, both of which can adversely affect the gastrointestinal tract. Finally, nicotine modestly increases metabolic rate and suppresses appetite, which accounts for why smokers typically gain weight after quitting.
- Nicotine is a toxic substance that can cause potentially dangerous symptoms such as nausea, salivation, abdominal pain, vomiting and diarrhea, confusion, and weakness. If a sufficient dose has been ingested, death may occur from respiratory failure. Treatment involves an attempt to remove the nicotine from the victim's stomach (if the nicotine has been swallowed), administration of artificial respiration, and dealing with drug-induced shock.
- Repeated exposure to nicotine can lead to tolerance and, in some cases, sensitization. Single doses of nicotine cause a rapid but transient form of acute tolerance that depends on desensitization of nicotinic receptors. Long-term nicotine exposure is associated with chronic tolerance, as a consequence of which smokers do not exhibit the adverse reactions to high doses of nicotine that are observed in nonsmokers. Smokers show an up-regulation of nAChR levels in many brain areas, seemingly as a compensatory response to the chronic receptor desensitization associated with repeated nicotine exposure.
- Chronic nicotine can also cause dependence, thereby leading to withdrawal symptoms upon abstinence. The *DSM-5* contains the category tobacco use disorder, which is diagnosed by finding at least two out three listed criteria. Compulsive cigarette use is characterized by successive stages of wanting, craving, and finally needing a cigarette. A number of questionnaires have additionally been developed to identify and quantify nicotine dependence. Prolonged abstinence in nicotine-dependent people evokes a complex syndrome of affective, somatic, and cognitive symptoms. Initial studies suggest that e-cigarette users are generally less dependent than tobacco cigarette users, but additional research is needed to confirm this finding.
- Animal models of nicotine dependence are important for studying the mechanisms of dependence and for testing new drug treatments. When rats are made dependent on nicotine by giving them continuous exposure to the drug (e.g., with osmotic minipumps), withdrawal symptoms can be observed if the dependent animals are administered a nicotinic receptor antagonist such as mecamylamine. This withdrawal syndrome has been related to resensitization of desensitized and up-regulated nAChRs, reduced activity of the mesolimbic dopaminergic pathway, increased CRF signaling in the central nucleus of the amygdala, and activation of the nicotine aversion pathway involving the medial habenula and interpeduncular nucleus.

Cigarette Smoking and Vaping

Useful information about the causes and consequences of smoking has been obtained by documenting changes in the prevalence of smoking over time and by identifying the characteristics of smokers and the pattern of progression from occasional to regular smoking. This information has been combined with knowledge about the mechanisms of nicotine action and data on the serious health consequences of smoking to develop smoking cessation programs. Although fewer data are available on vaping and how it compares to smoking, research continues to be performed and some of the results obtained thus far are presented below.

What percentage of the population are current users of tobacco and/or e-cigarettes?

The amount of cigarette smoking in the United States has varied tremendously over the past 100-plus years. As illustrated in **FIGURE 13.12**, yearly per capita cigarette consumption was quite low at the beginning of the twentieth century but then rose steeply until the mid-1950s. A significant cultural influence promoting cigarette smoking was the depiction of this behavior in films, which was quite common from the 1930s through the 1960s and was even used by advertisers to promote specific brands of cigarettes (Castaldelli-Maia et al., 2016). There was a brief dip in cigarette consumption following publication of the first studies linking smoking with lung cancer, but consumption rose again with the marketing of filtered cigarettes. The decline in cigarette consumption since the 1960s coincides with the Surgeon General's reports on the health consequences of smoking, the appearance of antismoking ads, large increases in cigarette taxes, and general disapproval of smoking in many parts of society.

More recent data on national use of nicotine-containing products can be obtained from the 2016 National Survey on Drug Use and Health (cigarettes and other tobacco products, but not including e-cigarettes) and the 2015 National Health Interview Survey (e-cigarettes). During 2016, approximately 24% of the population (corresponding to 64 million people) age 12 or older were current users of any tobacco product, and over 80% of those were cigarette smokers (Substance

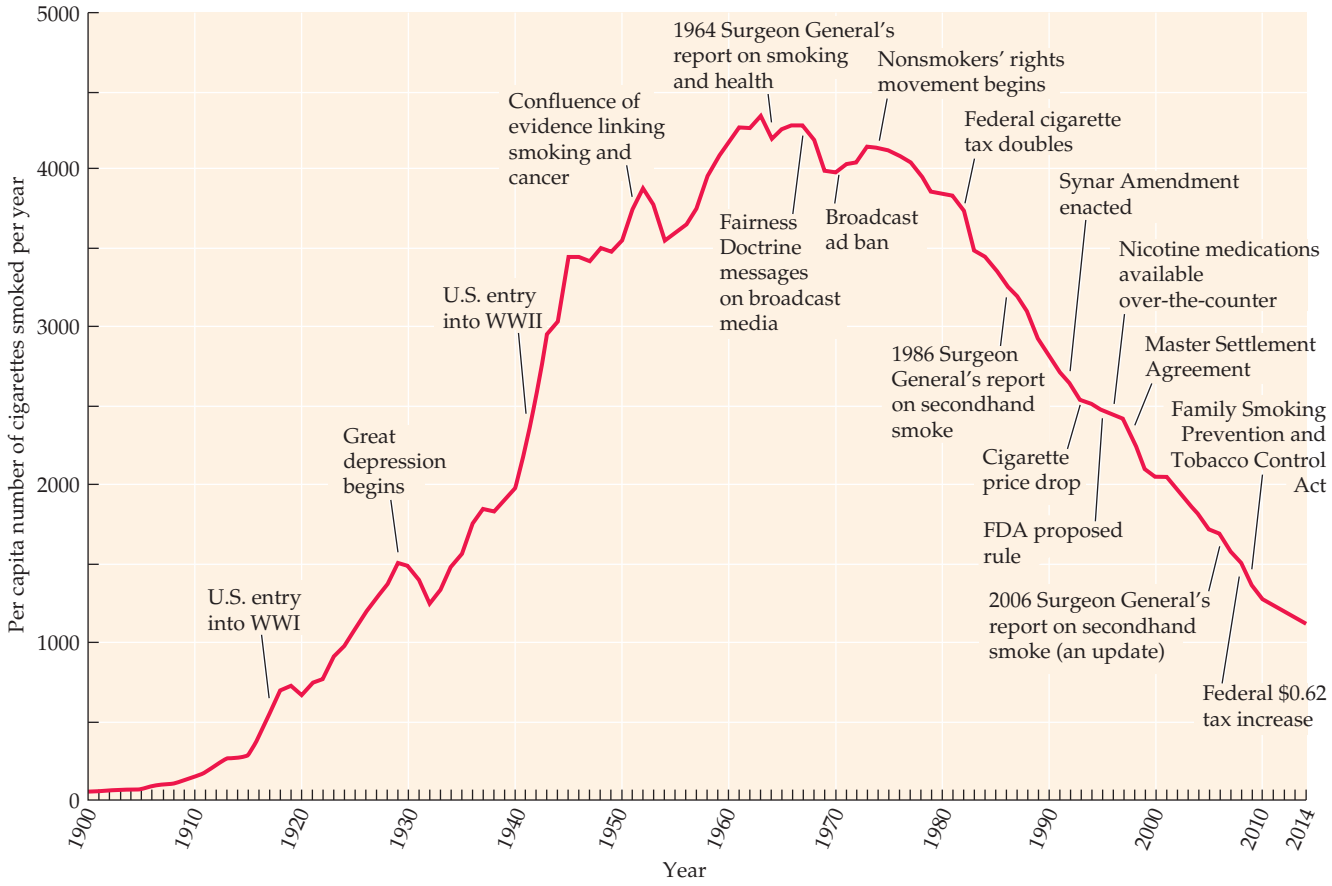


FIGURE 13.12 Yearly per capita cigarette consumption in the United States from 1900 to 2014 Data are for individuals 18 years of age or older. (After U.S. Department of Health and Human Services, 2014; modified from Warner, 1977, 1981, 1985.)

Abuse and Mental Health Services Administration, 2017). The National Health Interview Survey statistics apply to adults age 18 or older, and those data indicate that less than 4% of that age group were current e-cigarette users (Schoenborn and Gindi, 2015). For purposes of these surveys, current use is defined as use of the product at least once during the previous 30 days. The National Health Interview Survey also found that adult e-cigarette users were likely to be either current tobacco cigarette smokers or individuals who had quit tobacco cigarette smoking within the past year. Overall, the incidence of cigarette smoking is gradually declining across the population. In contrast, use of e-cigarettes by young people showed an increasing trend from 2011 to 2015, but a decrease from 2015 to 2016 (see Figure 13.3).

Nicotine users progress through a series of stages in their pattern and frequency of use

INITIATION AND SUBSEQUENT TRAJECTORY OF TOBACCO CIGARETTE SMOKING Most smokers pick up the habit during adolescence. Looked at another way, early smoking greatly increases the chances that one will smoke as an adult. For this reason, smoking and, more recently, vaping by teenagers have received a lot of attention from researchers as well as from policy makers interested in reducing the prevalence of these behaviors in our society. There are many theories about why teenagers take up smoking. Some of the hypothesized reasons include establishing feelings of independence and maturity (by defying parental wishes or societal norms), improving self-image and enhancing social acceptance (assuming that one's friends are already smokers), counteracting stress and/or boredom, and simple curiosity. Moreover, young people tend to emphasize the positive elements of smoking and vaping while disregarding or denying the negative aspects, including the health consequences. Longitudinal surveys have gone beyond self-reported reasons for smoking to examine empirically the various factors that predict smoking onset. A review of this research found that poor academic performance, rebelliousness, sensation seeking, receptivity to cigarette advertising/marketing, and smoking by family and/or friends were all significant predictors of smoking onset (Wellman et al., 2016).

Early adolescent experimentation with cigarettes can lead to a variety of outcomes. Many individuals

never develop any kind of smoking habit, whereas a small percentage become "chippers," smokers who maintain a pattern of regularly smoking a few cigarettes a day without becoming fully dependent on nicotine according to standard criteria (Shiffman and Paty, 2006). Finally, there are individuals who develop an established pattern of smoking characterized by compulsive use, cravings for tobacco, and difficulty stopping this behavior.

A considerable amount of research has focused on several important questions: What are the stages traversed in the development of the smoking habit? How rapidly do adolescents become "hooked" on smoking? And what is the role of nicotine dependence in adolescent smoking? There is a consensus among researchers that smokers go through several different stages from initial experimentation with cigarettes to a regular smoking habit, although there is less agreement about the exact definition and features of each stage. One staging system or model proposed by Mayhew and colleagues (2000) is shown in **TABLE 13.1**. The stages in this system are related to the individual's amount of cigarette smoking, and the system uses standard clinical criteria to suggest that only established smokers are nicotine dependent.

An alternative model has been proposed by DiFranza and coworkers (DiFranza et al., 2011; DiFranza, 2015), who derived their model from survey data, many thousands of interviews of adolescent and adult smokers, and other sources. This model differs from that of Mayhew et al. and other systems in a few important ways. First, DiFranza and coworkers point out that at least some symptoms of nicotine dependence, including difficulty quitting, have been demonstrated in adolescent smokers (Colby et al., 2000; DiFranza et al., 2010) and even in adult tobacco chippers who smoke no more than five cigarettes per day (Wellman et al., 2006). Second, the alternative model is based not only on changes in the number of cigarettes smoked but also on the latency between cigarettes and the growing compulsiveness of smoking: from no compulsiveness, to "wanting" a cigarette, to "craving" a cigarette, and finally to "needing" a cigarette, applying the terminology used by DiFranza and coworkers (2010)

First exposure to a cigarette is often an unpleasant experience. Inhalation of the tobacco smoke may cause coughing and throat irritation, and the smoker may also feel nausea and/or dizziness. However, some individuals experience relaxation even in their first smoking experience. The reinforcing efficacy of nicotine alone, without the irritating effects of cigarette smoke, was tested by administering the drug orally to volunteers who had never smoked (Duke et al., 2015). Half of the participants reported the experience to be rewarding, with relatively few adverse side effects such as nausea or dizziness, whereas the other half reported an overall aversive experience. These results suggest that nicotine reinforcement from the first cigarette could be an early contributor to the desire to smoke again in some individuals. From their first cigarette, those who are destined to become nicotine-dependent pass through recognizable stages in their feelings about smoking. Initially, the smoker feels an occasional wanting to smoke, which defines a low level of compulsiveness. As the smoking habit progresses in intensity, the individual begins to feel a craving to smoke, and then finally a need to smoke in order to feel normal. This last phase may well be accompanied by additional symptoms that meet the formal *DSM-5* diagnosis of tobacco dependence disorder. Not surprisingly, the latency between cigarettes decreases as the smoker passes through the stages of wanting, craving, and needing a cigarette.

A group of Canadian researchers determined the rate of progression through predetermined "milestones" from the first cigarette to clinically defined nicotine dependence by following over 1000 students prospectively for 5 years beginning at the seventh grade (Gervais et al., 2006). The researchers found that the first signs of craving, tolerance, and withdrawal symptoms often occurred before the smoker had smoked a total of 100 cigarettes (just five packs!) in her life. These findings are consistent with the model of DiFranza's group that hypothesizes an early onset of nicotine dependence. On the other hand, it's also important to note that the latency from first puff to the milestone of daily smoking ranged from 20 to almost 40 months for the study participants who reached that milestone (Gervais et al., 2006). Therefore, the progression of smoking

TABLE 13.1 Proposed Stages in the Development of a Smoking Habit

Stage	Definition
1a. Nonsmoking—precontemplation	Nonsmoker and doesn't intend to start smoking
1b. Nonsmoking—contemplation or preparation	Nonsmoker but is thinking about starting
2. Initiation or tried	Has smoked a few cigarettes only
3. Experimentation	Smokes occasionally/experimentally; not yet committed to smoking
4. Regular smoker	Smokes on a regular basis (for example, on weekends or at parties), but not too frequently and not daily
5. Established smoker	Smokes daily or almost daily, sometimes heavily; nicotine-dependent

Source: From Mayhew et al., 2000.

TABLE 13.2 Hypotheses about Why Adolescents Are More Likely to Initiate Use of E-cigarettes Than of Tobacco Cigarettes

Name of hypothesis	Description
Flavor hypothesis	Many more flavor additives are available in e-cigarettes.
Health hypothesis	E-cigarettes are presumed to be a healthier alternative.
Price hypothesis	E-cigarettes may be less expensive than tobacco cigarettes.
Concealment hypothesis	E-cigarette use is easier to conceal because of the lack of cigarette butts and acrid tobacco smoke.
Role model hypothesis	E-cigarette users rather than tobacco cigarette users are perceived as role models.
Acceptance hypothesis	E-cigarettes are more socially acceptable among peers.

Source: After Schneider and Diehl, 2016.

frequency can be fairly slow despite earlier signs of nicotine tolerance and dependence.

INITIATION AND SUBSEQUENT TRAJECTORY OF E-CIGARETTE USE We saw in Figure 13.3 that more adolescents now use e-cigarettes than tobacco cigarettes, which has given rise to various hypotheses that attempt to account for this choice. **TABLE 13.2** presents several of these hypotheses as summarized by Schneider and Diehl (2016). Although each of these hypotheses is supported by some empirical evidence, clearly more research is needed to determine the relative importance of each of the proposed factors.

A debate is currently ongoing among tobacco researchers and public health officials about the relative benefits and risks of e-cigarettes. The potential benefits are discussed in **BOX 13.1** and in the section on pharmacological interventions for tobacco dependence. Some consider the major risk to be the fact that e-cigarettes have now become the most significant route by which children and adolescents can become exposed to nicotine. Not only does this pose a risk for the later development of nicotine dependence in long-term vapers, it also allows for the progression from vaping to tobacco cigarette smoking in some cases. Because of the relative newness of e-cigarettes compared with tobacco cigarettes, researchers do not yet know what proportion of vapers will ultimately become nicotine-dependent. Nor do we know whether the trajectory and stages of e-cigarette use will be similar to those of tobacco cigarette smoking. However, several longitudinal studies found that adolescents and young adults who were e-cigarette users before ever smoking had significantly greater odds of subsequently using tobacco cigarettes or other combustible tobacco products (e.g., cigars or hookahs) than matched controls who were not e-cigarette users (Chatterjee et al., 2016).

BOX 13.1 The Cutting Edge

How Safe Are E-cigarettes?

The global debate over e-cigarettes and other ENDS revolves mainly around two contrary points of view. The argument *against* e-cigarettes is their potential for inducing nicotine dependence in young people who might otherwise not have taken up tobacco smoking. The argument *for* e-cigarettes is based on the notion that they are much safer than tobacco cigarettes, and therefore they are a healthier substitute for the latter and could even be used as a new tool for smoking cessation (see section on strategies for treating tobacco dependence). So what *is* the available evidence regarding the relative safety of e-cigarettes for vapers?

Information regarding the ability of tobacco smoking to increase the risk of developing cancers, COPD, and cardiovascular disease comes from large-scale epidemiological studies comparing long-term smokers with matched nonsmoking controls. The use of e-cigarettes is still too new for researchers to have performed comparable studies of vapers. However, an alternative approach that can be informative is to compare the chemical contents of e-cigarette vapor with the contents of tobacco smoke. We can then ask whether any of the harmful chemicals in tobacco smoke are also present in e-cigarette vapor, and if they are present, are the concentrations similar or different.

Results of chemical analyses of e-cigarette vapor can be obtained from a published review (Goniewicz et al., 2014) and a technical report available online (Wilson and Gartner, 2016). The first result is to be expected, namely that a much lower number of

BOX 13.1 The Cutting Edge (*continued*)

chemical compounds are detectable in e-cigarette vapor than in tobacco smoke. The second result is, perhaps, not as obvious. One might assume that the only substances present in this vapor are nicotine, solvents such as polyethylene glycol or glycerin that are used to dissolve the nicotine, and any added flavorant chemicals, if relevant. However, heating the solution in order to aerosolize the nicotine causes **pyrolysis**, which is a process of heat-induced chemical decomposition. For example, products of glycerin pyrolysis include toxic aldehydes such as formaldehyde, acetaldehyde, and acrolein. Therefore, e-cigarette vapor does contain some of the harmful chemicals previously found in tobacco smoke. On the other hand, risk assessments of vaping compared with tobacco smoking based on measurement of chemicals implicated in cancers, COPD, and cardiovascular disease concluded that the risk from vaping was much lower than that from smoking, because the concentrations of these chemicals were many times lower in e-cigarette vapor than in tobacco smoke (Goniewicz et al., 2014; Wilson and Gartner, 2016).

Nicotine itself can pose some risk for people with preexisting cardiovascular disease, so these individuals are advised to avoid using even e-cigarettes (Benowitz and Burbank, 2016).

To summarize, e-cigarettes appear to be much safer than tobacco cigarettes in the risk they pose for developing the chronic diseases associated with tobacco smoking. But the epidemiological studies needed to assess the actual risks from long-term vaping haven't yet been done. That is, even if chronic vapers are *less* likely to develop lung cancer later in life than chronic tobacco smokers, we don't yet know whether they are *more* likely to develop cancer than matched controls who neither smoke nor vape. Considering our present state of knowledge, it seems advisable that highly dependent smokers who have been unable to quit despite numerous attempts using available medications and psychosocial support services should consider switching to e-cigarettes. But, of course, the best approach is to avoid nicotine-containing products in the first place so that dependence never occurs.

Such findings have led to concerns that e-cigarettes are becoming a "gateway" to tobacco use (Kandel and Kandel, 2015; Chatterjee et al., 2016), although there are also critics of this view (Bell and Keane, 2014; Phillips, 2015). A general discussion of the gateway theory of substance use was provided in **Web Box 9.1**.

Why do smokers smoke and vapers vape?

Researchers have identified multiple factors that contribute to the development and maintenance of a smoking or vaping habit. As discussed below, these include not only nicotine but also other pharmacological and psychological factors.

THE ROLE OF NICOTINE IN SMOKING AND VAPING

Delivery of nicotine is obviously one of the key factors in smoking. As mentioned earlier, nicotine is intravenously self-administered by animals as well as by humans under some conditions. Over a 24-hour period, a regular smoker undergoes repeated elevations and drops in plasma nicotine, as shown in **FIGURE 13.13**. However, whereas cigarettes early in the day (beginning particularly with the first cigarette, which is smoked after the overnight period of abstinence) may elevate mood above the baseline level, later in the day even the peaks in plasma nicotine may not be sufficient to do more than merely maintain a neutral mood (by preventing withdrawal symptoms), because of the nicotinic receptor desensitization discussed previously (Benowitz, 2010). Overnight abstinence permits the receptors to resensitize and the cycle to begin again the following day. Although comparable research has not yet been performed with regular users of e-cigarettes, it's reasonable to conclude that the same patterns are present for those individuals because the same route of drug administration (inhalation) applies to both nicotine-containing vehicles.

Other evidence for an involvement of nicotine comes from an analysis of smoking and vaping behaviors. Smokers allowed to sample either nicotine-containing or denicotinized cigarettes preferred the former, even though the participants were blinded to the experimental conditions (Falcone et al., 2016). Furthermore, smoking intensity is increased when smokers of regular cigarettes switch to a brand that is low in nicotine and tar. This change in smoking behavior increases nicotine yields well beyond those specified by the Federal Trade Commission (FTC), which are based on standardized smoking by a machine. The history of e-cigarettes similarly shows that nicotine-free e-cigarettes have low popularity, and early nicotine-containing models that were inefficient in delivering the drug were quickly replaced by models with much greater efficiency. Obviously this change would not have occurred had there not been a demand for greater nicotine availability on the part of e-cigarette users.

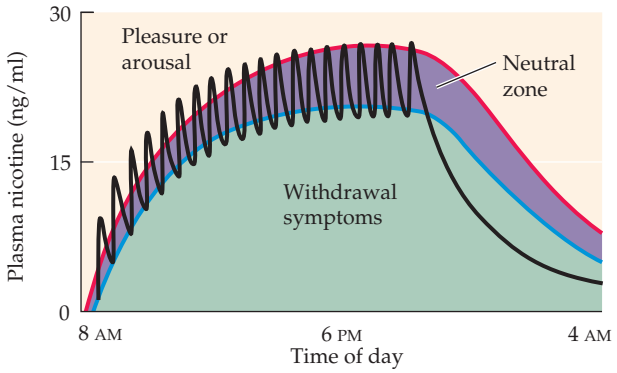


FIGURE 13.13 The daily smoking cycle and nicotine-induced mood changes Plasma nicotine is low in the morning as a result of overnight abstinence from smoking. Each cigarette over the course of the day leads to a spike followed by a decline in nicotine levels. The purple area depicts the zone of neutral affect (mood) between the pleasure and arousal produced by elevated nicotine levels (i.e., levels above the neutral zone) and the withdrawal symptoms produced by declining levels in a dependent smoker (i.e., levels below the neutral zone). Note that nicotine-induced positive effects diminish with repeated smoking episodes as a result of rapid tolerance to the drug's action, whereas withdrawal symptoms may become more pronounced. The nicotinic receptors resensitize significantly during overnight abstinence, allowing the cycle to repeat itself each day. (After Benowitz, 1992.)

Above we discussed the involvement of tolerance and dependence in the maintenance of smoking behavior. Although both processes contribute to smoking, we note that they are distinct from each other. In fact, chippers develop tolerance to nicotine despite their limited exposure to the drug (Perkins, 2002). The finding that nicotine dependence and tolerance can be at least partly separated from each together suggests that they are produced by different physiological processes. It would be very useful to understand why some individuals can maintain low levels of smoking and a minimal degree of dependence, but unfortunately we don't yet know what characteristics differentiate chippers from more typical smokers. Perhaps as time goes on, researchers will discover that "chipping" is more common in vapers than in tobacco smokers.

MOOD, STRESS, AND COGNITION Stress and negative affective states, especially anxiety, play significant roles in smoking (F. S. Hall et al., 2015; Holliday and Gould, 2016). Smokers routinely report that smoking causes relaxation and alleviation of stress. Yet another reported reason for smoking is an increased ability to concentrate. Consequently, some researchers have hypothesized that smoking (presumably through the delivery of nicotine) provides two specific advantages to the smoker: greater mood control (specifically with respect to stress reduction) and enhancement of concentration. This has been termed the **nicotine resource model**. An alternative model, sometimes called the **deprivation reversal model**, suggests that the positive effects of smoking actually represent the alleviation of irritability, stress, and poor concentration experienced by smokers between cigarettes. This model, therefore, proposes that having a smoking habit increases overall stress, which then must be countered by repeated smoking.

There is ample evidence for both of these models. Clearly, nicotine withdrawal causes a dysphoric mood and impaired attention, both of which are alleviated by nicotine consumption. Nevertheless, we cannot rule out the possibility that nicotine and other unconditioned or conditioned aspects of smoking behavior exert positive effects on these outcome measures. Advocates of the nicotine resource model can also point to the high rates of cigarette smoking among people with major depression, anxiety disorders, and schizophrenia (Featherstone and Siegel, 2015; Kutlu et al., 2015; Fluharty et al., 2017; Weinberger et al., 2017). It is possible that at least some of these individuals are engaged in self-medication to treat their negative affect and/or cognitive deficits. However, such a causal relationship is currently hypothetical, given the difficulty in providing experimental proof.

We mentioned earlier that women overall are more susceptible to smoking than men. Furthermore, women are more likely than men to smoke in order to alleviate negative mood states, especially anxiety. Based on these and other findings, Torres and O'Dell (2016) recently proposed a model emphasizing that anxiety is a more important factor in smoking initiation and relapse in women than in men. Ovarian hormones, particularly estrogens, are believed to play a key role in the increased vulnerability of women to tobacco use and addiction (Park et al., 2016; Torres and O'Dell, 2016).

THE ROLE OF OTHER FACTORS IN SMOKING AND VAPING Even though most of us think "nicotine" when we think about smoking, it cannot be the only factor responsible for maintaining this behavior. Indeed, given that many of the nicotinic receptors responsible for stimulating DA release in the NAcc are thought to be desensitized for most of the day in regular smokers, why do such individuals continue to smoke throughout the day with relatively little direct nicotine reinforcement? One reason, of course, is because they are nicotine-dependent and want to avoid nicotine withdrawal symptoms. However, there is also strong evidence that sensory stimuli associated with the act of smoking, such as the taste and smell of inhaling cigarette smoke, become conditioned

to the reinforcing effects of nicotine and are thus able to function as secondary reinforcers themselves (Rose, 2006; Rupprecht et al., 2015). Indeed, formerly secret internal documents obtained from major cigarette manufacturers reveal that these companies know about the importance of the sensory qualities of tobacco smoke and manipulate these qualities to enhance smoker satisfaction (Carpenter et al., 2007).

The great majority of studies on tobacco dependence focus on nicotine because of the substance's known reinforcing properties. However, cigarette smoke contains over 8000 other chemical constituents (Rodgman and Perfetti, 2013), and many chemicals are added to tobacco cigarettes and e-cigarette nicotine solutions as flavorings or for other purposes. Significantly, researchers have found evidence that some of these endogenous or added chemicals either have their own reinforcing properties or alter nicotine uptake, bioavailability, or neurophysiological effects (Brennan et al., 2014; van de Nobelen et al., 2016). Space limitations preclude a discussion of all of these effects; therefore, we will focus on two lines of research, one pertaining to inhibition of monoamine oxidase (MAO) enzymes by cigarette smoke and the other pertaining to the influence of the flavor additive menthol.

Researchers discovered some years ago that smokers have substantially lower levels of MAO-A and MAO-B activity in the brain as well as in several peripheral organs that express one or both of these enzymes. **FIGURE 13.14** shows PET scans demonstrating MAO-B inhibition in various organs of a smoker compared with a nonsmoker. MAO inhibition in smokers has been attributed to non-nicotine constituents of cigarette smoke, although it's not yet clear which of these substances is most important for this effect (Hogg, 2016). Because MAO is important in the breakdown of DA, it is plausible that MAO inhibition might contribute to the reinforcing effects of smoking. This hypothesis is supported by recent experiments demonstrating that pharmacological inhibition of MAO-A, the MAO subtype important for DA metabolism, enhances IV self-administration of low nicotine doses (Smith et al., 2016). The interaction between MAO and nicotine reinforcement indicates that inhibition of the enzyme is probably only important for people smoking low-nicotine cigarettes. Whether MAO inhibition also occurs in e-cigarette users remains to be determined.

A second line of research has focused on flavorings such as menthol, which is frequently used in both tobacco cigarettes and e-cigarettes. Menthol is a chemical extracted from the mint plant *Mentha arvensis* (see **FIGURE 13.15** for the structure of menthol). Most cigarettes contain at least some menthol, although only certain brands contain high enough levels to be marketed as mentholated cigarettes. Such brands comprise about 25% of cigarette sales overall but are especially appealing to adolescents and to new smokers generally. Menthol is used in cigarettes to provide a pleasant sensory experience that combats the harsh, irritating qualities of tobacco smoke. This effect is mediated by menthol-induced activation of TRPM8 receptors, a class of ion channels expressed by sensory neurons, that elicits a sensation of cooling (Wickham, 2015). Recent research has unexpectedly found that menthol also acts as a negative allosteric modulator of both high- and low-affinity nAChRs in vitro, which means that menthol reduces nicotine-mediated activation of these receptors (Kabbani, 2013; Wickham, 2015). This interaction might require the user to smoke or vape more frequently or more intensively (in terms of puff rate and/or volume) in order to experience the same effects of the nicotine. Additionally, a brain imaging study showed higher levels of $\alpha 4\beta 2$ subunit–containing receptors in the brains of menthol cigarette smokers than in smokers of nonmenthol brands (Brody et al., 2013). Together, these effects may contribute to an exceptionally high degree of dependence in smokers of menthol cigarettes. It remains to be seen whether menthol or other e-cigarette flavor additives contribute to the development of dependence in vapers.

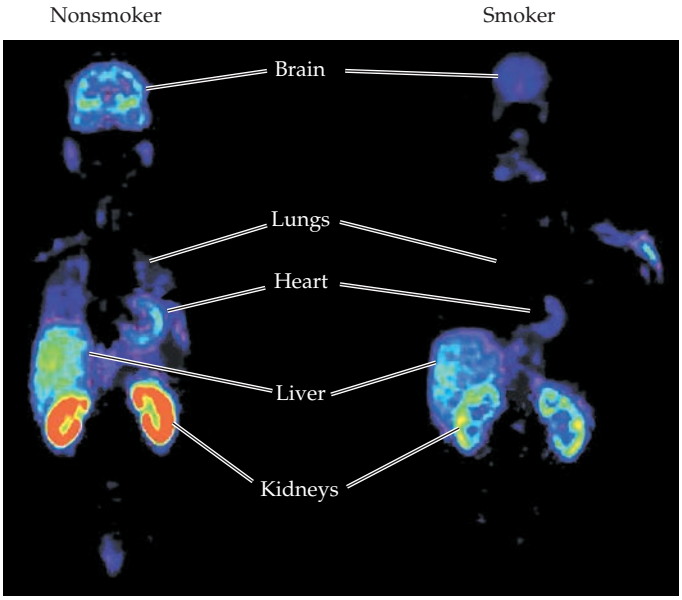


FIGURE 13.14 Whole-body PET scans illustrating reduced MAO-B activity The organs of a smoker are compared with those of a nonsmoker. (From Fowler et al., 2003.)

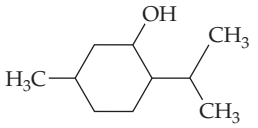


FIGURE 13.15 Chemical structure of menthol

Smoking is a major health hazard and a cause of premature death

The World Health Organization (WHO) collects global information on the prevalence of tobacco use and the consequent health effects. According to WHO, over 5 million people worldwide die each year from the direct consequences of tobacco use, whereas an estimated 600,000 nonsmokers die from exposure to secondhand smoke (World Health Organization, 2016a). In 1964, the Surgeon General of the United States released the first federally authorized report on the health consequences of tobacco smoking. There have been periodic updates of the Surgeon General's report, with the most recent version being published in 2014 to mark the fiftieth anniversary of the first release. Despite the recent decline in the prevalence of smoking, the latest report concludes that over 400,000 people in the United States continue to die annually from smoking-related causes, and the economic costs of smoking are estimated at approximately $300 billion due to a combination of smoking-related medical costs and lost productivity (U.S. Department of Health and Human Services, 2014). The remainder of this section is divided into two parts, the first dealing with the health consequences of smoking in adults and the second dealing with the consequences of developmental exposure to tobacco smoke and/or nicotine.

ADVERSE HEALTH EFFECTS OF SMOKING IN ADULTHOOD Cigarette smoking increases the long-term risk for many life-threatening illnesses, including several kinds of cancer, cardiovascular disease, and respiratory disease (Shah and Cole, 2010; Forey et al., 2011; Siegel et al., 2015; U.S. Department of Health and Human Services, 2014). The organs known to be adversely affected by direct exposure to tobacco smoke and the resulting cancers and chronic diseases are depicted in **FIGURE 13.16**. The deleterious effects of smoking stem from a combination of factors, including tar, carbon monoxide gas that is produced by the burning of tobacco, and nicotine. Tar contains a number of toxic substances, such as **nitrosamines**, that have been implicated in smoking-related carcinogenesis and other disease mechanisms (Yalcin and de la Monte, 2016). Besides the well-known strong association between cigarette smoking and lung cancer, smoking can also lead to other respiratory diseases such as emphysema and chronic bronchitis. The latter two conditions are sometimes combined into a single disorder called **chronic obstructive pulmonary disease**, or **COPD**. Although there is less public recognition of the relationship between smoking and cardiovascular disease, this relationship is actually quite strong. Smokers are at increased risk for heart attack, stroke, and atherosclerosis. Other disorders linked to long-term smoking are

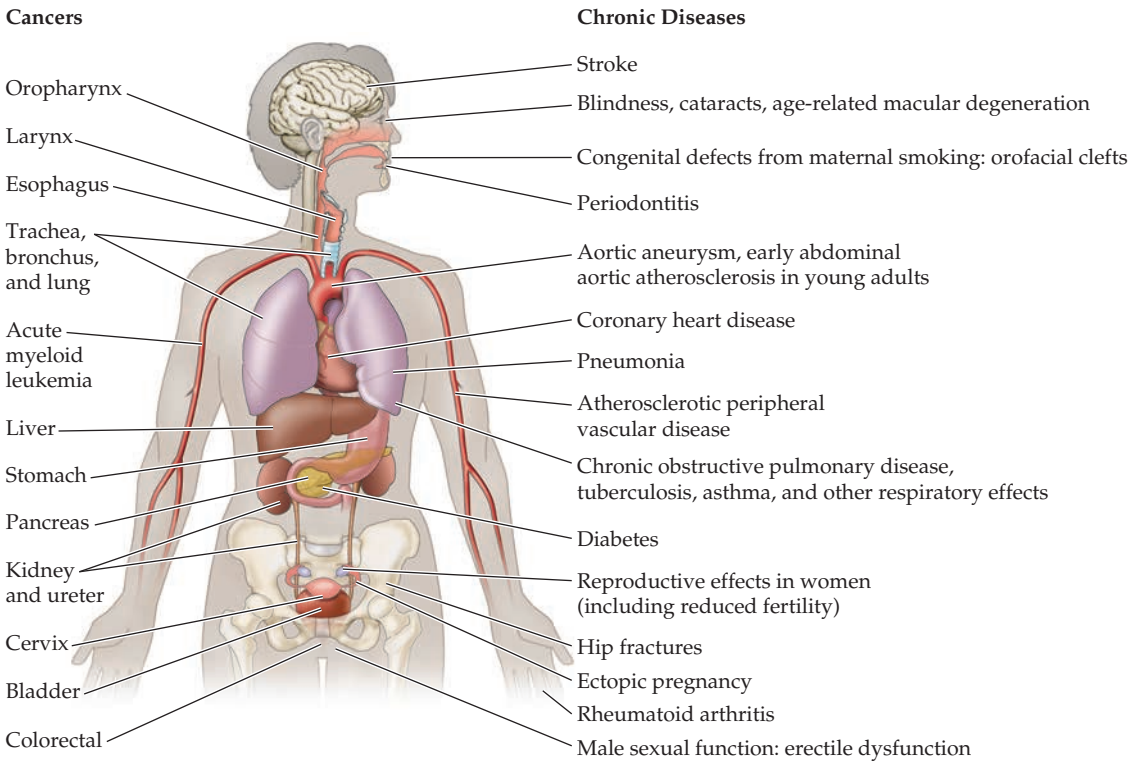


FIGURE 13.16 Cancers and chronic diseases that have been causally related to tobacco smoke exposure
(After U.S. Department of Health and Human Services, 2014.)

diabetes, rheumatoid arthritis, macular degeneration (deterioration of the central region of the retina), erectile dysfunction, and reduced immune system function (U.S. Department of Health and Human Services, 2014). Some of the mechanisms by which cigarette smoking predisposes people to later development of these diseases are well defined, while others are not. Important new information on this issue comes from a recent large-scale study of current smokers, former smokers, and never smokers showing that smoking produces epigenetic effects, expressed as changes in DNA methylation, on over 1000 genes (Joehanes et al., 2016). Such epigenetic effects, which can powerfully alter gene expression for long periods of time, may be a key mechanism linking smoking with disease risk later in life even in people who have stopped smoking.

The potential health risks of vaping have not been studied nearly as extensively as the risks associated with tobacco smoking. Nevertheless, there is some recent research on this topic that is presented in Box 13.1.

DEVELOPMENTAL CONSEQUENCES OF SMOKE OR NICOTINE EXPOSURE Researchers concerned with the developmental consequences of smoke or nicotine exposure have focused on two periods of exposure, namely fetal exposure and adolescent exposure. Fetal exposure occurs when a woman smokes during her pregnancy. When this happens, nicotine along with many other chemicals present in cigarette smoke pass through the placenta to reach all organs of the developing fetus, including the brain. Other impacts can occur through disruption of the mother's endocrine system and placenta (Marom-Haham and Shulman, 2016). Experimental studies involving administration of nicotine to pregnant animals have shown that prenatal exposure to this drug has long-lasting deleterious effects on somatic and neurobehavioral development of offspring (Winzer-Serhan, 2008; Bruin et al., 2010). Moreover, human clinical studies have consistently found an association of maternal cigarette smoking with **intrauterine growth restriction** (**IUGR**), which means that fetal growth is hampered, resulting in babies born underweight (commonly defined as below the tenth percentile for gestational age; Reeves and Bernstein, 2008). IUGR is significant because it puts the infant at a greatly elevated risk for perinatal mortality. Even if the affected infant survives to adulthood, he may still be susceptible later to developing cardiovascular disease, type 2 diabetes, or stroke. These long-term consequences of maternal smoking on the offspring are consistent with the **developmental origins of health and disease** hypothesis. This hypothesis, which originated from the work of David Barker and colleagues, postulates that characteristics of the intrauterine environment, such as nutrient availability and the presence of drugs, environmental toxins, or infectious agents, "program" the fetus in a way that determines subsequent vulnerability for developing chronic diseases (Wadhwa et al., 2009). Such diseases may be either somatic or neuropsychiatric, and there is growing evidence that the programming depends on epigenetic mechanisms within the fetus and, in some cases, the placenta (reviewed by Fernandez-Twinn et al., 2015; Kubota et al., 2015; Eriksson, 2016; Marsit, 2016). For these reasons, pregnant smokers are urged to quit their smoking habit. Because nicotine alone can exert adverse effects on the fetus, vapers as well as women using the nicotine patch or other kinds of nicotine replacement therapy for smoking cessation should likewise attempt to stop their exposure to the drug (Slotkin, 2008).

The influence of nicotine exposure on the adolescent brain has also been a significant area of concern. Adolescence is a vulnerable period because the brain is still developing during this time. Although most of the adult complement of neurons are formed by the beginning of adolescence, synaptic connections are changing rapidly (some synapses are strengthened significantly while others are pruned away), myelination of the cerebral cortex is not yet completed, and neurotransmitter systems are still maturing during the adolescent period. Because of the dynamic processes taking place during adolescence, it shouldn't be surprising that nicotine can perturb the ongoing developmental trajectory. Specifically, experimental animal studies have shown that even modest exposure to nicotine during adolescence leads to long-term changes in the cholinergic, serotonergic, and dopaminergic systems, altered dendritic branching in many brain areas, and various behavioral effects, including attention deficits, impulsivity, and increased self-administration of abused drugs (Slotkin, 2002; Counotte et al., 2011; Lydon et al., 2014; R. F. Smith et al., 2015; Yuan et al., 2015). These findings demonstrate that even though e-cigarette vapor doesn't contain most of the harmful chemicals found in tobacco cigarette smoke, use of vaping products by young people may pose a hazard to their neurobehavioral development.

Behavioral and pharmacological strategies are used to treat tobacco dependence

Surveys indicate that 70% to 75% of current smokers in the United States would like to quit smoking, and about 40% to 45% of daily smokers actually attempt to quit each year. Most attempts at quitting are performed without the use of medication or psychological counseling, although the trend globally is toward a lower rate of unassisted quit attempts (Edwards et al., 2014). This trend may reflect increased knowledge about and access to smoking cessation medications, particularly over-the-counter (OTC) medications. Smokers who try to quit on their own often believe that quitting is

their personal responsibility and that going it alone is the best approach for them (A. L. Smith et al., 2015). However, addiction to nicotine is so powerful that the success rate is quite low, even with medication, and multiple quit attempts may be required before success is ultimately achieved.

We will consider a variety of behavioral and pharmacological approaches for treating tobacco dependence. It is important to recognize that the success rate of any treatment approach is influenced by numerous variables, such as the duration of smoking behavior and the number of cigarettes smoked daily, the intensity of the abstinence syndrome, the motivation to quit, whether or not the smoker lives and/or works in a smoking environment, and so on. Additionally, even though the greatest benefit would be gained by getting adolescent smokers to quit before their habit has become ingrained by years of tobacco use, most smoking cessation programs and medications are developed and tested on adults. Fortunately, some researchers have begun to focus on the development and evaluation of treatment approaches specifically targeted at adolescent smokers (Simon et al., 2015; Towns et al., 2017).

BEHAVIORAL AND PSYCHOSOCIAL INTERVENTIONS A number of strategies are directed toward discouraging young people from beginning tobacco use or giving it up if it is already habitually used. For example, various state and federal agencies sponsor antismoking appeals in the media, and the Surgeon General's office has mandated health warnings on cigarette packages for many years. Another approach is the levying of high taxes on tobacco products. This may not prevent people from starting to use these products, but it does reduce the amount of use.

Years ago, many smokers attempted to quit by using various self-help programs published in books or manuals, none of which offered much benefit to the smoker. In recent years these written products have been largely supplanted by other media. For example, smokers now have available a variety of web-based cessation programs such as smokefree.gov, which is run by the National Cancer Institute. Another government-sponsored cessation tool is a toll-free telephone quitline at 1-800-QUIT-NOW. The phone line is staffed by experienced counselors who will help the smoker develop an individualized quit plan based on her personal history. Importantly, the medications that will be discussed in the next section are generally more successful when they are accompanied by some kind of counseling or behavioral intervention (Stead et al., 2015; 2016). Consequently, smokers who desire to quit are advised to seek behavioral or psychosocial (supportive) therapy along with medication to optimize their chance of succeeding.

PHARMACOLOGICAL INTERVENTIONS Medications development for smoking cessation has followed three main paths: (1) nicotine replacement therapy, (2) non-nicotine drugs aimed at reducing tobacco craving and withdrawal symptoms, and (3) antinicotine vaccines (Cahill et al., 2013; Prochaska and Benowitz, 2016). We will discuss each of these in turn, considering their current status and efficacy.

The most common pharmacological intervention for smoking cessation is **nicotine replacement therapy** (**NRT**). This approach is based on the following premises: that the difficulty associated with smoking cessation is significantly related to nicotine withdrawal symptoms; that blocking (or at least reducing) these symptoms by maintaining a certain circulating level of nicotine can assist in terminating smoking; that maintaining some nicotine in the smoker's system can blunt the reinforcing effectiveness of nicotine derived from smoking a cigarette; and that there are safer ways for individuals to obtain nicotine than by smoking. This last point is embodied by Russell's (1976) comment that "people smoke for nicotine but they die from tar." NRT was first made available after the formulation of a special nicotine-containing chewing gum (nicotine polacrilex), which has the advantage that nicotine can be absorbed more readily through the buccal mucosa (mucous membranes lining the mouth) than the gastrointestinal tract, where absorption is minimal and there is substantial first-pass metabolism in the liver. Nicotine gum was approved as a pharmacotherapeutic aid in the treatment of cigarette dependence in 1984 under the trade name Nicorette. This was later followed by the transdermal nicotine patch (Nicoderm, Habitrol, Nicotrol), nicotine nasal spray (Nicotrol NS), nicotine inhaler (Nicotrol Inhaler), and nicotine lozenge (Commit). Of these many nicotine-containing products, only the nasal spray and inhaler require a doctor's prescription. Making at least some OTC nicotine medications available was a significant advance in the battle against smoking, because some smokers are reluctant or unable financially to enter a formal treatment program yet may still be willing to try something they can obtain at a drug store without a prescription. **TABLE 13.3** lists some of the advantages and disadvantages of each nicotine delivery vehicle. It is worth noting that combination treatments such as nicotine gum plus the patch, or the nicotine inhaler plus the patch, may be more effective than either treatment alone in helping some smokers quit their habit (Prochaska and Benowitz, 2016).

Earlier in the chapter we discussed the negative side of e-cigarettes as a potential gateway to nicotine use and eventual dependence. But for current smokers, it is possible that e-cigarettes and other ENDS could serve as a new kind of NRT. Given the relatively recent

TABLE **13.3** Advantages and Disadvantages of Different Kinds of Nicotine Replacement Therapy

Treatment	Advantages	Disadvantages
Nicotine polacrilex (gum)	Ease of use; flexible dosing; OTC availability; rapid nicotine delivery	Need for frequent dosing; side effects such as jaw pain or mouth soreness
Nicotine lozenge	Ease of use; flexible dosing; OTC availability; rapid nicotine delivery	Need for frequent dosing; side effects such as heartburn or indigestion with rapid lozenge consumption
Transdermal nicotine patch	Ease of use; OTC availability; reduced morning craving with overnight use; few side effects except for possible skin irritation	Less flexible dosing and slower delivery of nicotine than with other treatments; possible insomnia with overnight use
Nicotine nasal spray	Flexible dosing; fastest nicotine delivery and reduction of cravings	Need for frequent dosing; initial side effects such as nose and eye irritation, sneezing, and coughing
Nicotine inhaler	Flexible dosing; similarity to hand-to-mouth feature of smoking; few side effects except for mild throat irritation and coughing	Need for frequent dosing

introduction of these products, relatively little information is available on their efficacy for smoking cessation. Recent reviews indicate that ENDS may work for some smokers who are trying to quit; however, the quality of the currently available evidence is poor (Lam and West, 2015; Khoudigian et al., 2016; Malas et al., 2016). Thus, better research is needed in order to evaluate properly the therapeutic benefit of ENDS for this purpose.

Although NRT continues to be an important tool for smoking cessation, two other pharmacological approaches have proven valuable in helping people quit smoking. The first is **bupropion**, a compound that was initially developed as an antidepressant and then later reformulated as a sustained-release preparation (trade name **Zyban**) for treatment of tobacco dependence. The efficacy of bupropion as a smoking cessation medication was discovered serendipitously when researchers noticed that depressed patients who were smokers and were given this compound for their depression reported reduced cigarette cravings and were able to quit smoking without additional therapeutic intervention. The antismoking properties of bupropion are thought to be related to its pharmacological profile as a reuptake inhibitor of DA and NE and as a weak nAChR antagonist. The second non-NRT medication is **varenicline** (**Chantix**), which acts as a partial agonist at high-affinity $\alpha 4\beta 2$ nAChRs that are expressed in the VTA as well as in other brain areas. This partial agonism elicits a moderate amount of receptor activation, in contrast to the full receptor agonism produced by cigarette-derived nicotine. The resulting effect is a reduction of nicotine cravings and of adverse withdrawal reactions in the abstinent smoker (Prochaska and Benowitz, 2016).

Finally, you may recall that in Chapter 12 we discussed efforts by researchers to develop a vaccine against cocaine. Similar work targeting nicotine has led to the development of vaccines that, in animal studies, reduce nicotine availability to the brain and block reinstatement of nicotine-seeking behavior (Zalewska-Kaxzubska, 2015). Unfortunately, clinical trials found that the first generation of antinicotine vaccines were largely ineffective (van Schayck et al., 2014). Nevertheless, researchers are continuing to work on this problem, since a vaccine approach has the potential to be much more effective than existing pharmacotherapies.

Section Summary

- Approximately 24% of the population of this country (corresponding to 64 million people) were current users of tobacco products at the time of the 2015 National Survey on Drug Use and Health. Less than 4% of adults age 18 or older were current users of e-cigarettes according to the 2014 National Health Interview Survey. Overall, cigarette smoking is declining across the population, whereas the use of e-cigarettes is growing.
- Smokers typically begin during adolescence, and many different reasons have been given for teenage smoking. Longitudinal surveys found that poor academic performance, rebelliousness, sensation seeking, receptivity to cigarette advertising/marketing, and smoking by family and/or friends are significant predictors of smoking onset.

- Individuals go through several stages on the way from nonsmoking through occasional smoking and then eventually to established smoking. Several different kinds of trajectories have been reported for people progressing through these stages. DiFranza and coworkers have proposed a model of adolescent nicotine dependence in which smokers pass through successive stages of "wanting," "craving," and finally "needing" a cigarette. Once nicotine dependence has developed, cigarettes become compulsory (i.e., smoked in order to alleviate withdrawal symptoms) instead of elective.
- Although first exposure to a cigarette is aversive in most cases, a subset of individuals experience relaxation at their first smoking experience. This response is likely related to the rewarding effects of nicotine.
- Various hypotheses have been offered to explain why young people are more attracted to e-cigarettes than tobacco cigarettes. These hypotheses are related to flavor, health, price, concealment, role models, and acceptance of e-cigarettes compared with tobacco cigarettes. Because e-cigarettes are now the most common route of nicotine exposure among young people, concerns have been raised that e-cigarettes are becoming a gateway to nicotine dependence and, in some cases, use of combustible tobacco products.
- Several factors contribute to the development and maintenance of a smoking habit. Delivery of nicotine and, for many, the development of nicotine dependence are obviously key factors in smoking and vaping. Repeated cigarette smoking during the day leads to nicotinic receptor desensitization, which is partially reversed by overnight abstinence. Because smokers commonly report that smoking causes relaxation, a reduction in stress, and increased concentration, some researchers have proposed a nicotine resource model hypothesizing that the nicotine obtained through smoking has the beneficial effects of increasing mood control (in relation to stress reduction) and enhancing concentration. An alternative model, the deprivation reversal model, argues that the positive effects experienced during smoking actually constitute the alleviation of withdrawal effects such as irritability, anxiety, and poor concentration. Both models are supported by certain evidence, with mood regulation (i.e., alleviation of anxiety) perhaps being more important in female than male smokers. In addition, stimuli associated with cigarette smoke, such as the taste and smell of cigarette smoke, are thought to function as secondary reinforcers after being repeatedly paired with the primary reinforcing effects of nicotine.
- Other constituents of cigarette smoke or e-cigarette vapor may also influence smoking or vaping behavior. Certain non-nicotine constituents of tobacco inhibit MAO-A, an enzyme important for DA metabolism. Researchers have hypothesized that this effect is likely to be most important for enhancing the reinforcement of low-nicotine cigarettes. Menthol, a well-known flavorant used in both tobacco cigarettes and e-cigarettes, acts as a negative allosteric modulator of nAChRs. The net effects of this interaction may be to increase the frequency and/or intensity of smoking or vaping by the user.
- Chippers are long-term smokers who smoke a few cigarettes daily on a regular basis but do not become dependent. However, chippers do develop nicotine tolerance, which suggests that tolerance and dependence are produced by different physiological mechanisms.
- Although withdrawal symptoms undoubtedly play an important role in maintaining the smoking habit in dependent smokers, other factors also contribute to this habit, including the taste and smell of cigarette smoke.
- Chronic use of tobacco results in many adverse health consequences, including cancer, cardiovascular disease, and respiratory diseases such as emphysema and bronchitis. The deleterious effects of inhaling cigarette smoke have been related not only to nicotine but also to tar and carbon monoxide gas that is produced by the burning of tobacco.
- Smoking by a pregnant woman has adverse consequences for her fetus due to a combination of nicotine exposure, exposure to other components of cigarette smoke, and disruption of the woman's endocrine system. Infants born to smokers are at elevated risk for intrauterine growth restriction and later susceptibility to developing cardiovascular disease, type 2 diabetes, or stroke. The long-term consequences of smoking during pregnancy are consistent with the developmental origins of health and disease hypothesis.
- The brain is still developing during adolescence, and animal studies have found that exposure of the adolescent brain to nicotine can have deleterious effects on dendritic branching and on neurotransmitter development. Behavioral processes such as attention, impulse control, and

vulnerability to drug self-administration are also influenced by adolescent nicotine exposure.

- Many different treatment strategies have been developed to assist smokers in quitting. Individuals who are trying to quit can take advantage of various behavioral and psychosocial interventions, some of which are web based and available at no cost. Pharmacological interventions take three forms: nicotine replacement therapy (NRT), non-nicotine drugs aimed at reducing craving and withdrawal symptoms, and antinicotine vaccines. Nicotine replacement can be accomplished by means of nicotine gum, lozenges, patches, nasal spray, or an inhaler. For some, e-cigarettes may represent yet another potential kind of NRT. Currently available non-nicotine drugs for smoking cessation are bupropion (Zyban), which is a mixed DA and NE reuptake inhibitor and weak nAChR antagonist, and varenicline (Chantix), which is a partial agonist at high-affinity $\alpha4\beta2$ nAChRs. Both NRT and these alternative pharmacological approaches are more effective when paired with some type of behavioral or psychosocial intervention. Finally, a nicotine vaccine that reduces nicotine availability to the brain has been under development for a number of years; however, clinical testing has not yet demonstrated significant therapeutic efficacy of this approach. Because breaking the smoking habit is almost always a difficult proposition, it is much better never to become dependent in the first place.

Caffeine

Background

If you are like most people living in Western societies, you probably had at least one cup of coffee (or perhaps tea) this morning, possibly followed by additional cups as the day progressed. Whether you like the taste of coffee (which is bitter when taken black) or not, you are probably consuming it at least partly for its pharmacological properties as a stimulant. This, of course, brings us to the subject of **caffeine**, the principal psychoactive ingredient in coffee.

FIGURE 13.17 The coffee plant, *Coffea arabica* (© istock.com/elenaleonova.)

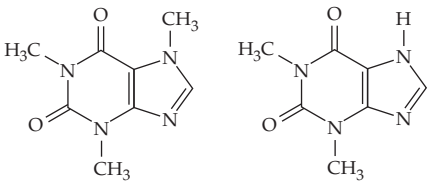


FIGURE 13.18 Chemical structures of caffeine and theophylline

The major source of caffeine is coffee beans, which are the seeds of the plant *Coffea arabica* (**FIGURE 13.17**). Tea leaves contain significant amounts of both caffeine and a related compound called **theophylline** (which is Greek for "divine leaf") (**FIGURE 13.18**). Caffeine and theophylline are members of a wider group of plant-derived chemicals called **methylxanthines**.

Coffee is believed to have been discovered in Ethiopia around 850 CE, and indeed the names of the plant genus *Coffea* and the beverage made from its seeds are derived from the Arabic word *quahweh* (Gonzalez de Mejia and Ramirez-Mares, 2014). Coffee was brought to Europe by Venetian merchants in 1615, after which its consumption spread rapidly. The stimulating effects of coffee have been known for hundreds of years. For example, the nineteenth-century French author Honoré de Balzac, who reportedly consumed immense amounts of the beverage, wrote an article in 1830 entitled "Pleasures and Pains of Coffee" in which he stated, "Coffee slips into the stomach and you immediately feel a general commotion. Ideas begin to move like the battalions of the Grand Army on the field of battle and the battle takes place. Memories come at a gallop, carried by the wind" (cited in Cappeletti et al., 2015).

Caffeine is currently one of the most widely used drugs in the world. In the United States, for example, a large survey found that about 85% of the population (excluding children younger than 2 years of age) consume at least one caffeinated beverage per day (Mitchell et al., 2014). The same survey estimated the mean daily caffeine consumption to be 165 mg across all age groups. The typical caffeine content of various beverages, foods, and OTC drugs is shown in **TABLE 13.4**. Not surprisingly, coffee is the largest contributor to overall caffeine intake, with tea, caffeinated soft drinks, and energy drinks being the other significant sources of dietary caffeine. Information about the history and

TABLE 13.4 Caffeine Content of Selected Beverages, Foods, and Drugs

Source	Serving size	Caffeine content (mg)*
Coffee		
Regular brewed coffee	8 oz	95–330
Brewed decaffeinated coffee	8 oz	3–12
Instant coffee	8 oz	30–70
Espresso	1 oz	50–150
Tea		
Black, brewed or tea bag	8 oz	40–74
Black, decaffeinated	8 oz	2–5
Green, brewed or tea bag	8 oz	25–50
Oolong, brewed or tea bag	8 oz	21–64
White, brewed or tea bag	8 oz	15
Instant tea	8 oz	33–64
Yerba mate, brewed or tea bag	8 oz	65–130
Iced tea	12 oz	27–42
Other beverages		
Carbonated beverages with caffeine	12 oz	22–69
Alcoholic beverages with caffeine	1 oz	3–9
Energy drinks with caffeine	8.2–23.5 oz	33–400
Water with caffeine	16.9–20.0 oz	42–125
Foods		
Chocolates	8 oz	0–6
Sweets	Various	1–122
Snacks	1 oz or 1 bar	3–41
Gums and mints	Various	20–400
Fast foods	Various	1–49
Drugs		
NoDoz or Vivarin	1 tablet	200
Extra Strength Excedrin	2 tablets	130

Sources: Data for beverages and foods from Gonzalez de Mejia and Ramirez-Mares, 2014; for drugs, from Burke, 2008.
*Typical range in milligrams per serving.

current status of caffeine-containing energy drinks is provided in **Web Box 13.2.**

Basic Pharmacology of Caffeine

Caffeine is normally consumed orally through the beverages in which it is present. Under this condition, it is virtually completely absorbed from the gastrointestinal tract within 30 to 60 minutes. Caffeine absorption begins in the stomach but takes place mainly within the small intestine. The plasma half-life of caffeine varies substantially from one person to another, but the average value is about 4 hours. Consequently, people who drink coffee repeatedly over the course of a day experience gradually rising plasma caffeine concentrations. Most of this caffeine is then cleared from the circulation during sleep. The rate of plasma clearance is stimulated by smoking and is reduced when smoking is terminated (Doepker et al., 2016). The resulting increase in plasma caffeine levels could contribute to cigarette withdrawal symptoms in heavy coffee drinkers, particularly since caffeine is anxiogenic (anxiety provoking) at high doses (see the next section). In contrast, pregnant women metabolize caffeine more slowly and, therefore, should consider moderating their caffeine intake accordingly.

Caffeine is converted to a variety of metabolites by the liver. These metabolites account for almost all caffeine excretion, as only 1% to 2% of an administered dose is excreted unchanged. In humans, approximately 95% of caffeine metabolites are eliminated through the urine, 2% to 5% through the feces, and the remainder through other bodily fluids such as saliva.

Behavioral and Physiological Effects

Caffeine is best known for its ability to increase arousal; however, it can also enhance cognitive function and athletic performance when taken at appropriate doses. Regular use of caffeine can produce tolerance and dependence, and excessive ingestion poses significant health risks. Nevertheless, caffeine also has a few therapeutic uses that are mentioned below.

Acute subjective and behavioral effects of caffeine depend on dose and prior exposure

In laboratory animals such as rats and mice, caffeine has biphasic effects related to dose. At low doses, it has stimulant effects as shown by increased locomotor activity. At high doses, this effect is reversed and animals actually show reduced activity. This interesting dose–response function has been related to the neurochemical actions of caffeine, as discussed later in this chapter.

People ingest caffeine mostly for its ability to stimulate arousal, increase concentration, and reduce fatigue.

According to a recent survey of college students, caffeine-containing beverages or foods are also consumed for reasons of taste, social factors, mood enhancement, and stress reduction (Doepker et al., 2016). The arousing effects of caffeine can disrupt sleep, particularly in older adults and when the drug is consumed shortly before going to bed (Clark et al., 2017). On the other hand, these same effects can be helpful to people under conditions in which alertness has been compromised, such as working late in the day or into the night, trying to function after a period of sleep deprivation, or feeling fatigued while operating a motor vehicle. Caffeine and even stronger stimulants are also used by military personnel when they must remain alert during a long, sustained operation. As with any drug, there are significant individual differences in sensitivity to caffeine. But in general, doses that exceed 400 mg cause feelings of tension, jitteriness, and anxiety (Nehlig, 2016). Interestingly, patients suffering from panic disorder appear to be hypersensitive to caffeine's anxiogenic effects and may even suffer panic attacks in response to caffeine administration (Vilarim et al., 2011).

Caffeine does more than just increase our arousal. In controlled laboratory studies, humans receiving low or intermediate doses of caffeine report a variety of positive subjective effects, including feelings of well-being, enhanced energy or vigor, increased alertness and ability to concentrate, self-confidence, increased work motivation, and enhanced sociability (Glade, 2010). Caffeine is particularly effective in improving concentration in fatigued individuals and in the elderly (Nehlig, 2010). Additionally, recent work indicates that a caffeine dose of approximately 200 mg given after a learning task can enhance memory consolidation (Borota et al., 2014).

Because chronic caffeine consumption can lead to dependence and abstinence-related withdrawal symptoms (see later in this chapter), some investigators have raised concerns that cognitive enhancement may be due to alleviation of withdrawal symptoms rather than improvement over baseline levels (James, 2014). However, there are experimental findings that argue against this interpretation (Nehlig, 2010). For example, **FIGURE 13.19** presents some of the results from a study examining the influence of a single 2 mg/kg dose of caffeine on a variety of cognitive measures in both chronic caffeine consumers (tested after overnight abstinence) and nonconsumers (Smith et al., 2006). Note that on a measure of performance on a vigilance (i.e., sustained attention) task, there was no difference between the two groups (caffeine consumers and nonconsumers) when administered a placebo instead of caffeine. This finding suggests a lack of performance decrement due to caffeine withdrawal. More importantly, both groups benefited significantly from the caffeine treatment, supporting the interpretation that an appropriate dose of caffeine can increase attentiveness over baseline levels.

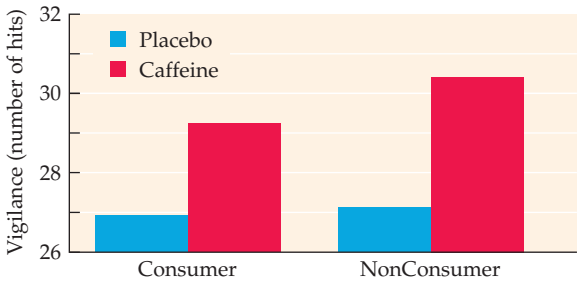


FIGURE 13.19 Effects of caffeine on attention
Caffeine (2 mg/kg) or a placebo was administered to regular caffeine consumers who had undergone overnight withdrawal (Consumer) and to nonconsumers (NonCon) of caffeine. The same individuals were tested under both drug conditions on separate days. The figure shows performance on a vigilance task of sustained attention measured as the number of correct detections (hits) of a digit signal on a computer screen. Caffeine positively affected performance in both treatment groups compared with placebo administration. (After Smith et al., 2006.)

Caffeine consumption can enhance sports performance

The influence of caffeine on athletic performance has been a topic of research for many years. Most studies have examined the acute effects of low to moderate doses of caffeine (typically 3 to 6 mg/kg, which corresponds to about 200 to 400 mg for a 70 kg person) administered before and sometimes also during performance of the sport. Caffeine may be delivered through coffee, through a preweighed amount of caffeine powder dissolved in a caffeine-free liquid, or through caffeine-containing gum. This research has generally demonstrated small but statistically significant benefits of caffeine both in endurance sports (e.g., distance running, cycling, swimming, rowing, cross-country skiing) and in short-term high-intensity activities (e.g., sprint running, sprint swimming, weight lifting) (Ganio et al., 2009; Astorino and Roberson, 2010; Higgins et al., 2016). Variability of results within and across studies suggests that some individuals benefit more than others from caffeine, although the reason for such variability is not yet known. Tarnopolsky (2008) has proposed that several mechanisms may underlie the ergogenic (performance-enhancing) effects of caffeine, including increased force of muscle contraction, enhanced arousal and alertness as discussed earlier, and reduced pain perception. Spriet (2014) more recently argued that the ergogenic properties of the abovementioned doses of caffeine more likely result from CNS-related cognitive and mood enhancement than from peripheral (i.e., muscular) effects of the drug.

Regular caffeine use leads to tolerance and dependence

What happens when people are chronically exposed to caffeine through regular coffee, tea, or soda consumption? For many years, it was believed that tolerance to the stimulating action of caffeine did not occur. However, several studies have shown that tolerance does develop to at least some of caffeine's subjective effects as well as its ability to disrupt sleep (Griffiths and Mumford, 1995). This may, for example, enable a heavy coffee drinker to consume caffeine shortly before bedtime and still fall asleep, whereas a late-night cup of coffee is likely to cause insomnia in someone who normally consumes little caffeine. Chronic caffeine use also produces tolerance to the cardiovascular and respiratory effects of the drug (see next section).

Perhaps those of you who are regular caffeine users have occasionally been forced to miss your morning cup of coffee or tea. It is likely that you experienced at least a few psychological and/or physical symptoms, including headache and lethargy or fatigue. If so, then you are dependent on caffeine. Don't worry though, as this is a very common type of drug dependence and is harmless except in a small percentage of individuals who have extremely high levels of caffeine intake. Controlled studies have demonstrated a range of caffeine withdrawal symptoms, including headache, drowsiness, fatigue, impaired concentration and psychomotor performance, and, in some cases, mild anxiety or depression. An intense craving for coffee may also be experienced. Symptoms of caffeine withdrawal can occur in individuals who are consuming as little as 100 mg/day, which is the equivalent of one 6-ounce cup of regular coffee or three cans of caffeinated soft drink (Juliano and Griffiths, 2004). If caffeine is withheld for a prolonged period of time, the abstinence syndrome lasts for a few days but then dissipates (**FIGURE 13.20**). It is important to note that despite the ability of caffeine to produce physical dependence, this compound does not meet the overall criteria necessary to be considered an addictive drug (Satel, 2006).

Researchers believe that relief from withdrawal is a major factor in chronic coffee drinking, particularly with regard to the first cup in the morning. This hypothesis is supported by controlled studies showing that physical dependence plays an important role in the reinforcing effects of caffeine and the choice to consume caffeinated beverages (Garrett and Griffiths, 1998). Caffeine withdrawal symptoms can be severe enough to cause occupational and/or social dysfunction in heavy users who have severe physical dependence on the drug. There is even evidence that some schoolchildren become dependent on caffeine as the result of heavy intake of cola and other caffeine-containing beverages or food. Indeed, frequent headaches have been reported in children and adolescents who were consuming at least

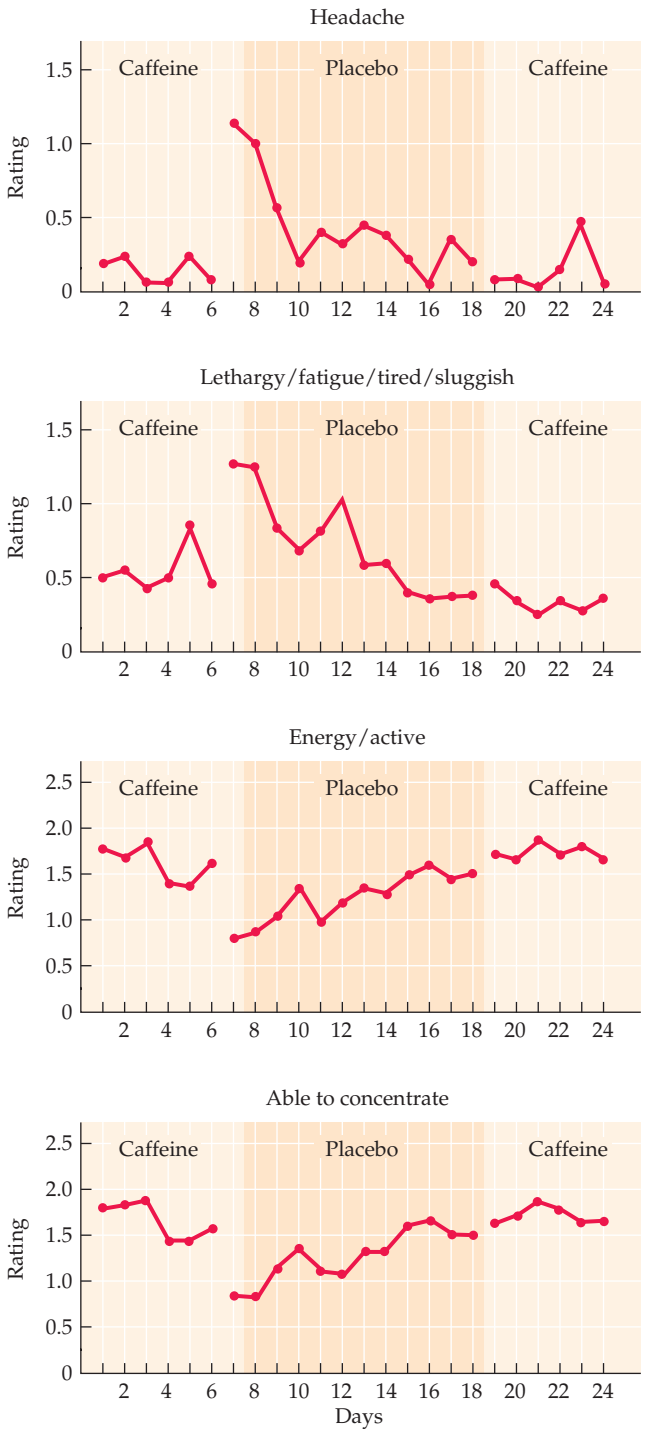


FIGURE 13.20 Time course of caffeine withdrawal in regular users During the first phase of the study (left panel in each graph), participants were maintained on 100 mg of caffeine daily in capsule form while abstaining from all dietary sources of caffeine. During the second phase (middle panel), placebo capsules were substituted for caffeine without the knowledge of the participants. In the third phase (right panel), caffeine administration was reinstated. Caffeine withdrawal symptoms rapidly appeared during abstinence; however, the symptoms gradually disappeared over the course of several days. (After Griffiths et al., 1990.)

1.5 liters of cola drinks (containing about 200 mg of caffeine) per day (Hering-Hanit and Gadoth, 2003). In almost all cases, the headaches completely disappeared following gradual cessation of caffeine consumption.

Caffeine and caffeine-containing beverages pose health risks but also exert therapeutic benefits

Acute caffeine administration leads to several effects on peripheral physiology, including increased blood pressure and respiration rate, enhanced water excretion (diuresis), and stimulation of catecholamine release from the adrenal medulla (Dews, 1982). These effects are most evident in people who are not regular caffeine consumers, indicating the production of tolerance under conditions of chronic caffeine intake.

Health organizations have reached a consensus that daily caffeine consumption up to 400 mg poses little or no risk to healthy individuals, except perhaps to pregnant women (Nehlig, 2016). As mentioned earlier, pregnant women are advised to moderate their caffeine consumption not only for their own health but also for that of their unborn children. Although chronic caffeine consumption within the indicated range is usually not harmful, problems can occur at higher doses. Clinicians and researchers have identified two disorders related to excessive caffeine use: (1) **caffeine intoxication** and (2) **caffeine dependence syndrome** or **caffeine use disorder**. Caffeine intoxication is associated with recent high-dose caffeine use (sometimes 1000 mg or more) and is characterized by symptoms of restlessness, nervousness, insomnia, and physiological disturbances including tachycardia (increased heart rate), muscle twitching, and gastrointestinal upset. This disorder is recognized both by the *DSM-5* of the American Psychiatric Association and by the *International Classification of Diseases*, tenth edition, (*ICD-10*) of the World Health Organization (Meredith et al., 2013). The idea of caffeine dependence is somewhat more controversial, even though evidence for such a disorder has been available for some time (Striley et al, 2011). In recognition of such evidence, the *ICD-10* contains a category called caffeine dependence syndrome that is contained within the general category of substance dependence syndromes. The *ICD-10* defines a substance dependence syndrome as "a cluster of behavioural, cognitive, and physiological phenomena that develop after repeated substance use and that typically include a strong desire to take the drug, difficulties in controlling its use, persisting in its use despite harmful consequences, a higher priority given to drug use than to other activities and obligations, increased tolerance, and sometimes a physical withdrawal state" (World Health Organization, 2016b). The *DSM-5* contains a category called caffeine use disorder that is designated for additional research before (potentially) being included as a recognized diagnostic category. The listed criteria for caffeine use disorder are similar, though not identical, to the *ICD-10* criteria for caffeine dependence syndrome. Reviews by several different researchers agree that problematic caffeine use occurs in some individuals but additionally concur that more research is required to confirm that the problems arising from excessive caffeine use rise to the level of a mental disorder, to determine whether the proposed criteria for caffeine use disorder are appropriate, and to ascertain the etiology and prevalence of the disorder (Addicott, 2014; Budney et al., 2015; Meredith et al., 2013).

Caffeine is not usually regarded as a medicinal agent; however, it does have a few therapeutic uses. First, caffeine has mild analgesic effects, and it can also potentiate the analgesic properties of aspirin and acetaminophen (Shapiro, 2008). For this reason, caffeine is a constituent of several OTC analgesic agents, including Anacin and Excedrin. Second, an even more important clinical use of caffeine is in the treatment of newborn infants suffering from apneic episodes (periodic cessation of breathing). Premature infants whose respiratory systems have not yet matured are particularly vulnerable to this disorder, and caffeine can be lifesaving for these babies by regularizing their breathing (Abdel-Hady et al., 2015). In addition, pediatric patients suffering from asthma or persistent cough may benefit from treatment with two other methylxanthines, theophylline and theobromine, respectively (Oñatibia-Astibia et al., 2016).

Although people primarily consume coffee for its caffeine content, its flavor, and/or for other reasons mentioned earlier, you may have come across claims in the popular media that moderate coffee consumption has health benefits. The type of research needed to test such claims involves large-scale epidemiological studies in which the prevalence of a disease in regular coffee drinkers is compared with the prevalence in noncoffee drinkers who are matched as closely as possible to the first group. Such research entails certain pitfalls, notably the difficulty encountered in matching all potentially relevant lifestyle variables in the two groups being compared. Despite this problem, a consensus is emerging that regular consumption of coffee in amounts of three to four cups per day exerts some protective effect against the development of type 2 diabetes (Santos and Lima, 2016). This effect occurs even with consumption of decaffeinated coffee; therefore, the active substances are thought to be other constituents of coffee such as **chlorogenic acids** (although caffeine may provide some additional benefit when present).[3] Substantial research has also been performed to determine whether

[3]Brewed coffee contains over 1000 identifiable chemicals of varying concentrations. This complex chemical mixture is responsible for the distinctive tastes and aromas associated with different blends of coffee and different methods of coffee roasting, grinding, and brewing.

coffee consumption exerts either harmful or beneficial effects on the development of cardiovascular diseases such as atherosclerosis, coronary heart disease, congestive heart failure, and stroke. Evidence accumulated to date suggests that coffee may reduce risk for some cardiovascular diseases but increase risk for others, especially in people who are slow caffeine metabolizers (Whayne, 2015; Zulli et al., 2016). Finally, some studies indicate that lifelong coffee/caffeine consumption reduces the risk of developing Parkinson's disease as well as age-related cognitive decline (Nehlig, 2016; Panza et al., 2015). Although these findings are encouraging, they are not yet sufficiently compelling for physicians to recommend caffeine consumption to their aging patients. Indeed, we don't yet know how long caffeine must be consumed for this putative benefit to be obtained.

Mechanisms of Action

Although the mechanism by which caffeine exerts its stimulant effects is not yet completely understood, substantial progress has been made through intensive research efforts. It is clear that caffeine does not directly influence catecholamine systems in the manner of the psychomotor stimulants amphetamine and cocaine. How else might it work? When the biochemistry of the second messenger cyclic adenosine monophosphate (cAMP) was first being studied, investigators discovered that caffeine and theophylline are inhibitors of cAMP phosphodiesterase, the enzyme that breaks down cAMP. This led to the theory that the behavioral activation produced by caffeine or theophylline was caused by a buildup of brain cAMP concentrations. Subsequent research, however, demonstrated that caffeine can be arousing at brain concentrations that are insufficient to inhibit phosphodiesterase. In addition, phosphodiesterase inhibitors that differ in their chemical structure from caffeine or theophylline are actually behavioral depressants rather than stimulants. These findings cast serious doubt on the relevance of this phosphodiesterase inhibition for the stimulant properties of caffeine and theophylline.

Subsequent research identified several other cellular actions of caffeine. These include blockade of $GABA_A$ receptors, stimulation of Ca^{2+} release within cells, and blockade of A_1 and A_{2A} receptors for a substance called **adenosine**. **FIGURE 13.21** illustrates idealized concentration–response functions for the various cellular effects of caffeine. We can see that at the levels of caffeine associated with one or a few cups of coffee, only the adenosine receptor blockade would come into play. The other effects require much higher doses, even into the toxic range associated with caffeine intoxication.

There is now overwhelming evidence that blockade of adenosine receptors, particularly the A_{2A} subtype,

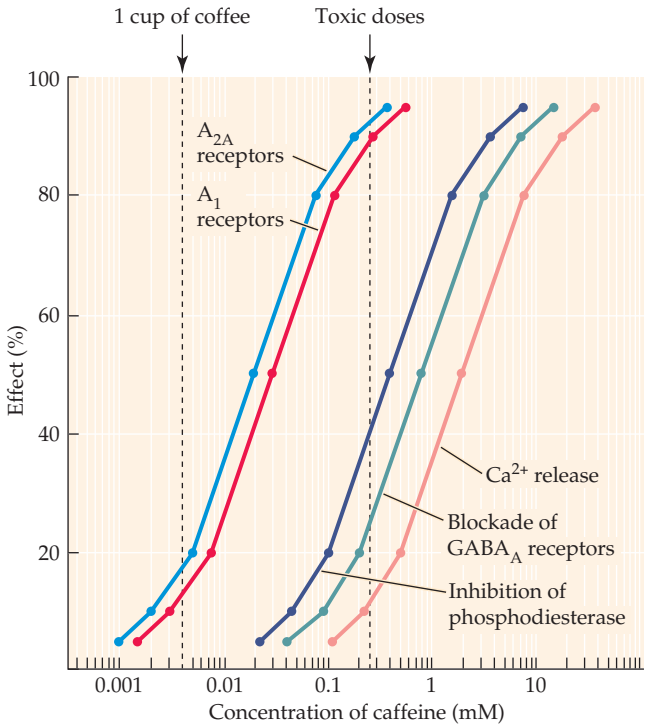


FIGURE 13.21 Concentration–response curves for caffeine's effects on various neurochemical processes Partial blockade of adenosine A_1 and A_{2A} receptors occurs at caffeine concentrations produced by doses like those typically consumed in one or a few cups of coffee. In contrast, other effects of caffeine require concentrations in the toxic range. (After Daly and Fredholm, 1998.)

underlies caffeine-induced behavioral stimulation (Huang et al., 2005). To understand this mechanism, therefore, we first need to provide necessary information about adenosine and its role in the nervous system. In biology and biochemistry classes, students learn about the role of adenosine as a constituent of the vital energy-containing compound adenosine triphosphate (ATP) and as one of the nucleosides in RNA. However, adenosine in the brain also seems to serve a neurotransmitter-like function. As in the case of other neurotransmitter receptors, the adenosine receptor is located within the cell membrane and possesses a binding site that is accessible from the extracellular space. For this reason, only *extracellular*, not intracellular, adenosine has access to the receptors. Interestingly, most extracellular adenosine comes from the breakdown of ATP that has been released from cells through channels called **pannexins**. Once outside of the cell, some of the ATP excites its own purinergic receptors, some of which are ligand-gated channels (**P2X receptors**) and others of which are metabotropic receptors (**P2Y receptors**). ATP can also be broken down enzymatically either directly to adenosine or in successive steps that yield the intermediates adenosine diphosphate (ADP)

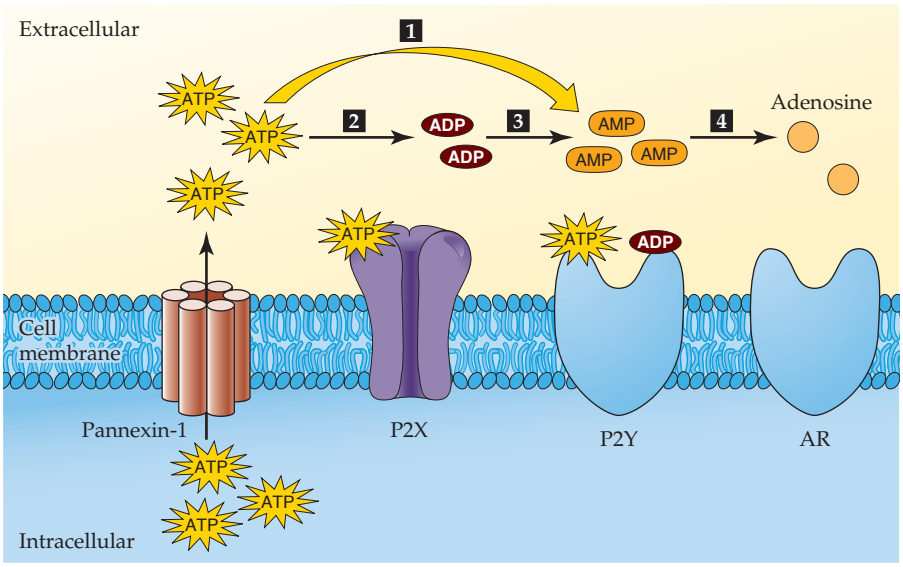


FIGURE 13.22 Extracellular release of ATP and activation of purinergic receptors ATP is released from inside cells by pannexin channels such as pannexin-1. Once it reaches the extracellular space, ATP can directly activate its own receptors (P2X and P2Y). ATP can also be enzymatically converted directly to adenosine (pathway 1) *or* sequentially metabolized to ADP, AMP, and then adenosine (pathways 2–4). Extracellular adenosine activates metabotropic adenosine receptors (AR) in the cell membrane. (After Velasquez and Eugenin, 2014, CC-BY 3.0.)

and adenosine monophosphate (AMP). ATP release, its breakdown to adenosine, and the receptors for ATP and adenosine are illustrated in **FIGURE 13.22**.

Four different adenosine receptor subtypes have been identified: A_1, A_{2A}, A_{2B}, and A_3. Of these subtypes, the A_1 and A_{2A} receptors are responsible for mediating most of adenosine's effects in the brain and therefore are the major adenosine receptor targets of caffeine (Ribeiro and Sebastião, 2010). The A_1 receptor is the predominant subtype in the cerebral cortex, hippocampus, and cerebellum, whereas the A_{2A} subtype is enriched in the striatum and olfactory bulb (Ribeiro et al., 2003). Importantly, one of the primary locations of the A_{2A} receptor is the GABAergic output neurons of the dorsal striatum (caudate–putamen) and ventral striatum (NAcc), cells that are also rich in DA receptors. Researchers have demonstrated that striatal A_{2A} receptors form heteromers (complexes containing more than one type of molecule) with D_2 receptors (Ferré et al., 2007; Casadó-Anguera et al., 2016). Current evidence suggests that the structure is a heterotetramer consisting of two A_{2A} and two D_2 receptors. Furthermore, the interaction between these different receptors helps account for caffeine's stimulatory effects in the following way. Occupancy of the A_{2A} receptor by adenosine exerts an allosteric influence on the D_2 receptor, reducing its affinity for DA and, therefore, decreasing the arousing and behaviorally activating effects of DA (Ferré, 2016). Consequently, under conditions when extracellular adenosine levels are low, D_2 receptor signaling is unimpeded and the organism is alert and aroused (**FIGURE 13.23**, left). When adenosine levels are higher, however, adenosine inhibition of D_2-mediated signaling leads to lower levels of arousal and psychomotor activation (Figure 13.23A, right). Consumption of caffeine releases this inhibition by blocking the adenosine receptors and enhancing D_2 signaling, thus leading to mild arousal and psychomotor activation. Note that the degree of activation is much less pronounced than the activation produced by cocaine- or amphetamine-mediated increases in extracellular DA levels, which are sufficient to overcome the negative allosteric influence of adenosine (see Chapter 12). There are additional actions of caffeine related to its blockade of adenosine A_1 receptors that will not be discussed here because of space limitations. Finally, adenosine has been proposed as a key neurotransmitter/neuromodulator in the production of sleep, and studies on cats have shown that extracellular adenosine levels in the basal forebrain are significantly elevated during prolonged wakefulness (Ribeiro and Sebastião, 2010). This finding makes clear why caffeine blockade of adenosine receptors is especially beneficial for promoting wakefulness in drowsy individuals.

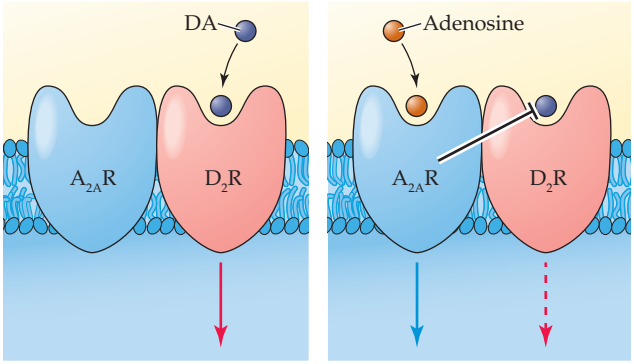


FIGURE 13.23 Activation of A2A receptors allosterically inhibits D2 receptor signaling $A_{2A}R$, A_{2A} receptor; D_2R, D_2 receptor. (After Ferré et al., 2007.)

Section Summary

- Caffeine and theophylline are members of a class of plant-derived substances called methylxanthines. Caffeine, especially, is contained in a number of foods such as coffee and tea. When consumed orally, it is readily absorbed from the gastrointestinal tract and is gradually metabolized and excreted with a typical half-life of approximately 4 hours.
- Caffeine and other ingredients (e.g., sugar, amino acids, and sometimes alcohol) are constituents of so-called energy drinks. These beverages are consumed for the same stimulating effects as other caffeine-containing foods and beverages. While energy drinks can serve a useful function by combating fatigue and inattention, they can also contribute to excessive caffeine consumption and may be dangerous when the caffeine is combined with significant amounts of alcohol in an effort to counteract alcohol's intoxicating effects.
- In rodents, caffeine has locomotor stimulant effects at low doses but actually reduces activity at high doses.
- Humans generally experience heightened attention and arousal, reduced fatigue, and reduced sleep in response to normal amounts of caffeine. Higher doses, typically greater than 400 mg, can lead to feelings of tension and anxiety.
- Laboratory studies have demonstrated enhanced mood, improved psychomotor performance, and increased memory consolidation following caffeine administration. When used by athletes, caffeine also enhances performance in both endurance sports and short-term high-intensity activities.
- Regular caffeine use leads to tolerance and physical dependence. Symptoms of caffeine withdrawal include headache, drowsiness, fatigue, impaired concentration, and reduced psychomotor performance.
- Caffeine acutely produces various physiological effects, such as increased blood pressure and respiration rate, diuresis, and increased catecholamine release.
- Daily caffeine use up to 400 mg is generally considered to be safe, except perhaps in pregnant women. Consumption of high doses, however, has been associated with two psychiatric disorders: caffeine intoxication, and caffeine dependence syndrome or caffeine use disorder. Caffeine intoxication, caused by recent excessive caffeine consumption, is characterized by symptoms of restlessness, nervousness, insomnia, and physiological disturbances such as tachycardia, muscle twitching, and gastrointestinal upset. Caffeine dependence syndrome is a syndrome of substance dependence (see Chapter 9) associated with chronic, maladaptive caffeine use. This syndrome is currently accepted by the *ICD-10* but needs further confirmation according to the *DSM-5*.
- Caffeine has several clinical uses, including pain relief and the treatment of newborn infants with apnea.
- Epidemiological studies indicate that regular coffee consumption of three to four cups per day reduces the risk of developing type 2 diabetes. This protective effect may be due to the presence of chlorogenic acids in coffee, rather than its caffeine content. Additional research is ongoing to determine whether coffee/caffeine consumption reduces the risk of developing Parkinson's disease as well as age-related cognitive decline.
- Although caffeine has a number of biochemical effects on the brain, its psychological and behavioral properties are mediated primarily by its ability to block A_1 and A_{2A} receptors for the neurotransmitter/neuromodulator adenosine. Extracellular adenosine is derived largely from the breakdown of ATP that has been released into the extracellular space. The behavioral stimulant properties of caffeine have been linked especially to antagonism of A_{2A} receptors, which form heteromeric complexes with DA D_2 receptors in the striatum. Under drug-free conditions, adenosine activation of striatal A_{2A} receptors exerts a negative allosteric effect on the D_2 receptors, thereby weakening cellular signaling through those receptors. Caffeine blockade of the adenosine receptors releases the D_2 receptors from this negative effect, thereby enhancing dopaminergic transmission in the striatum.

STUDY QUESTIONS

1. How much nicotine is contained within a typical tobacco cigarette, what factors influence how much nicotine available from the cigarette reaches the bloodstream, and how does this nicotine eventually make its way into the brain?
2. Describe the components of a typical e-cigarette.
3. (a) Name the principal metabolite of nicotine and the liver enzyme that produces this metabolite. (b) What is the average elimination half-life of nicotine? How do differences in the rate of nicotine metabolism influence smoking behavior and the risk for nicotine dependence?
4. Discuss the principal mechanism of action of nicotine on nicotinic cholinergic receptors. How do differences in receptor subunit composition affect the affinity for nicotine? What happens to high-affinity nicotinic receptors when they are persistently exposed to nicotine?
5. Nicotine administration has been found to produce different mood changes in recently abstinent smokers than in nonsmokers. What are these mood changes, and what is the reason for the difference?
6. (a) Describe experimental evidence in support of the idea that nicotine enhances cognitive function. What is the subunit composition of the nicotinic receptors that are believed to be most important for such enhancement? (b) What other neurotransmitter system(s) is/are involved in mediating nicotine-induced cognitive improvement?
7. Discuss the mechanisms involved in nicotine reinforcement. Include relevant experimental evidence in your answer.
8. Nicotine can exert not only reinforcing but also aversive effects. What is the evidence for this statement, and what is known about the mechanism of nicotine aversion?
9. Describe the peripheral physiological effects of nicotine that are mediated by the sympathetic and parasympathetic branches of the autonomic nervous system.
10. Describe the symptoms of nicotine poisoning. Why can a person smoke several packs of cigarettes over the course of a day without perishing from nicotine's toxic effects?
11. Discuss the processes of acute and chronic nicotine tolerance.
12. Compare and contrast the different ways of assessing nicotine dependence, including the *DSM-5* tobacco use disorder diagnostic criteria and the various questionnaires that have been developed for use in tobacco research.
13. Describe the features of nicotine abstinence syndrome.
14. Discuss the use of animal models to study nicotine dependence.
15. Nicotine users progress through a series of stages in their pattern and frequency of use. (a) Discuss the different stages that have been proposed to encompass this progression. (b) How is the initial reaction to nicotine exposure thought to influence the risk for subsequent development of nicotine dependence?
16. Discuss the roles of nicotine, mood and stress, cognitive enhancement (especially with respect to the ability to concentrate), and other factors in smoking and vaping. Include in your answer a comparison of the nicotine resource model and the deprivation reversal model as explanations for the involvement of nicotine in the maintenance of a smoking habit.
17. Describe the adverse effects of smoking on (a) adult health, (b) prenatal development, (c) development of the adolescent brain.
18. Are e-cigarettes safer than tobacco cigarettes? Justify your answer.
19. Compare and contrast the various pharmacological interventions used to treat nicotine dependence.
20. Name the group of plant-derived chemicals to which caffeine and theophylline belong.
21. Describe the pharmacokinetics of caffeine.
22. Discuss the acute subjective and behavioral effects of caffeine. Make sure to consider dose-related differences in such effects.

(*Continued*)

STUDY QUESTIONS (*continued*)

23. Some athletes consume caffeine to improve their performance. Is this justified by experimental findings? Which kinds of sports are known to benefit from caffeine, and which are not?
24. Describe the phenomena of caffeine tolerance and dependence. Can an individual who only consumes one cup of caffeinated coffee per day become caffeine-dependent?
25. Name and describe the features of disorders related to excessive caffeine use.
26. Why have researchers and policy makers raised concerns over the increasing consumption of energy drinks, especially among children and adolescents?
27. What are the demonstrated therapeutic/health benefits of caffeine and/or coffee consumption?
28. Discuss the mechanism of action underlying caffeine's effects on behavior. Include in your answer a description of the adenosine signaling system, including the source of neurotransmitter-related adenosine, the principal receptors for this substance, and interactions between adenosine and dopamine signaling.

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CHAPTER 14

"Spice," which is a street term for potent synthetic marijuana, can cause severe adverse reactions in users. (Spencer Platt/Getty Images.)

Marijuana and the Cannabinoids

A MAN COMES INTO THE EMERGENCY ROOM in Jackson, Mississippi. Six-foot-four, 240 lbs. "Solid, brick muscle," recalls Dr. Robert Galli, a professor of emergency medicine and toxicology at the University of Mississippi medical center (UMMC) in Jackson. "This big guy was fumbling around in the street, he was rolling around in the grass, he had no shirt on, his pants and underwear were down to his shoes, and he's flopping around in the rain with about 15 people taking videos of him." Someone called 911. First the fire department arrived, followed by police, then paramedics, who ascertained from the surrounding crowd that the man smoked "Spice."

—Glenza, 2016

This account, published in May 2016 in the British newspaper *The Guardian*, is just one of many recent stories warning of the dangers of "synthetic marijuana" (also known as Spice, K2, and many other street names). Typical headlines are "The Dangers of Synthetic Marijuana" (Forman, 2015), "Major Misnomer: Synthetic Marijuana's Dangerous Highs" (Kort, 2016), and "Why Synthetic Marijuana Is More Dangerous Than Ever" (Kroll, 2016b). These headlines raise many important questions: just what *is* synthetic marijuana, where does it come from, how does it act on the body, and is it as dangerous as portrayed in the popular media?

The present chapter deals with cannabis, an ancient drug that continues to be the center of much controversy. You will first learn about the history of cannabis, from early times through the anti-marijuana campaign of the 1930s and into the current era in which medical marijuana and recreational marijuana legalization either have already been implemented or are under discussion in many states. This will be followed by the pharmacology and mechanism of action of cannabinoids, including both the biologically active compounds present in the cannabis plant and the so-called endocannabinoids, which are cannabinoid substances made by one's own body. The psychological and behavioral effects of cannabis use will be covered, including current information about excessive use and abuse of this drug. Finally, you will learn about the current status of marijuana and cannabinoids as therapeutic agents, and importantly, some answers to the questions raised in the chapter opening will be offered. ■

Background and History of Cannabis and Marijuana

Forms of cannabis and their chemical constituents

Marijuana (alternately spelled "marihuana") is produced from the flowering hemp, a weed-like plant given the botanical name *Cannabis sativa* by Linnaeus in 1753 (**FIGURE 14.1**). Historically, hemp has served an important function in many cultures as a major source of fiber for making rope, cloth, and even paper. At times, its seeds have been used for their oil content and as bird feed. More important for neuropharmacologists is that cannabis plants contain 70 unique compounds that are collectively known as **phytocannabinoids** (meaning compounds with a cannabinoid structure that are found in the cannabis plant), as well as more than 400 other identified compounds (ElSohly and Slade, 2005; Andre et al., 2016). The psychoactive properties of some of these compounds, particularly a substance called **Δ^9-tetrahydrocannabinol** (**THC**), account for the use of cannabis as a drug. Although cannabinoids can be found to some extent in all parts of the plant, they are concentrated in a sticky yellowish resin that is secreted in particularly large amounts by the flowering tops of female plants.

Cannabis can be obtained in a number of different forms for the purpose of consumption. The most familiar to us is **marijuana**, which is derived from the Mexican word *maraguanquo*, meaning "an intoxicating plant." *Marijuana* refers to a crude mixture of dried and crumbled leaves, small stems, and flowering tops. Although marijuana can be consumed orally, as in cookies or brownies, it is usually smoked in rolled cigarettes known as "joints" or in various kinds of pipes and bongs. If desired, marijuana and tobacco can be consumed together in "blunts," which are hollowed-out cigars filled with marijuana, or in "spliffs" or "mulled cigarettes," which are joints containing a mixture of marijuana and tobacco (Schauer et al., 2017). Marijuana potency in terms of THC content varies widely, depending on the genetic strain of the plant and its growing conditions. One method of significantly increasing potency is to prevent pollination and hence seed production by the female plants. Marijuana produced by this method is called **sinsemilla** (meaning "without seeds"). Analysis of cannabis samples seized by the U.S. Drug Enforcement Administration (DEA) has shown a marked rise in THC content, and therefore marijuana potency, between 1995 and 2014 (**FIGURE 14.2**; ElSohly et al., 2016). This change is attributed to a shift of marijuana production from "regular" marijuana to sinsemilla.

FIGURE 14.1 Cannabis plants (© iStock.com/OpenRangeStock.)

Cannabis plant material can also be processed using methods that concentrate its psychoactive ingredients. One example of this is a cannabis extract called **hashish**. Like marijuana, hashish may be smoked or eaten (**FIGURE 14.3**). The potency of hashish greatly depends on how it has been prepared. In the Middle and Far East, for example, hashish generally refers to a relatively pure resin preparation with a very high cannabinoid content. Whereas hashish has a long history, there is a recent trend toward a new form of high-potency cannabis consumption called **dabbing** (Loflin and Earleywine, 2014; Miller et al., 2016). This practice typically involves extraction of cannabis with butane followed by evaporation of the solvent. The result is a waxy residue that, like hashish, contains an extremely high concentration of THC. Some common street names for this material are "dabs," "earwax," "butter," "shatter," "honey oil," and "butane hash oil." A "dab" refers to a single dose of the extract that is heated with a blowtorch or an e-cigarette/vape pen in order to vaporize and inhale the THC (Giroud et al., 2015). Over the past few years, information about dabbing has spread rapidly via social media such as Twitter and YouTube (Daniulaityte et al., 2015; Krauss et al., 2015). Informal interviews with young users revealed that dabbing is preferable to smoking marijuana because it's easier to do surreptitiously and it produces a much stronger "high" (Nirmay, 2016).

History of cannabis

Cannabis is believed to have originated in central Asia, probably in China. There is archeological evidence for the use of hemp fibers 8000 years ago (**FIGURE 14.4**). Medical and religious use of cannabis can be traced back to ancient China, India, and the Middle East (Russo, 2007; Ligresti et al., 2016). From those places, the substance spread to the Arab world, where the consumption of hashish became commonplace. Indeed, hashish is frequently mentioned in the Arabian folk stories that constitute *The Thousand and One Nights*. However, Western interest in this substance did not

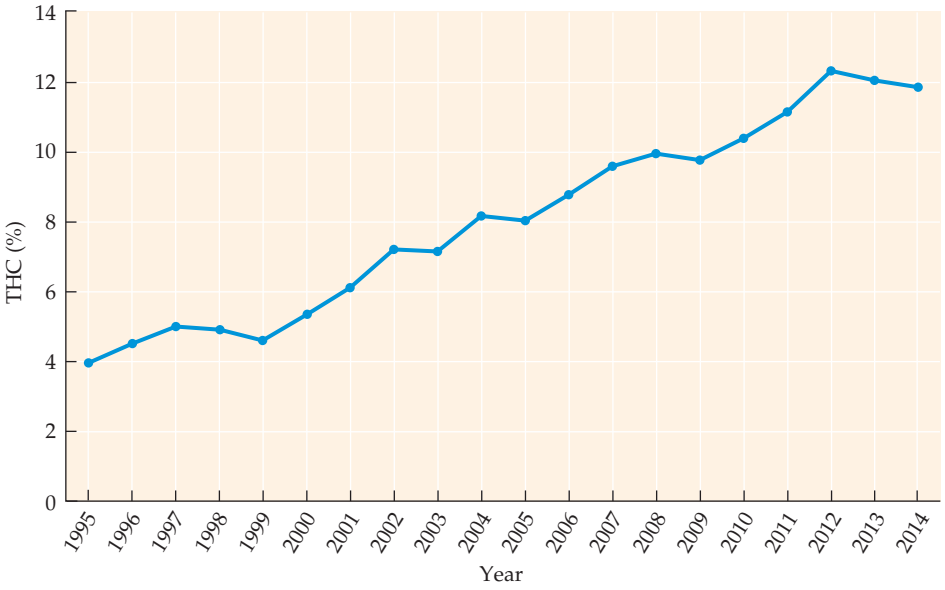


FIGURE 14.2 Yearly mean THC content of marijuana specimens seized by the DEA from 1995 to 2014 (After ElSohly et al., 2016.)

begin until the early to mid-nineteenth century, when some of Napoleon's soldiers reportedly brought hashish from Egypt back with them to France. Around the same time, a French physician named Jacques-Joseph Moreau encountered the intoxicating effects of hashish in the course of several trips to the Middle East. After returning to Paris, Moreau helped found a notorious association of French writers and artists known as *Le Club des Haschischins* ("club of the hashish eaters"), which included such notables as Victor Hugo, Alexandre Dumas, Théophile Gautier, and Charles Baudelaire.

The history of cannabis in the United States dates back to the colonial era, when hemp was an important agricultural commodity. No less than George Washington himself was a hemp farmer, which is ironic in view of the patriotic fervor associated with the contemporary "War on Drugs." Yet domestic hemp growers of the seventeenth and eighteenth centuries apparently had little awareness of the plant's intoxicating properties. Rather, historians believe that the social practice of consuming cannabis (mainly marijuana smoking) was brought into the United States in the early 1900s by Mexican immigrants crossing the Mexican–American border, and by Caribbean seamen and West Indian immigrants entering the country by way of New Orleans and other ports on the Gulf of Mexico.

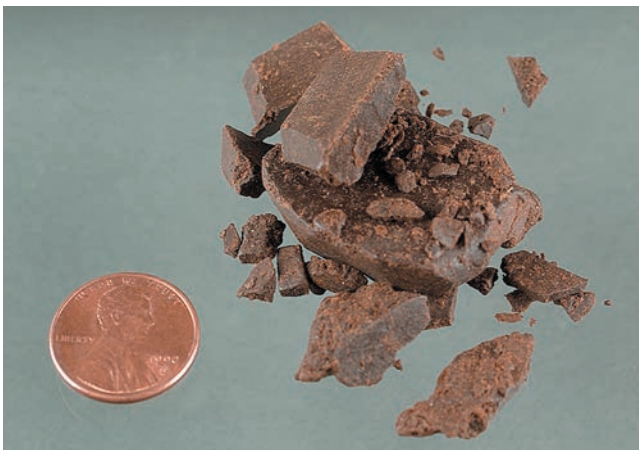


FIGURE 14.3 The potent form of cannabis called hashish (Courtesy of the U.S. DEA.)

Marijuana use rapidly spread outward from these points of origin, resulting in its coming to the attention of the federal government. In the 1930s, a man named Harry Anslinger spearheaded a public relations campaign to portray marijuana as a social menace capable of destroying the youth of America. Anslinger had been appointed in 1930 to be the first commissioner of narcotics in the Bureau of Narcotics of the United States Treasury Department. In congressional hearings, Anslinger testified as follows: "Those who are habitually accustomed to use of the drug [marijuana] are said to develop a delirious rage after its administration, during which they are temporarily, at least, irresponsible and liable to commit violent crimes. The prolonged use of this narcotic is said to produce mental deterioration. It apparently releases inhibitions of an antisocial nature which dwell within the individual" (Schaffer Library of Drug Policy). At the same time, Anslinger's Bureau of Narcotics was feeding information to the popular media about the evils of marijuana use. This stream of propaganda resulted in magazine articles with titles such as "Marihuana: Assassin of Youth" (*American Magazine*) and "Sex Crazing Drug Menace" (*Physical Culture*), as well as anti-marijuana movies such as *Reefer Madness* (**FIGURE 14.5**) that are now regarded as cult classics.

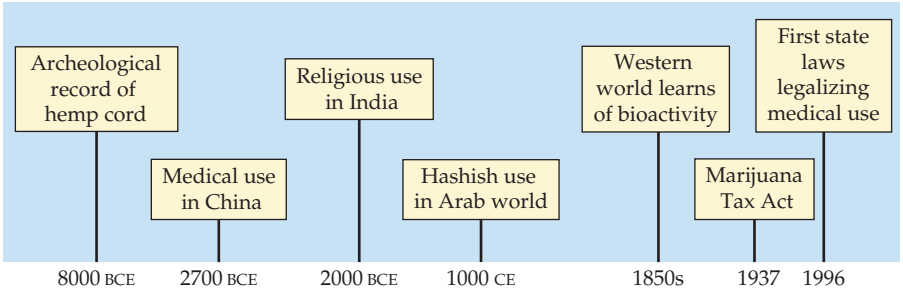


FIGURE 14.4 An 8000-year time line of cannabis use around the world (After Childers and Breivogel, 1998.)

As a consequence of Anslinger's anti-marijuana campaign, the federal government in 1937 passed its first legislation designed to control marijuana sales, called the Marijuana Tax Act. This legislation instituted a national registration and taxation system aimed at discouraging all use of cannabis for commercial, recreational, and medical purposes. Although the Marijuana Tax Act was overturned as unconstitutional by the U.S. Supreme Court in 1969, marijuana and other forms of cannabis remain regulated by state laws and by the federal Controlled Substances Act of 1970.

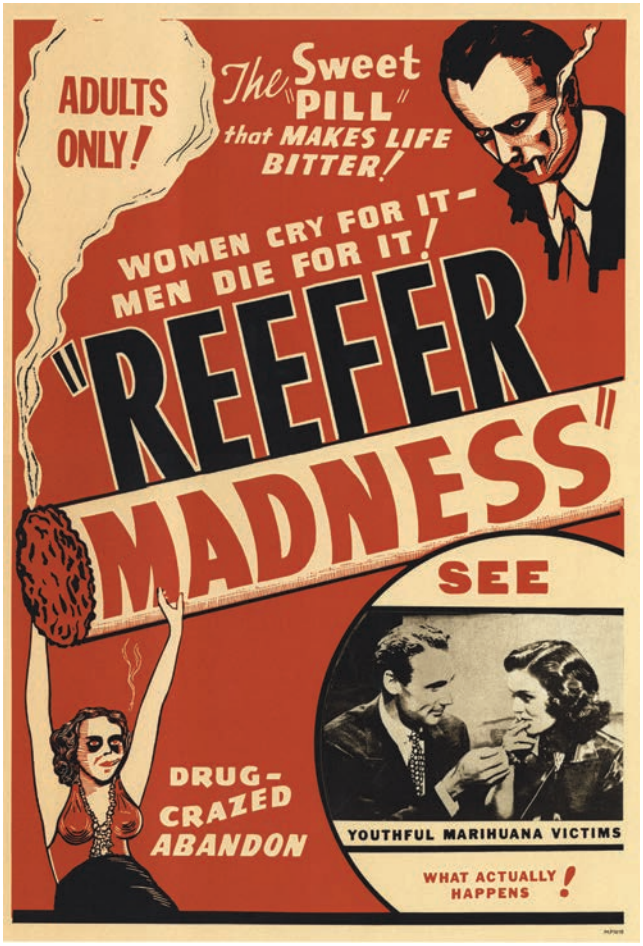


FIGURE 14.5 Poster advertising the 1936 film *Reefer Madness*

Basic Pharmacology of Marijuana

THC

Modern cannabinoid pharmacology began in 1964, when two Israeli researchers named Yehiel Gaoni and Raphael Mechoulam identified THC as the major active ingredient of *C. sativa* (**FIGURE 14.6**). Many other phytocannabinoids are also present, such as cannabidiol (see next section), but these compounds are not thought to contribute to the psychoactive properties of cannabis, because they have low activity at the neuronal cannabinoid receptor (see Mechanisms of Action section below). Later on, we will discuss the brain's own chemicals that mimic the effects of THC, and we will also consider the development of selective cannabinoid antagonists that have contributed significantly to research in this area.

A typical hand-rolled marijuana cigarette ("joint") consists of about 0.5 to 1 g of cannabis. If the THC content is 4% (although it can be even higher depending on the strain and growing conditions), then a 1 g joint contains 40 mg of active ingredient that is available to the smoker. As in the case of nicotine in tobacco leaves (see Chapter 13), burning of the marijuana causes the THC to vaporize and to enter the smoker's lungs in small particles. But because of a variety of factors, only about 20% to 30% of the original THC content is absorbed in the lungs. In practice, the amount of THC absorbed is affected not only by the initial amount of plant material used and the potency of this material but also by the pattern of smoking. The effective dose and latency to onset of effects of smoked marijuana are influenced by puff volume, puff frequency, inhalation depth, and breath-hold duration (Gorelick and Heishman, 2006).

Smoked THC is quickly absorbed through the lungs, resulting in rapidly rising levels in the blood plasma of the smoker (**FIGURE 14.7**). After peak levels are reached, plasma THC concentrations begin to decline as a result of a combination of metabolism in the liver and accumulation of the drug in the body's fat stores. In contrast, oral consumption of marijuana leads to prolonged but poor absorption of THC, thus resulting in low and variable plasma concentrations. The reduced bioavailability of THC following oral consumption compared with smoking probably results from both degradation

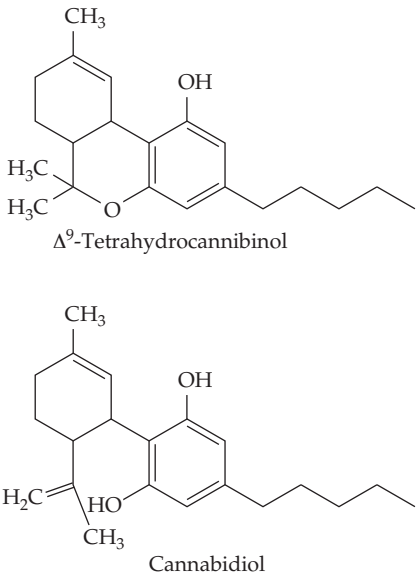


FIGURE 14.6 Chemical structures of Δ⁹-tetrahydrocannabinol (THC) and cannabidiol (CBD)

in the stomach and first-pass hepatic metabolism; that is, once orally ingested THC has been absorbed from the gastrointestinal tract, it must pass through the liver, where much of it is metabolized before it can enter the general circulation. THC metabolism is quite complicated, with over 80 metabolites having been identified (Dinis-Oliveira, 2016). Two of the major metabolites are 11-hydroxy-THC and 11-nor-9-carboxy-THC (THC-COOH). These substances as well as various minor metabolites are excreted primarily in the feces (about two-thirds of the administered dose) and the urine

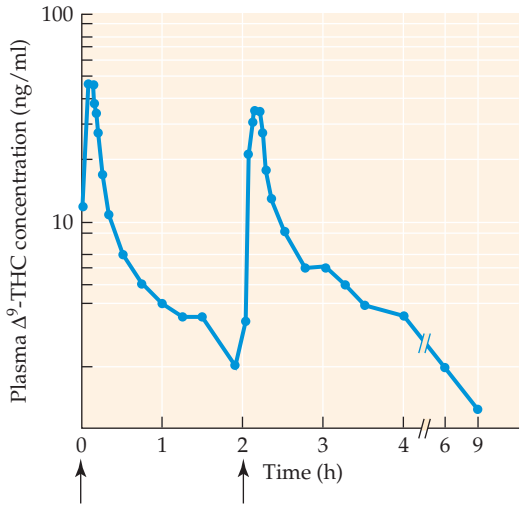


FIGURE 14.7 Mean time course of plasma THC concentrations Data were obtained from individuals who smoked a marijuana cigarette containing approximately 9 mg of THC at the two time points indicated by the arrows. (After Agurell et al., 1986, and Barnet et al., 1982.)

(about one-third of the administered dose). Even though THC levels in the bloodstream decline fairly rapidly after one smokes marijuana, complete elimination from the body is much slower because of persistence of the drug in fat tissue. Consequently, the elimination half-life ($t_{½}$) of THC is generally estimated at about 20 to 30 hours. Furthermore, the gradual movement of THC and fat-soluble metabolites back out of fat stores means that sensitive urine screening tests for THC-COOH can detect the presence of this metabolite more than 2 weeks after a single marijuana use.

Cannabidiol

Recent advances in cannabinoid research have shown that other phytocannabinoids besides THC have biological activity despite their low affinity for the neuronal cannabinoid receptor. The best studied of these compounds is **cannabidiol** (**CBD**), which is similar in structure to THC (see Figure 14.6). Interest in the therapeutic potential of CBD has been growing, in part because this cannabis constituent lacks the intoxicating and dependence-producing effects of THC. Among the disorders being considered as possible targets of CBD pharmacotherapy are epilepsy, neurodegenerative diseases, anxiety disorders, substance use disorders, and psychosis (Ligresti et al., 2016). These disorders and the potential mechanisms of action of CBD are discussed in later sections of the chapter.

Section Summary

- *Cannabis sativa*, the flowering hemp plant, exudes a resin containing a number of unique compounds known as phytocannabinoids.
- Cannabis can be obtained in several different types of preparations, including marijuana and hashish, both of which may be smoked or taken orally. Dabbing refers to inhalation of the heated vapors of a newer kind of cannabis extract. Hashish and dabs are preferred by some users because of their greater potency compared with marijuana and the resulting increase in subjective "high."
- The consumption of cannabis for its intoxicating effects is thought to date back thousands of years in Eastern cultures. The practice of marijuana smoking was introduced into the United States in the early 1900s by Mexican and West Indian immigrants.
- An anti-marijuana campaign instituted in the 1930s led to the first federal regulations controlling this substance.
- The most important psychoactive phytocannabinoid is Δ⁹-tetrahydrocannabinol (THC). A non-psychoactive constituent, cannabidiol (CBD), is also of interest because of its emerging potential for

treating a variety of neuropsychological and neuropsychiatric disorders.

- Inhaled THC is rapidly absorbed from the lungs into the circulation, where it is almost completely bound to plasma proteins. Oral THC consumption yields slower absorption and a lower plasma peak than occurs following smoking.
- THC is extensively metabolized in the liver, and the metabolites are excreted mainly in the feces and urine. Following a single dose of THC, total clearance of the drug and its metabolites may take days because of sequestration of these compounds in fat tissue.

Mechanisms of Action

For many years, researchers interested in how THC and other cannabinoids work in the brain were hampered by the lack of an identified cellular receptor for these compounds. Subsequent discovery of cannabinoid receptors permitted the synthesis of selective cannabinoid agonists and antagonists, as well as elucidation of the endogenous cannabinoid system.

Cannabinoid effects are mediated by cannabinoid receptors

Pharmacological characterization of a central nervous system (CNS) **cannabinoid receptor** was announced in 1988 by a group of researchers that included William Devane and Allyn Howlett, at St. Louis University, and Lawrence Melvin and M. Ross Johnson, at the Pfizer pharmaceutical company (Devane et al., 1988).[1] This initial characterization was quickly followed by other studies showing significant expression of cannabinoid receptors in many brain areas such as the basal ganglia (including the striatum, globus pallidus, entopeduncular nucleus, and substantia nigra pars reticularis), cerebellum, hippocampus, and cerebral cortex (**FIGURE 14.8**). As discussed later, localization of cannabinoid receptors in these areas is consistent with the recognized behavioral effects of these compounds on locomotor activity, coordination, and memory.

Around the same time that the St. Louis University and Pfizer researchers were first characterizing the cannabinoid receptor pharmacologically, another group of scientists at the National Institute of Mental Health (NIMH) including Lisa Matsuda and Tom Bonner cloned a novel gene from rat cerebral cortex that coded for a membrane protein with the characteristics of a G protein–coupled (metabotropic) receptor. Further studies revealed that these investigators, who were working on an unrelated problem, had actually cloned

[1]Readers interested in more information on the history of the discovery and characterization of cannabinoid receptors are referred to the excellent review by Mackie (2007).

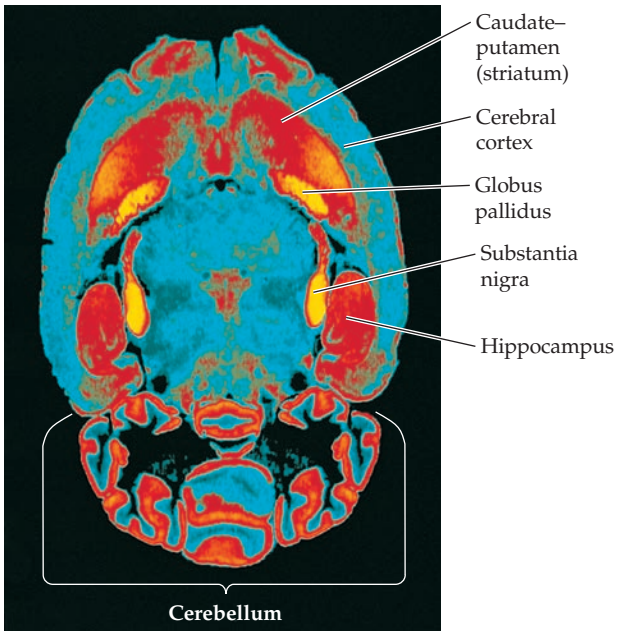


FIGURE 14.8 Autoradiogram of a horizontal section through a rat brain Distribution of CB_1 cannabinoid receptors is shown, with color coding of receptor density: yellow > orange > red > blue. (Courtesy of Miles Herkenham, National Institute of Mental Health.)

the gene for the rat brain cannabinoid receptor (Matsuda et al., 1990). This is a good example of an approach that is sometimes called *reverse pharmacology*, namely, the cloning of a novel receptor gene, the identity of which must then be determined by more classical pharmacological methods. The CNS cannabinoid receptor is currently designated **CB_1**.

The cellular and subcellular localization of CB_1 receptors within the CNS has been studied extensively using a variety of experimental approaches (reviewed by Hu and Mackie, 2015). Most of these receptors are expressed by various neuronal populations. However, low but meaningful CB_1 receptor expression has also been found in astrocytes (Metna-Laurent and Marsicano, 2015). The functional role of these astroglial cannabinoid receptors is briefly discussed later. Within neurons, CB_1 receptors are primarily found in presynaptic structures, specifically nerve terminals and axon segments near the terminals. On the other hand, some neocortical pyramidal neurons seem to possess somatodendritic CB_1 receptors that mediate self-inhibition by these cells (Marinelli et al., 2009).

A second cannabinoid receptor, **CB_2**, was discovered after the identification of the CB_1 subtype. CB_2 receptors were initially found in the immune system (Malfitano et al., 2014) and then later in other tissues such as bone, adipose (fat) cells, and the gastrointestinal tract (Atwood and Mackie, 2010). CB_2 receptors are also expressed by microglia (the brain's immune cells),

especially when those cells have been activated by inflammatory or degenerative processes occurring within the brain. Finally, CB_2 receptors may be expressed by neurons in some areas of the brain, though at lower levels than the CB_1 receptor (Lu and Mackie, 2016).

Cannabinoid receptors belong to the large family of metabotropic receptors. The cellular effects of the CB_1 receptor are mediated primarily through coupling to the G proteins G_i and G_o, which leads to inhibition of cyclic adenosine monophosphate (cAMP) formation, inhibition of voltage-sensitive Ca^{2+} channels, and activation of K^+ channel opening (Howlett, 2005; Ronan et al., 2016). In the case of presynaptic CB_1 receptors on nerve terminals, these signaling events combine to exert a powerful inhibitory effect on neurotransmitter release. Indeed, cannabinoids can inhibit the release of many different neurotransmitters, including acetylcholine, dopamine, norepinephrine, serotonin, GABA (γ-aminobutyric acid), and glutamate (Iversen, 2003). An additional effect of cannabinoid receptors is to modulate gene expression by several different mechanisms. One such mechanism is activation of the MAP kinase system, which plays an important role in synaptic plasticity, learning, and memory (see Chapter 3). Additional mechanisms involve epigenetic changes such as DNA methylation that may contribute to the long-term consequences of cannabis use (Szutorisz and Hurd, 2016). This topic will be addressed later in the Cannabis Abuse and the Effects of Chronic Cannabis Exposure section.

Pharmacological and genetic studies reveal the functional roles of cannabinoid receptors

Various synthetic cannabinoid agonists and antagonists were developed initially for research use, but some of these compounds are now being investigated for potential therapeutic applications. Two of the earliest-developed compounds are CP-55,940 and WIN 55,212-2, which are full agonists at both CB_1 and CB_2 receptors (Svíženská et al., 2008). Interestingly, THC is known to be a partial rather than a full CB_1 and CB_2 receptor agonist, since this substance produces lower peak receptor-mediated effects than the abovementioned synthetic agonists. The first selective CB_1 antagonist was **SR 141716A**, also known as **rimonabant**, which was developed by the French pharmaceutical firm Sanofi Recherche. Rimonabant and other antagonists have played a significant role in elucidating the involvement of either CB_1 or CB_2 receptors in specific actions of cannabinoid compounds.

Administration of THC to mice leads to a classical "tetrad" of effects consisting of (1) reduced locomotor activity, (2) hypothermia (a decrease in core body temperature), (3) catalepsy as indicated by immobility in the ring test (a test that measures the animal's behavior after it is placed on a horizontal wire ring), and (4) hypoalgesia (reduced pain sensitivity) measured using the hot plate or tail-flick test. These effects are mediated primarily by CB_1 receptors, because they can be blocked by a CB_1 antagonist, they can be duplicated by administration of a selective CB_1 agonist, and they are largely absent in CB_1 knockout mice given THC (Valverde et al., 2005; Pertwee, 2008). More specifically, the receptors that mediate the tetrad of cannabinoid effects are located mainly in the principal output neurons of the neocortex (glutamatergic pyramidal neurons) and the striatum (GABAergic medium spiny neurons) (Monory et al., 2007). CB_1 receptors also play an important role in the reward system, as shown by the reinforcing effects of CB_1 agonists in both animals and humans. This topic is discussed in greater detail later in the chapter.

We shall also see later that cannabinoids adversely affect human cognitive function, which has led to considerable interest in the effects of these compounds on learning and memory in laboratory animals. The results of this work indicate that cannabinoids disrupt memory in several different kinds of learning tasks, including the radial arm maze, the Morris water maze, and the delayed non-match-to-position task (Riedel and Davies, 2005). Furthermore, such effects rely on activation of CB_1 receptors in the hippocampus. This was demonstrated pharmacologically by showing that either systemic administration or microinjection of THC or CP-55,940 directly into the dorsal hippocampus produced significant memory deficits in the radial arm maze (Wise et al., 2009). Moreover, these effects could be completely blocked by intrahippocampal infusion of rimonabant (**FIGURE 14.9**). Cannabinoid interference with memory

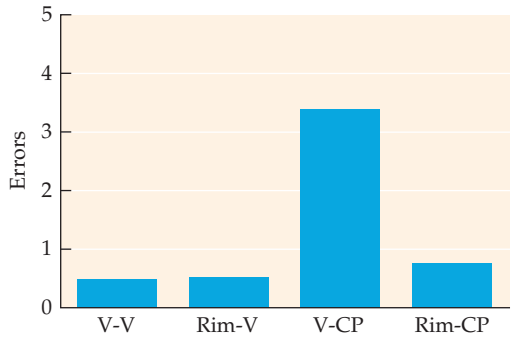


FIGURE 14.9 Hippocampal CB_1 receptors are responsible for cannabinoid-induced memory impairment Rats were trained to a high degree of performance on an eight-arm radial arm maze (see Chapter 4). After training was completed, intraperitoneal injection of the cannabinoid agonist CP-55,940 (0.05 mg/kg) along with a vehicle microinjection into the hippocampus (V-CP) produced a significant number of errors on the task compared with the control group (V-V), indicating an impairment in working memory. This effect was completely blocked by microinjection of rimonabant (0.06 μg) into the dorsal hippocampus (Rim-CP), whereas rimonabant by itself had no effect on maze performance (Rim-V). (After Wise et al., 2009.)

for spatial tasks additionally involves inhibition of long-term potentiation (LTP) in the hippocampal CA1 area (Puighermanal et al., 2012). Such interference may additionally depend on a reduction in oscillatory (i.e., rhythmic) electrical activity within the hippocampal circuitry (Holderith et al., 2011). Electroencephalographic (EEG) studies in humans along with studies on LTP in hippocampal slices have shown that gamma and theta oscillations play an important role in memory encoding and retrieval (Nyhus and Curran, 2010).

CB_2 agonist effects can be observed using the immune system as an example. In this case, such drugs inhibit the release of cytokines, which are immune cell signaling molecules such as interleukins and interferons. Among the effects of CB_2 agonist-mediated cytokine suppression are alterations in migration of immune cells toward the site of an inflammatory reaction (Miller and Stella, 2008). Space limitations preclude a more extensive discussion of the CB_2 receptor subtype in immune system regulation. However, CB_2 receptors are involved in other functions discussed below, and they are also potential therapeutic targets of cannabinoid-type drugs.

Endocannabinoids are cannabinoid receptor agonists synthesized by the body

ENDOCANNABINOID BIOCHEMISTRY AND PHARMACOLOGY The discovery and characterization of cannabinoid receptors finally enabled pharmacologists to study the cellular mechanisms by which marijuana produces its behavioral effects. Yet why should our brain possess receptors for substances made by a plant? This situation is reminiscent of the quandary faced by opiate researchers when opioid receptors were first identified as mediating the actions of morphine, which comes from a poppy plant (see Chapter 11). Accordingly, the same assumption was made that there must be an endogenous neurotransmitter-like substance that acts on the newly discovered receptors. Within a few years, a group headed by Raphael Mechoulam, the same Israeli scientist involved in the discovery of THC almost 30 years earlier, announced that they had isolated a substance with cannabinoid-like activity from pig brain (Devane et al., 1992). Chemical analysis revealed the substance to be a lipid with a structure related to that of arachidonic acid. The formal chemical name of this substance is **arachidonoyl ethanolamide** (**AEA**), but the researchers gave it the additional name **anandamide**, from the Indian Sanskrit word *ananda*, meaning "bringer of inner bliss and tranquility" (Felder and Glass, 1998, p. 186). Later studies demonstrated the existence of other arachidonic acid derivatives, such as **2-arachidonoylglycerol** (**2-AG**), that also bind to and activate CB_1 receptors (**FIGURE 14.10**). Together, these substances came to be known as **endocannabinoids**, meaning endogenous cannabinoids.

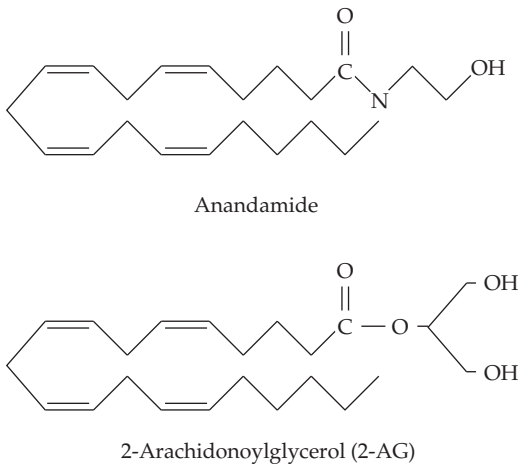


FIGURE 14.10 Chemical structures of the endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG)

The endocannabinoids are generated from inositol phospholipids in the membrane that contain the fatty acid arachidonic acid within their structure. Unlike the classical neurotransmitters, however, endocannabinoids are too lipid soluble to be stored in vesicles, since they would just pass right through the vesicle membrane. Consequently, these substances are synthesized and released when needed. Endocannabinoid synthesis and release are usually triggered by a rise in intracellular Ca^{2+} levels, which follows from the fact that some of the enzymes involved in the generation of these compounds are Ca^{2+} sensitive. This rise can be caused by the opening of voltage-gated Ca^{2+} channels or NMDA receptor channels in the membrane or by release of Ca^{2+} from intracellular storage sites due to the action of a second-messenger system such as the phosphoinositide system.

After being released, endocannabinoids are removed from the extracellular fluid by an uptake mechanism that is still under debate. Most of the research on endocannabinoid uptake has come from studies on anandamide. Because of its high lipid solubility, some anandamide uptake probably occurs by simple passive diffusion across the cell membrane. On the other hand, there is substantial experimental evidence for a membrane protein carrier (provisionally termed the **endocannabinoid membrane transporter**) that binds anandamide (and perhaps also 2-AG) and transports it across the membrane into the cell (Fowler, 2013). Despite the current interest in this hypothesis, it still requires validation by cloning of the relevant gene for the transporter and characterization of the gene product as a true endocannabinoid carrier protein.

Once inside the cell, the endocannabinoids can be metabolized by several different enzymes. For anandamide, the best-known enzyme involved in its degradation is called **fatty acid amide hydrolase** (**FAAH**).

In contrast, 2-AG is broken down primarily by a different enzyme known as **monoacyl-glycerol lipase** (**MAGL**) (Lu and Mackie, 2016). Interestingly, both endocannabinoids can also be metabolized by **cyclooxygenase-2** (**COX-2**), an enzyme that plays an important role in the process of inflammation. Thus, even though interaction with COX-2 is a minor pathway for endocannabinoid metabolism, it is possible that repeated use of COX-2 inhibitors (known as **nonsteroidal anti-inflammatory drugs**, or **NSAIDs**; e.g., ibuprofen) for pain relief could alter endocannabinoid activity in the brain and elsewhere.

Despite the discovery of anandamide before 2-AG, the latter substance is present in the brain at much higher levels than anandamide and is considered to be the more important synaptic signaling molecule. Furthermore, pharmacological studies have shown that 2-AG is a full agonist at both CB_1 and CB_2 receptors, whereas anandamide is only a partial agonist at CB_1 and has relatively little efficacy at CB_2. Differences in synthesis, metabolism, localization, and receptor activity of 2-AG and anandamide are consistent with the view that these two endocannabinoids are independently regulated and have different functional roles (Di Marzo and De Petrocellis, 2012).

ENDOCANNABINOID SIGNALING Mechanisms of endocannabinoid signaling have been studied extensively since the discovery of these substances. **FIGURE 14.11** illustrates three different signaling mechanisms discovered thus far (Castillo et al., 2012). In all three mechanisms, the endocannabinoid is synthesized in a postsynaptic element such as a dendritic spine; however, the location of the receptor and the effects of receptor activation vary in each case. The most common endocannabinoid signaling mechanism is **retrograde signaling** (Figure 14.11A), in which the endocannabinoid activates CB_1 receptors on nearby nerve terminals. This signaling mechanism, which is usually mediated by 2-AG, inhibits Ca^{2+}-mediated neurotransmitter release from the terminal (Sugiura, 2009). As discussed previously in Chapter 3 for nitric oxide, retrograde signaling is so named because it involves a signaling molecule carrying information in the opposite direction from normal (i.e., postsynaptic to presynaptic). There is now overwhelming evidence that endocannabinoids are retrograde messengers at synapses in a number of brain regions, such as the hippocampus and the cerebellum (Lu and Mackie, 2016). The second type of endocannabinoid signaling, which is usually mediated by anandamide, is shown in Figure 14.11B. In this case, the endocannabinoid remains within the postsynaptic cell and activates either a cannabinoid receptor or a different type of receptor called **TRPV1**. TRPV1 receptors are nonspecific cation channels that were first discovered in sensory neurons, where they play a key role in the heat and pain sensations produced by capsaicin, the "hot" ingredient in chili peppers. Subsequent research has shown that TRPV1 receptors are also expressed in some neurons within the brain, where they participate in regulating pain processing, mood, motor function, and learning and memory (Martins et al., 2014). Finally, Figure 14.11C illustrates an unusual mode of signaling in which endocannabinoids activate cannabinoid receptors in the membranes of nearby astrocytes, which results in release of glutamate from the glial cells.

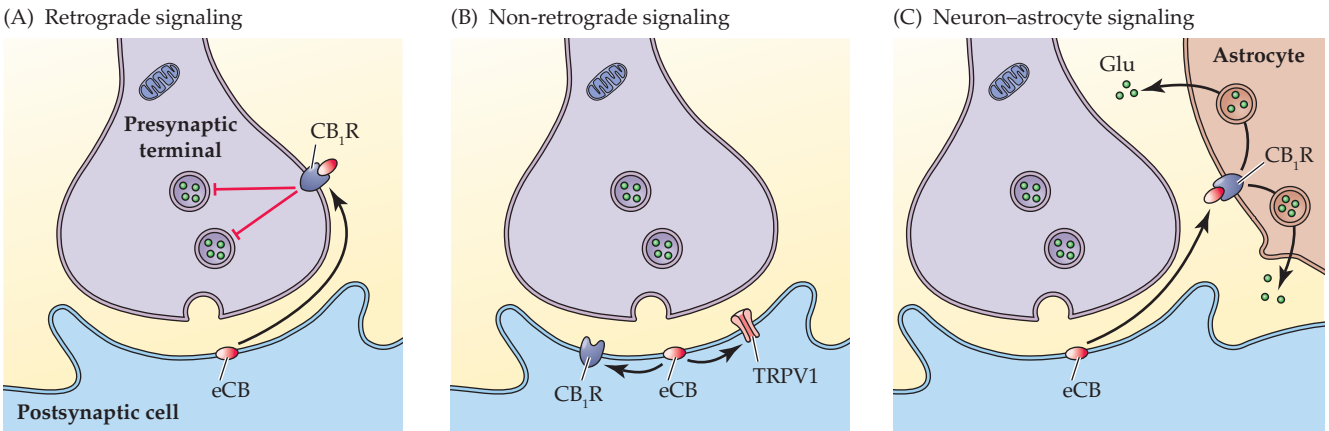


FIGURE 14.11 Mechanisms of endocannabinoid signaling (A) The most common and well-understood mechanism of endocannabinoid action is retrograde signaling. This mechanism involves release of the endocannabinoid (eCB), typically 2-AG, which diffuses from its site of synthesis in a dendritic spine to a presynaptic element where it activates CB_1 receptors (CB_1R). Such activation inhibits Ca^{2+}-mediated vesicular neurotransmitter release. (B) In the second mechanism, an endocannabinoid such as anandamide remains within the dendritic spine to activate either postsynaptic CB_1 receptors or TRPV1 cation channels. (C) The third mechanism involves endocannabinoid activation of astrocyte CB_1 receptors, which has been shown to provoke glutamate (Glu) release from the cells. (After Castillo et al., 2012.)

Because of the importance of retrograde signaling in the actions of endocannabinoids, it is worth exploring this mechanism in more detail. One well-characterized example of endocannabinoid retrograde signaling involves excitatory glutamatergic synapses in several brain areas, including the hippocampus (Olmo et al., 2016). At these synapses, glutamate release from the nerve terminal activates metabotropic glutamate receptor 5 (mGluR5) in the membrane of the dendritic spine. The mGluR5 receptors turn on PLCβ, a key enzyme of the phosphoinositide second-messenger system described in Chapter 3. The effect of PLCβ is to elevate intracellular levels of diacylglycerol (DAG), the precursor of 2-AG. The intracellular enzyme DGLα converts DAG to 2-AG, which is released into the extracellular space and diffuses to the nerve terminal where it activates presynaptic CB_1 receptors. Finally, the CB_1 receptors inhibit opening of presynaptic voltage-gated Ca^{2+} channels, thus reducing glutamate release from the terminal. This negative feedback on the glutamatergic neurons is an important regulator of brain excitability and can help protect the brain against the excitotoxic effects of excessive glutamate release (Chiarlone et al., 2014; Batista et al., 2016).

FUNCTIONAL EFFECTS OF THE ENDOCANNABINOID SYSTEM The ability of exogenous cannabinoids such as THC to disrupt short- and long-term memory of hippocampal-dependent tasks is well established. Surprisingly, the role of the endogenous cannabinoid system in memory processes is not as clear (reviewed in Marsicano and Lafenêtre, 2009). Researchers have used several different approaches to investigate this issue. One approach has been to eliminate (most) CNS cannabinoid signaling either by chronic administration of rimonabant or by knocking out the *CB1* gene and then comparing the knockout mice to wild-type controls. Other studies have tested the effects of elevating either anandamide or 2-AG levels by knocking out the *Faah* or *Magl* genes, respectively. Although some of these studies are consistent with the idea that endogenous cannabinoid signaling inhibits learning or memory consolidation, other research found that in the Morris water maze spatial navigation task, the endocannabinoid system plays a more important role in the ability of animals to extinguish the memory of the original location of the hidden platform in order to learn a new platform location (Varvel and Lichtman, 2002; Varvel et al., 2005; 2007). The discrepant results produced by genetic manipulations of the endocannabinoid system compared with administration of an exogenous cannabinoid may be related to temporal differences in these experimental approaches. Treating animals with an exogenous agonist produces large but temporary activation of CB_1 receptors, whereas gene knockout methods cause long-lasting changes associated with either a loss or an accentuation of cannabinoid signaling.

Acute consumption of cannabis by humans produces feelings of relaxation, reduced anxiety, elevated mood, and stress relief (see Acute Behavioral and Physiological Effects of Cannabinoids section below). This pattern of subjective responses led to the hypothesis that the endocannabinoid system helps regulate fear, anxiety, and reactions to stress. Indeed, this hypothesis has been confirmed in a large number of rodent studies (reviewed in McLaughlin and Gobbi, 2012). For example, enhancement of endocannabinoid signaling by inhibiting endocannabinoid metabolism or uptake has anxiolytic effects in the elevated plus-maze, elevated zero-maze, light–dark test, and social interaction test. Although most studies have used unstressed animals, Rossi and coworkers (2010) demonstrated that pharmacological inhibition of FAAH prevented the usual anxiety-like behaviors seen in mice subjected to repeated social defeat stress. In contrast, *CB1*-knockout mice that lack most CNS endocannabinoid signaling show increased anxiety-like behaviors (McLaughlin and Gobbi, 2012). Tests of depressive-like behaviors such as the forced swim and tail suspension tests are also sensitive to manipulations of the endocannabinoid system. Consistent with the results described for anxiety, elevated endocannabinoid activity exerts an antidepressant effect, whereas reduced endocannabinoid activity produces greater depressive-like behavior (McLaughlin and Gobbi, 2012). A specific role for 2-AG in the regulation of both anxiety- and depressive-like behaviors was recently shown by Shonesy and coworkers (2014), who found increases in both behavioral phenotypes in mice with genetic disruption of the 2-AG–synthesizing enzyme DGLα.

Researchers have used an auditory fear conditioning task to assess the potential role of endocannabinoids in learned fear and anxiety. This task involves the pairing of an auditory stimulus such as a tone with a foot shock, as illustrated in **FIGURE 14.12A**. Foot shock is an unconditioned fear stimulus (US) that elicits the unconditioned response (UR) of freezing behavior (i.e., no movement except for breathing), which is the species-typical response to a fearful situation in rats and mice. By means of a neural circuit involving the amygdala, pairing the tone with the foot shock causes the tone to become a conditioned stimulus (CS) that elicits a conditioned freezing response (CR). A number of studies have shown that the endocannabinoid system is not required for acquisition of auditory fear conditioning. However, prevention of CB_1 receptor signaling either by gene knockout or by rimonabant administration impairs normal extinction of the freezing response when the tone is presented alone without further shock pairing. Extinction paradigms may involve several daily sessions of tone presentation or a single prolonged stimulus presentation (see **FIGURE 14.12B**). Analysis of many fear extinction studies

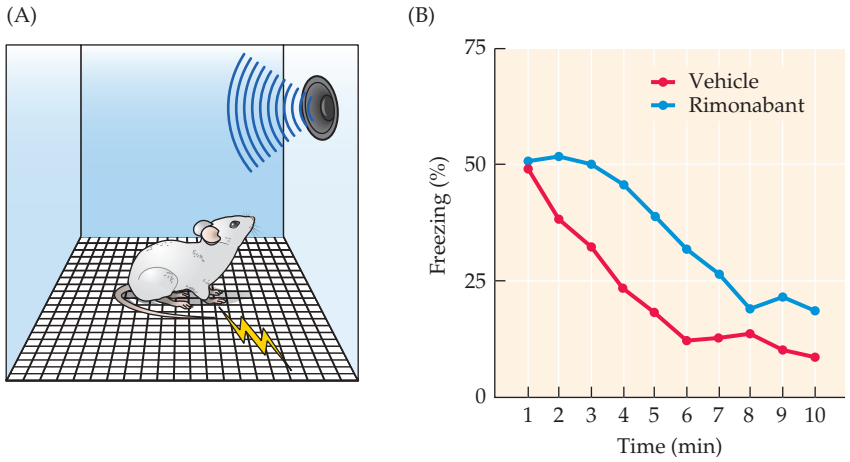


FIGURE 14.12 Participation of the endocannabinoid system in fear extinction (A) In auditory fear conditioning, a sound such as a tone is paired with a foot shock, thereby inducing a fear response (e.g., freezing behavior in rodents) to subsequent presentation of the same tone. (B) In the present study, male C57BL/6N mice were subjected to a single session of auditory fear conditioning by pairing a 2-second foot shock with a 9 kHz tone. The following day, half the animals were randomly assigned to receive a SC injection of rimonabant (3 mg/kg), whereas the other half received drug vehicle 1 hour before being returned to the conditioning apparatus. The figure illustrates the time course of freezing over a 10-minute period during which the 9 kHz tone stimulus (but no shock) was continuously presented. The rapid reduction in freezing behavior in the vehicle-treated group demonstrates normal extinction of the fear response, whereas the relative persistence of this behavior in the rimonabant-treated group suggests that endocannabinoid release helps facilitate extinction of learned fear responses. (B after Riebe et al., 2012.)

has led to the hypothesis that the endocannabinoid system is involved in the alleviation of fear, thereby functioning to prevent fear responses from becoming too pervasive (Riebe et al., 2012; Lutz et al., 2015). Future application of this idea could lead to the treatment of patients with post-traumatic stress disorder (PTSD) with cannabinoid agonist drugs or with compounds that block endocannabinoid metabolism or uptake (Berardi et al., 2016).

Endocannabinoids have additionally been shown to play a significant role in hunger, eating behavior, and energy metabolism. CB_1 receptor antagonists reliably reduce food consumption in both animal subjects and human study participants, and more detailed studies suggest that endocannabinoid activity within the hypothalamus, the mesolimbic dopamine (DA) pathway, and the olfactory system combine to enhance the motivation to eat, the hedonic properties of food (especially highly palatable food), and food-mediated reward (Cristino et al., 2014; Jager and Witkamp, 2014; Soria-Gomez et al., 2014). Additional evidence has shown that endocannabinoids in the gut provide a signal that controls dietary intake of fat (DiPatrizio, 2016). Because of the early success of CB_1 antagonism in reducing food intake, rimonabant (under the trade name Accomplia) was approved and released by Sanofi-Aventis in the European Union in June 2006 as an anti-obesity agent. Unfortunately, reporting of adverse psychiatric side effects by some users resulted in voluntary suspension of Accomplia marketing by the company in December 2008, followed shortly by withdrawal of approval of the medication by the European Medicines Agency. Nevertheless, targeting the cannabinoid system for the treatment of obesity has continued to be of interest to researchers and pharmaceutical companies, particularly since endocannabinoids are believed to contribute to development of the so-called metabolic syndrome that precedes outright obesity and is characterized by elevated blood sugar and lipids, high blood pressure, and excess body fat (Quarta et al., 2011; Mazier et al., 2015). Based on the hypothesis that gut endocannabinoids play a role in the onset of obesity and the metabolic syndrome, researchers have begun to develop and test cannabinoid antagonists that have limited penetration across the blood–brain barrier, thus bypassing adverse psychological side effects assumed to depend on CB_1 receptor blockade in the CNS. Although a few such drugs have shown efficacy in animal models of obesity, none have yet been licensed for use in human patients.

The last functional role of endocannabinoids to be discussed here is pain regulation. A number of studies have demonstrated that CB_1 and CB_2 knockout mice, as well as genetically normal animals given rimonabant, exhibit **hyperalgesia** (increased pain sensitivity) to several different types of pain stimuli. These findings clearly demonstrate a role for endocannabinoids acting on both cannabinoid receptor subtypes in the modulation of pain perception (Buckley, 2008; Calignano et al., 1998; Valverde et al., 2005). **FIGURE 14.13**

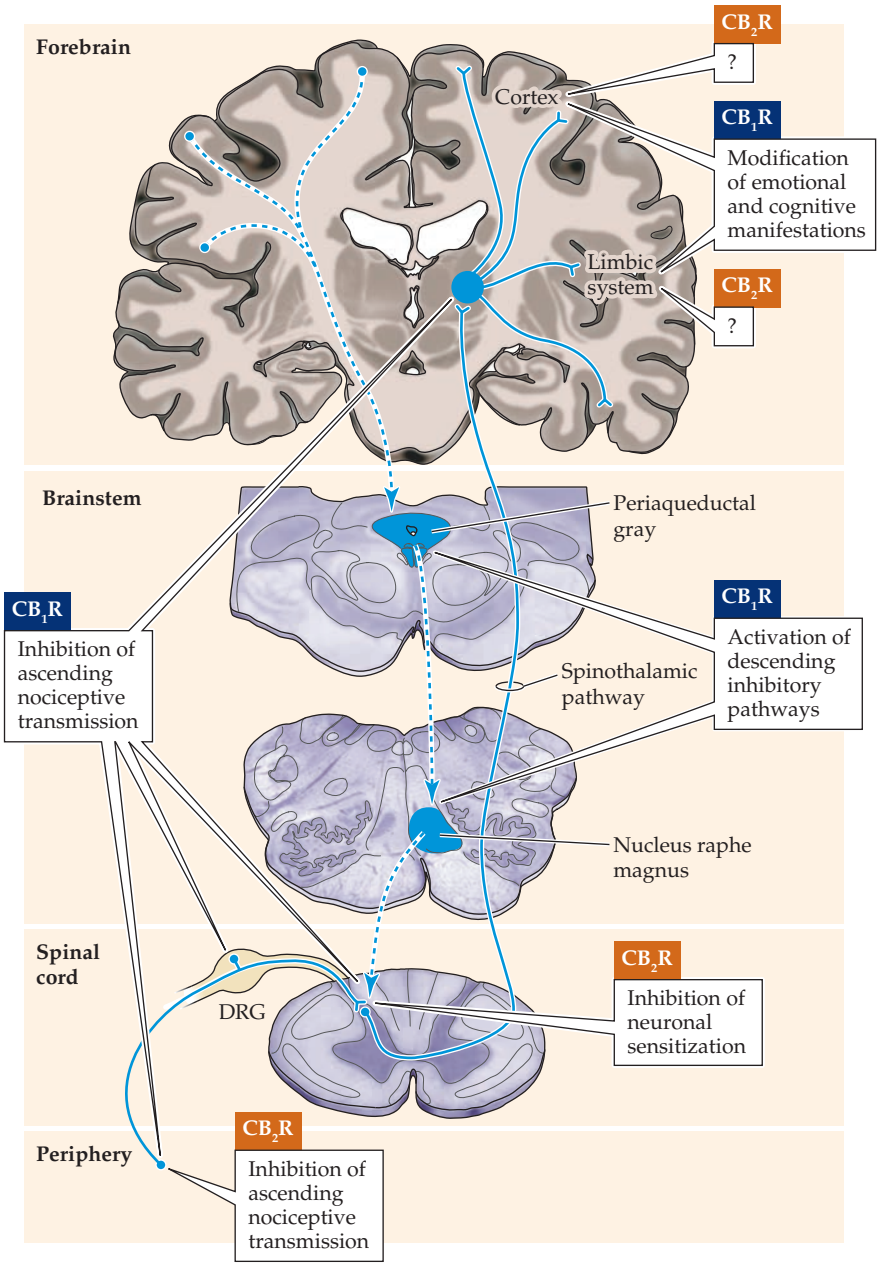


FIGURE 14.13 Location of CB_1 and CB_2 receptors involved in the modulation of pain perception Both cannabinoid receptor subtypes (CB_1R and CB_2R) participate in pain stimulus processing and perception at multiple levels of the nervous system. Activation of these receptors inhibits ascending pain information originating from peripheral nociceptive fibers, enhances descending pain-inhibitory pathways, and modulates the emotional and cognitive components of perceived pain. The presence of pain-modulating CB_2 receptors in the forebrain is currently uncertain, which accounts for the "?" in that part of the figure. DRG, dorsal root ganglia. (After Maldonado et al., 2016.)

illustrates the location of CB_1 and CB_2 receptors in the forebrain, brainstem, spinal cord, and periphery that participate in regulating both pain perception and the cognitive–affective responses to pain. Researchers and clinicians are especially interested in the role of the endocannabinoid system in chronic pain syndromes such as **neuropathic pain**. Neuropathic pain refers to a type of pain produced within the nervous system itself, not in direct response to a nociceptive (pain-inducing) stimulus like a stab wound or burn. Opioid drugs are relatively ineffective in alleviating neuropathic pain compared with nociceptive pain, which accounts for the search for alternative medications that might be more efficacious. Research with laboratory animals has found an up-regulation of cannabinoid receptors in models of neuropathic pain, suggesting that the endocannabinoid system may be a prime target for new therapeutic approaches to this problem (Maldonado et al., 2016).

Involvement of endocannabinoids in so many different systems suggests an immense potential for therapeutic interventions. Pure preparations of THC, or THC plus CBD, are already used medically for a few different disorders. Furthermore, prescription of marijuana itself (i.e., "medical marijuana") has become legal in an increasing number of states. **Web Box 14.1** discusses current and possible future use of marijuana as well as cannabinoid-based drugs in medicine.

Section Summary

- Two cannabinoid receptors, CB_1 and CB_2, have been identified and their genes cloned.
- The CB_1 receptor is the principal cannabinoid receptor in the brain, where it is expressed at a high density in the basal ganglia, cerebellum, hippocampus, and cerebral cortex.
- The CB_2 receptor was first identified in the immune system, but it is also found in a number of other tissues, including the brain, where it is mainly localized in microglial cells.
- Cannabinoid receptors belong to the G protein–coupled receptor superfamily. Receptor activation can inhibit cAMP formation, inhibit voltage-sensitive Ca^{2+} channels, and activate K^+ channels.
- CB_1 receptors are typically located on axon terminals, where they act to inhibit the release of many different neurotransmitters.
- Agonists at the CB_1 receptor include the synthetic full agonists CP-55,940 and WIN 55,212-2 and the partial agonist THC. The first selective CB_1 antagonist was SR 141716A, also known as rimonabant.
- THC administration to mice causes a classical tetrad of CB_1 receptor–mediated effects that consist of reduced locomotor activity, hypothermia, catalepsy, and hypoalgesia.
- CB_1 agonists also impair learning and memory consolidation in several different kinds of tasks. Interference with memory of hippocampal-dependent spatial tasks has been linked to inhibition of LTP in the hippocampal CA1 area and a reduction in oscillatory activity within the hippocampal circuitry.
- CB_2 receptor activation in the immune system causes cytokine release and changes in immune cell migration toward an inflammatory site.
- The brain synthesizes several substances, called endocannabinoids, that are neurotransmitter-like agonists at cannabinoid receptors. Anandamide was the first endocannabinoid to be discovered; however, another endocannabinoid called 2-AG is present in the brain at higher levels than anandamide.
- Endocannabinoids are generated on demand from arachidonic acid–containing membrane lipids by a Ca^{2+}-dependent mechanism and are released from the cell by a process that does not involve synaptic vesicles. They are believed to be removed from the extracellular space by a carrier protein called the endocannabinoid membrane transporter.
- Anandamide and 2-AG are degraded primarily by FAAH and MAGL, respectively.
- Endocannabinoids usually function as retrograde messengers that are synthesized and released from postsynaptic cells to activate CB_1 receptors on nearby nerve terminals. This process leads to an inhibition of voltage-gated Ca^{2+} channel opening and a consequent reduction in neurotransmitter release from the terminals. However, two other modes of endocannabinoid signaling have been discovered. First, in some cases the endocannabinoid (usually anandamide) remains within the postsynaptic cell where it activates either a cannabinoid receptor or an excitatory ion channel called TRPV1. Second, endocannabinoids can activate cannabinoid receptors on astrocytes, thus causing release of glutamate from those glial cells.
- A well-characterized example of endocannabinoid retrograde signaling involves excitatory glutamatergic synapses in the hippocampus along with some other brain areas. Glutamate release from the nerve terminal activates mGluR5 receptors in dendritic spines, which elevates intracellular Ca^{2+} levels and triggers 2-AG synthesis and release. 2-AG diffuses back to the terminal where it activates CB_1 receptors, resulting in inhibition of voltage-gated Ca^{2+} channels and a reduction in glutamate release. This negative feedback system regulates brain excitability and helps protect the brain against glutamate excitotoxicity.
- The endocannabinoid system plays a complex role in learning and memory of hippocampal-dependent tasks. Some studies indicate that endocannabinoid signaling inhibits learning or memory processes, whereas other study findings are more consistent with an involvement in extinction of an already learned spatial task.
- Enhancing endocannabinoid signaling has anxiolytic effects in both stressed and unstressed laboratory animals, and it also leads to an antidepressant profile in standard rodent tests of depressive-like behavior. In contrast, reduced endocannabinoid levels are associated with increased anxiety- and depressive-like behaviors. In auditory fear conditioning tasks, endocannabinoids facilitate extinction of the CR (freezing) when the CS (tone) is no longer paired with the US (foot shock). This finding is consistent with a theorized role for the endocannabinoid system in alleviating fear.
- In recent years, marijuana has been legalized for medical use in many states despite the lack of well-controlled clinical studies demonstrating its therapeutic efficacy. Oral preparations of synthetic

THC or a THC analog are currently licensed for treating nausea and vomiting in cancer chemotherapy patients, as well as the wasting syndrome in patients with AIDS. An oral spray containing both THC and CBD is also approved in many countries (not including the United States) for neuropathic pain and spasticity in patients with multiple sclerosis. The endocannabinoid system is being actively studied as a target in the treatment of many other kinds of disorders, including headaches, visceral pain, neurodegenerative disorders and stroke, and a variety of neuropsychiatric disorders. Finally, a strong initiative is underway to develop CBD-based medications for pediatric epileptic syndromes that are refractory to standard antiseizure drugs.

Acute Behavioral and Physiological Effects of Cannabinoids

Cannabinoid use produces a range of behavioral and physiological effects that vary depending on the dose, the frequency of use, the characteristics of the user, and the setting in which use occurs.

Cannabis consumption produces a dose-dependent state of intoxication

The earliest recorded clinical studies on the intoxicating properties of cannabis were performed by Moreau, the French physician mentioned earlier who introduced hashish to nineteenth-century Parisian literary society. Moreau, who is sometimes called the "father of psychopharmacology," became interested in the possible relationship between hashish intoxication and the characteristics of mental illness. Consequently, he and his students meticulously recorded their subjective experiences after consuming varying amounts of hashish. Because of the potency of their preparation, these individuals reported profound personality changes and perceptual distortions, even frank hallucinations.[2] Hallucinogenic responses have also been reported either following a high dose of pure THC administered to volunteers in a research setting, or as an occasional side effect of ingesting a synthetic cannabinoid for medicinal purposes (Koukkou and Lehmann, 1976; Timpone et al., 1997).

The lower cannabis doses associated with smoking one or two marijuana cigarettes produce a somewhat more modest reaction, although many of the same *kinds* of effects are found across the dose–response curve. The subjective and behavioral effects of marijuana have been studied using approaches ranging from the distribution of questionnaires to experienced marijuana users (Tart, 1970) to controlled laboratory studies involving administration of oral THC to infrequent users (e.g., Curran et al., 2002). As summarized in Iversen (2000), these effects can be separated into four stages: the "buzz," the "high," the stage of being "stoned," and finally the "come-down." The "buzz" is a brief period of initial responding during which the user may feel light-headed or even slightly dizzy. Tingling sensations in the extremities and other parts of the body are commonly experienced. The marijuana "high" is characterized by feelings of euphoria and exhilaration, as well as a sense of disinhibition that is often manifested as increased laughter. If the user has taken a sufficiently large amount of marijuana, his level of intoxication progresses to the stage of being "stoned." In this stage, the user usually feels calm, relaxed, perhaps even in a dreamlike state. Indeed, relaxation is the most common effect reported by cannabis users in self-report studies involving open-ended questions (Green et al., 2003). Sensory reactions experienced by users in the stage of being stoned include floating sensations, enhanced visual and auditory perception, visual illusions, and a tremendous slowing of time passage. Sociability can undergo different types of changes, in that the user may experience either an increased desire to be with others or a desire to be alone. The "come-down" stage is the gradual cessation of these effects, which varies in length depending on the THC dose and the individual's rate of THC metabolism.

Marijuana and other forms of cannabis also produce several physiological responses. There is increased blood flow to the skin, which leads to sensations of warmth or flushing. Heart rate is stimulated, which may be experienced by the user as a pounding pulse. Finally, marijuana increases hunger (the infamous "munchies"), an effect that is more than just street lore but has actually been documented in controlled laboratory studies of both humans (Foltin et al., 1988) and rats (Williams et al., 1998; also see Kirkham, 2009). Indeed, appetite stimulation is one of the recognized therapeutic uses for cannabinoids (see **Web Box 14.1**).

Not surprisingly, the marijuana "high" and its other subjective and physiological effects are dose-dependent, in that the concentration of THC in a smoked marijuana cigarette has a direct relationship to the intensity of these effects (Cooper and Haney, 2008). Moreover, these effects are at least partially mediated by CB_1 receptors. Huestis and colleagues (2001; 2007) found that according to self-reported ratings, intoxication following the smoking of a single marijuana cigarette was significantly although not completely inhibited by prior treatment with rimonabant (**FIGURE 14.14**). Similar results were found for the heart rate–elevating effects of the drug. Either a higher dose of the antagonist is needed to fully block the effects of marijuana or some

[2]Moreau's work culminated in a book entitled *Du Hachich et de l'aliénation mentale* (*Hashish and Mental Alienation*), major excerpts of which can be found in Nahas (1975).

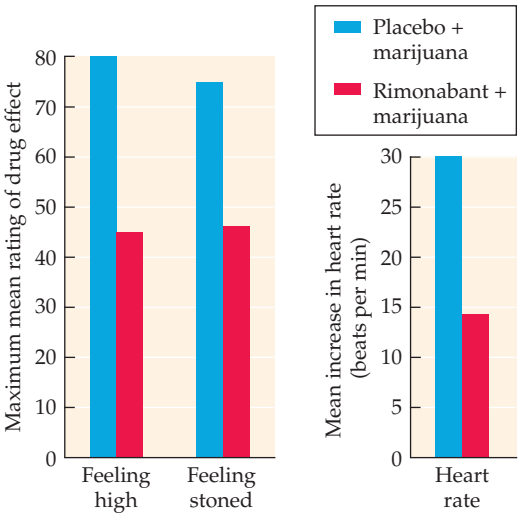


FIGURE 14.14 Reduction in the subjective and physiological effects of smoked marijuana by rimonabant pretreatment. Study participants received either 90 mg of the CB_1 cannabinoid receptor antagonist rimonabant or a placebo orally, after which they smoked either an active (2.64% THC content) or a placebo marijuana cigarette. Self-reported subjective effects and heart rate were measured over the next 65 minutes. The data shown represent the maximum mean effects of the active marijuana cigarette in the presence or absence of the receptor antagonist. (After Huestis et al., 2001.)

other mechanism in addition to CB_1 receptor activation (e.g., activation of central CB_2 receptors) is involved in producing these effects.

Smoking marijuana can also transiently evoke psychotic symptoms such as depersonalization (feeling separated from the self), derealization (feeling that the external world is unreal), agitation, and even paranoia (Sewell et al., 2009). Adverse reactions are most likely to occur in first-time users, although regular users may also experience these effects if they consume an unusually high dose. Flashbacks, which are widely known to occur in LSD users, have occasionally been reported for marijuana as well (Iversen, 2000). Although some authors have suggested the existence of a specific cannabis-induced psychosis, that concept remains questionable.

As with most psychoactive drugs, the psychological effects of marijuana vary greatly as a function not only of dose but also of the setting, the individual's past exposure to the drug, and her mental set, which refers to the expectation of what effects the drug will produce. The influence of expectancy was demonstrated by Kirk and coworkers (1998), who gave volunteers capsules containing either THC or a placebo. The volunteers were separated into an "informed" and an "uninformed" group. All were instructed beforehand that the capsules would contain placebo or one of several types of psychoactive compounds, but only for the informed group were cannabinoids included in the list of possible drugs. When asked to rate their responses to the substance they had consumed, the informed group gave higher ratings than the uninformed group in the categories of "like drug" and "want more drug." The expectation of consuming cannabinoids not only enhanced the pleasurable effects of actual cannabinoid administration, it also elicited a more positive reaction when the volunteers were given placebo instead.

Plasma THC levels peak much more rapidly following intravenous (IV) THC injection or marijuana smoking than after oral ingestion. Consequently, users reach the peak "high" sooner with the first two routes of administration. Nevertheless, users who are smoking marijuana do not reach this peak until some time after the cigarette has been finished. This delay means that the maximum level of intoxication occurs when plasma THC concentrations are already declining, suggesting that the brain and plasma THC concentrations are not yet equilibrated at the time when the plasma level is peaking. Another possible factor is the contribution of active THC metabolites (whose peak does not coincide with that of THC itself) to the psychoactive properties of marijuana.

Marijuana use can lead to deficits in memory and other cognitive processes

Clinical accounts of marijuana intoxication have often noted deficits in thought processes and in verbal behavior. These may include illogical or disordered thinking, fragmented speech, and difficulty in remaining focused on a given topic of conversation. The early descriptive work later gave rise to quantitative experimental assessments of marijuana's effects on learning, memory, and other cognitive processes such as attention and executive function. Different studies have assessed the effects of either acute or chronic cannabinoid exposure. In acute studies, marijuana or pure THC is administered to participants who typically have a history of previous cannabis use. The results are compared with those obtained from matched participants given a placebo instead of active drug. Chronic effects of marijuana on cognition are assessed by comparing regular, sometimes heavy, cannabis users against nonusers, with neither group being given a drug prior to testing. The effects of chronic marijuana use on cognitive function are discussed later in the chapter.

Among the different cognitive processes mentioned above, memory impairment is the most consistent finding in studies of acute cannabinoid exposure (Broyd et al., 2016). Impairment has been seen in many different kinds of tests, including tests of verbal, visual, and visuospatial episodic memory, semantic memory, and working memory (Schoeler and Bhattacharya, 2013). Categorization of memory into episodic, semantic,

and working memory is explained as follows: "Most of the memory tests that have been employed when investigating the acute effects of cannabis assess the declarative (explicit) memory system, which requires the conscious recollection of events and facts and is further divided into episodic (facts about the world) and semantic memory (storage of elements that have an autobiographical context), while working memory is defined as a temporary storage and manipulation of episodic information" (Schoeler and Bhattacharya, 2013, p. 12). Acute cannabinoid exposure has consistently been found to impair episodic verbal memory and working memory. In addition to memory impairment, deficits in verbal learning, attention, inhibitory control, and psychomotor function (e.g., reaction time) have also been reliably associated with acute cannabinoid exposure. Interestingly, pretreatment with CBD has been reported to protect against THC-induced deficits in verbal learning and memory. Moreover, amount of prior marijuana use has been shown to influence the results on some cognitive tests, with heavier use reducing the adverse effects of acute cannabinoid exposure. This finding has led to the hypothesis that behavioral ("cognitive") tolerance develops in heavy marijuana smokers (Hart et al., 2001).

The ability of marijuana to impair psychomotor performance has real-world implications for situations such as driving an automobile. The adversely affected processes that are relevant for driving include reaction time, ability to perform divided attention tasks, and critical tracking. Not surprisingly, therefore, acute cannabis exposure has also been demonstrated to produce deficits in driving ability, using both driving simulators and tests of on-road performance (Bondallaz et al., 2016). Even more pronounced effects can occur when marijuana is combined with alcohol. These findings are not just of academic interest, as cannabis intoxication has been shown to increase the risk of being in a motor vehicle crash (Rogeberg and Elvik, 2016). Although this increased risk is only of low to medium magnitude, it is certainly prudent for individuals who have just smoked marijuana to avoid driving and other activities requiring operation of heavy machinery until the intoxicating effects have dissipated.

Rewarding and reinforcing effects of cannabinoids have been studied in both humans and animals

Cannabinoids are obviously reinforcing to users who smoke marijuana recreationally or who consume cannabis by other means. However, cannabinoid reinforcement in humans has also been studied under controlled laboratory conditions. For example, Chait and Zacny (1992) found that regular marijuana users could discriminate THC-containing marijuana cigarettes from placebo cigarettes containing no THC, and that all their study participants preferred the marijuana with THC when given a choice. In the same study, pure THC taken orally in capsule form was also preferred over a placebo. Chait and Burke (1994) subsequently related marijuana preference to THC content, as users reliably selected marijuana with a 1.95% THC content over marijuana containing only 0.63% THC.

We saw in earlier chapters that most drugs that are abused by humans are rewarding and reinforcing in experimental animal paradigms such as drug-induced place conditioning and IV drug self-administration. Psychostimulants like cocaine or methamphetamine and opiates like heroin or morphine are particularly efficacious in such paradigms. In contrast, there has been significant difficulty demonstrating consistent rewarding and reinforcing properties of THC in rodents and in non-human primates (Vlachou and Panagis, 2014; Tanda, 2016). One exception to this general conclusion comes from a series of studies demonstrating reliable self-administration of THC by squirrel monkeys (reviewed by Panlilio et al., 2010). One key factor in these experiments was the use of low drug doses that are within the range of estimated human THC intake from a single puff on a typical marijuana cigarette (**FIGURE 14.15**). Lever pressing for THC was completely blocked by pretreatment with rimonabant, indicating that the reinforcing effect was dependent on CB_1 receptor activation. These same investigators showed that THC can induce drug-seeking behavior (a model of relapse in human drug users) in monkeys (Justinová et al., 2008) and that the endocannabinoid 2-AG is also self-administered (and thus reinforcing) in the squirrel monkey model (Justinová et al., 2011).

Researchers have hypothesized that the inability to establish reliable THC place conditioning and self-administration in rodents or rhesus monkeys compared with squirrel monkeys is due to multiple factors. These factors might include experimental design issues, species differences, drug doses used, drug bioavailability and rate of clearance, and the fact that THC is only a partial agonist at the CB_1 receptor. The notion that properties of the drug itself may be important for the failure of THC to support place conditioning and IV self-administration is strengthened by numerous findings that WIN 55,212-2, a synthetic cannabinoid that is a full agonist at the CB_1 receptor, is self-administered by both rats and mice (Vlachou and Panagis, 2014; Tanda, 2016). Administration of WIN 55,212-2 additionally can produce both a conditioned place preference and a reduced threshold for electrical self-stimulation of the brain.

The ability of WIN 55,212-2 to enhance electrical self-stimulation suggests an involvement of CB_1 receptors in the brain's reward system. This has been confirmed in studies demonstrating (1) reductions in the rewarding effects of natural substances such as food

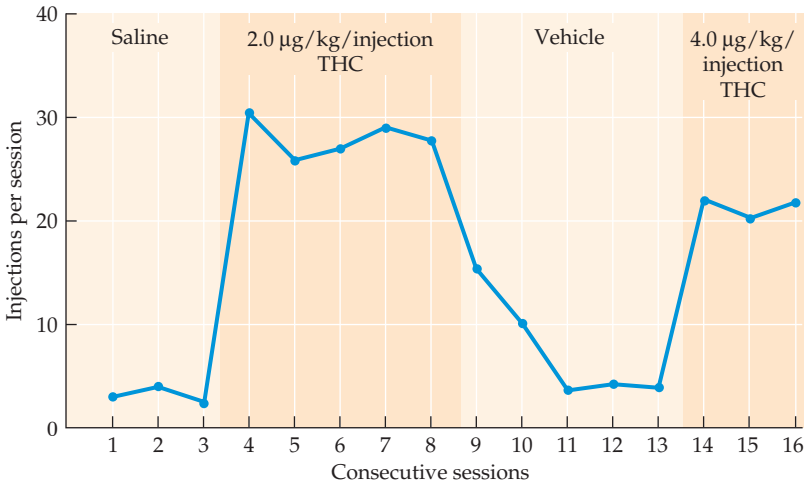


FIGURE 14.15 Acquisition of THC self-administration by squirrel monkeys Monkeys were initially trained in drug self-administration on a fixed-ratio-10 (FR-10) schedule using cocaine as the reinforcer (not shown). They were then switched to saline, which led to a nearly complete elimination of lever-pressing behavior. When THC (2.0 μg/kg/injection) was substituted for saline, lever pressing immediately increased to an amount sufficient to deliver approximately 30 drug injections per 1-hour session. Substitution with the vehicle again reduced operant responding until the active drug was made available once again. (After Tanda et al., 2000.)

or sweetened solutions in animals that lack normal endocannabinoid activity, and (2) enhanced reward due to pharmacological manipulations that increase endocannabinoid signaling (Vlachou and Panagis, 2014). Interestingly, studies using mutant mice lacking either CB_1 receptors or μ-opioid receptors have shown an interaction between the endocannabinoid and endogenous opioid systems. Thus, the rewarding and reinforcing effects of opiates are reduced or eliminated in CB_1 knockout mice, whereas these same effects of cannabinoids are similarly blunted in mice with a null mutation of the μ-opioid receptor (Befort, 2015). Additional evidence has implicated the endocannabinoid system in the processes of reinforcement, dependence, and/or relapse for a number of other drugs, including ethanol (Colombo et al., 2005), nicotine (Castañé et al., 2005; Maldonado and Berrendero, 2010), and psychostimulants such as cocaine (Arnold, 2005; Wiskerke et al., 2008).

Once cannabinoids were shown to be reinforcing under the appropriate experimental conditions and choice of species, researchers began to investigate the mechanisms underlying the reinforcing effects. One factor in cannabinoid reinforcement seems to be activation of the mesolimbic DA system, as cannabinoids have been found to stimulate the firing of dopaminergic neurons in the ventral tegmental area (VTA) and to enhance DA release in the nucleus accumbens of laboratory animals (Wenzel and Cheer, 2014). Both tonic and phasic (burst) firing of the neurons are increased by cannabinoid administration. Although most studies have been performed using exogenous cannabinoid substances, recent work by De Luca and colleagues (2014) found a similar increase in accumbens DA release in rats self-administering 2-AG. The effects of cannabinoids on the dopaminergic system are mediated by CB_1 receptors, as they can be blocked by rimonabant. However, the underlying mechanism must be indirect, as CB_1 receptors are not expressed by the dopaminergic neurons themselves. Current evidence indicates that cannabinoids activate CB_1 receptors in GABAergic interneurons of the VTA, resulting in a reduction in local GABA release. This reduction releases the dopaminergic neurons from the inhibitory effects of GABA, thus causing increased cell firing. Finally, several studies have used brain imaging methods such as positron emission tomography (PET) to ascertain whether cannabinoids stimulate DA release in the human brain. The majority of studies have found negative results (reviewed in Sami et al., 2015), although positive findings have been reported in a few cases (e.g., Bossong et al., 2015). While the same imaging methods have shown increased extracellular DA following administration of psychostimulants such cocaine or amphetamine, it is likely that the smaller effects of cannabinoids are too difficult to detect reliably until the methodology has become more sensitive.

Section Summary

- The subjective characteristics of cannabis intoxication include feelings of euphoria, disinhibition, relaxation, altered sensations, and increased appetite. The euphoric effects produced by smoking marijuana appear to be mediated at least partly by CB_1 receptors. Psychopathological reactions can occur, particularly at high doses or in inexperienced users.
- Cannabis acutely causes impairment in episodic verbal memory and working memory, attention, inhibitory control, and psychomotor performance. Some of these effects are reduced in magnitude when participants have been pretreated with CBD.
- Researchers have had difficulty demonstrating reliable IV self-administration of THC in rodents and rhesus monkeys; however, both self-administration and drug-seeking behavior for this compound have been shown in squirrel monkeys. Certain properties of THC may underlie its lack of clear

reinforcing properties in rats and mice, as these species more readily self-administer the synthetic cannabinoid WIN 55,212-2, which is a full CB_1 receptor agonist. Rodent studies have additionally demonstrated the ability of WIN 55,212-2 to produce a conditioned place preference and a reduced threshold for electrical self-stimulation of the brain. These effects are all mediated by activation of CB_1 receptors.

- Endocannabinoids are involved in the brain's reward system. These substances help mediate the rewarding and reinforcing effects of natural rewards like sweetened solutions as well as the effects of various abused drugs such as opiates.
- Cannabinoid reinforcement has been shown to depend on the CB_1 receptor and may also involve DA, since cannabinoids stimulate the firing of DA neurons in the VTA and enhance DA release in the nucleus accumbens. The mechanism underlying cannabinoid enhancement of dopaminergic activity involves activation of CB_1 receptors expressed by inhibitory VTA GABAergic interneurons, thereby resulting in a disinhibition of dopaminergic cell firing.

Cannabis Abuse and the Effects of Chronic Cannabis Exposure

The United Nations (UN) regularly reports on trends of drug use around the world. Based on UN data, anywhere from 128 million to 232 million people worldwide have used cannabis at least once (Anthony et al., 2016). These figures correspond to approximately 2.7% to 4.9% of the world's population. Across countries, the greatest prevalence of cannabis use is in North America, Australia, and New Zealand. Indeed, marijuana is the most widely used illicit drug in the United States. According to the 2016 National Survey on Drug Use and Health, more than 20 million Americans age 18 or older were current marijuana users at the time of the survey (Substance Abuse and Mental Health Services Administration, 2017). Moreover, the survey estimated an additional 1.6 million users between 12 and 17 years of age.

Initial marijuana use typically occurs in adolescence and peaks during young adulthood. If an individual has not yet tried marijuana by her mid-20s, she is unlikely to begin at a later age. This is shown in **FIGURE 14.16**, which is derived from a longitudinal study of 976 people drawn from upstate New York. In this cohort, the peak age for initiating marijuana use was 17, although a few children began as early 10 or 11 years of age. It is also the case that the prevalence of illicit drug use (including marijuana) declines with age. In the 2016 National Survey, for example, the percentage of responders who were current users of at least one illicit drug (typically marijuana) was 8.8% between 12 and 17 years of age, 22.3% from 18 to 25 years of age (the peak age range for illicit drug use), and only 1.9% at 65 years or older.

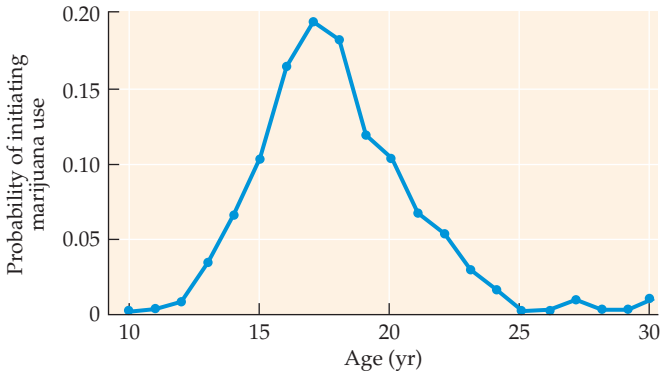


FIGURE 14.16 Probability of initiating marijuana use as a function of age (After Brook et al., 1999.)

Several factors seem to influence the likelihood of early marijuana use, including lax parental monitoring and early behavioral problems. For example, a study by Falls and colleagues (2011) found a significant association between early conduct problems and early initiation of marijuana use (i.e., before 15 years of age) in a large sample of college students. In addition, most adolescents have prior experience with alcohol and/or cigarettes before trying marijuana. For this reason, alcohol and tobacco have been hypothesized as "gateway" drugs to marijuana use, although not all findings are consistent with this hypothesis (e.g., see van Leeuwen et al., 2011). Some evidence also exists that marijuana, in turn, may serve as a gateway to other illicit drugs (e.g., cocaine) or to prescribed psychoactive drugs such as sedatives (Mayet et al., 2012). However, it is difficult to determine whether marijuana actually facilitates the progression to "hard drugs," or whether certain users are already predisposed to seek out these more dangerous substances because of some combination of personality traits, life circumstances, and other factors independent of their exposure to marijuana (see **Web Box 9.1**).

A further issue to consider is the progression from initial to regular (i.e., daily or near daily) marijuana use. Risk factors in the development of heavy marijuana use by adolescents include emotional problems in the family, heavy drug use in the household and/or by peers, dislike of school and poor school performance, and an early age of first use of marijuana (Gruber and Pope, 2002). On the other hand, rates of marijuana use tend to be lower among adolescents from stable families with close parental supervision, as well as those who have strong career aspirations or assume adult responsibilities such as marriage and parenthood. Another important factor may be the degree to which the young person experiences positive

reactions to his or her early use of cannabis. Researchers in New Zealand examined the relationship between the subjective responses to early cannabis use at 14 to 16 years of age and the likelihood of becoming cannabis-dependent by the age of 21, according to criteria of the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-IV*) (Fergusson et al., 2003b). Individuals who reported more positive responses (i.e., feeling happy, feeling relaxed, laughing a lot, doing silly things, or getting very "high") to their early experience with cannabis were at greater risk of later dependence than those who reported fewer of these positive reactions. Finally, Brook and coworkers (2016) examined much longer-term trajectories of marijuana use frequency in a study cohort from upstate New York from age 14 to approximately 43 years of age. The investigators identified six different patterns that they called (1) nonusers/experimenters, (2) chronic/occasional users, (3) chronic/heavy users, (4) increasing users, (5) decreasers, and (6) quitters. Use trajectories and percentage of users within each pattern are shown in **FIGURE 14.17**. Programs aimed at reducing marijuana use and the development of dependence clearly need to identify and specifically target the factors that influence the usage patterns of the groups 3 and 4.

Once daily use has begun, the pattern becomes relatively stable. Among a group of daily marijuana users studied by Hughes and colleagues (2014), the number of uses per day averaged 3.2. The drug was usually taken in several different ways, including bongs, pipes, and vaporizers. Study participants reported severe intoxication on about 25% of the days they used marijuana, and over 70% of participants also binge drank alcohol or used tobacco. These findings not only highlight the dangers associated with daily cannabis use, they point to the risk of developing a cannabis use disorder, as discussed in the next section.

Chronic use of cannabis can lead to the development of a cannabis use disorder

The fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-5*) specifies diagnostic criteria for cannabis intoxication, withdrawal, and use disorder (American Psychiatric Association, 2013). Earlier in the chapter we described the main characteristics of intoxication on cannabis/marijuana. The present section summarizes our current knowledge about cannabis use disorder, tolerance, withdrawal, and treatment approaches.

CANNABIS USE DISORDER Based on epidemiological studies, researchers have estimated that approximately 10% of individuals who have ever used cannabis will eventually become dependent (Copeland and Smith, 2009). Not surprisingly, the risk of dependence

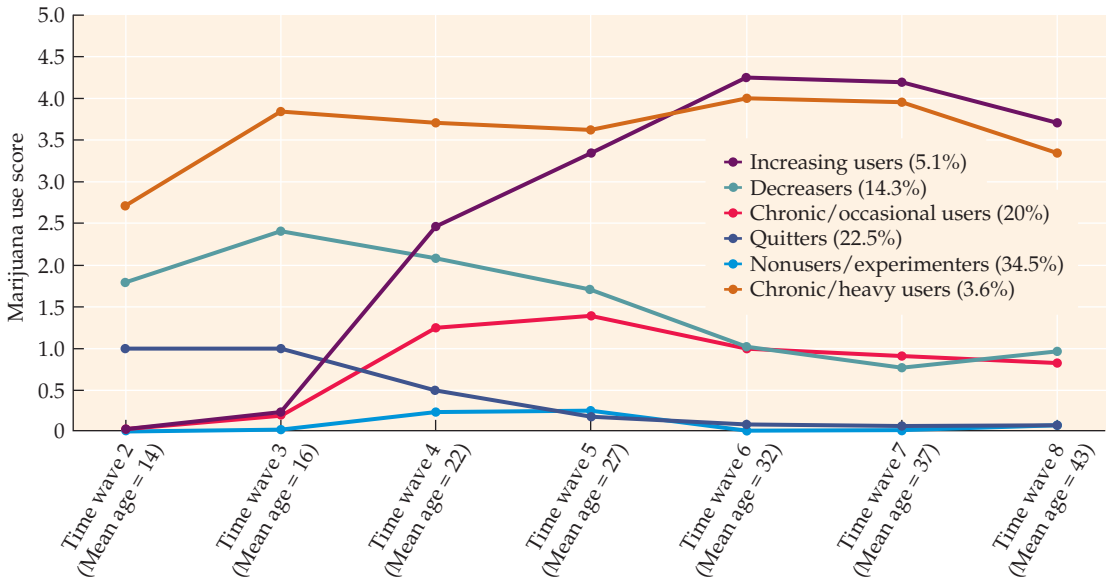


FIGURE 14.17 Trajectories of marijuana use from the adolescent period to approximately age 43
Study participants were recruited in 1975 at a mean age of 6.3 years (T1, not shown). Follow-up assessments of marijuana use were conducted in 1983 (T2; mean age, 14.1 years), 1985 to 1986 (T3; mean age, 16.3 years), 1992 (T4; mean age, 22.3 years), 1997 (T5; mean age, 27.0 years), 2002 (T6; mean age, 31.9 years), 2007 (T7; mean age, 36.6 years), and 2012 to 2013 (T8; mean age, 43.0 years). Marijuana use scores were as follows: 0 = none; 1 = no more than a few times a year; 2 = once a month; 3 = several times a month; 4 = once a week; 5 = several times a week; and 6 = daily. The figure depicts six patterns of stable or changing marijuana use obtained from 548 participants, with the percentage of participants shown for each pattern. T, Time wave. (After Brook et al., 2016.)

is related to drug use patterns. Thus, people who progress to daily use have a 50% probability of become dependent. We saw in Chapter 9 that the *DSM-5* has replaced the previous terms *substance abuse* and *substance dependence* with a single term, *substance use disorder*. To define cannabis use disorder, *DSM-5* applies the overall list of criteria for a substance use disorder to cannabis users. These criteria entail significant impairment and/or distress across several functional areas, including problematic behaviors resulting from persistent cannabis use; failed attempts to control or decrease cannabis use; excessive amount of time spent procuring the drug; adverse impact of cannabis use on performance at work, at school, or in social roles; persistent use of cannabis in potentially hazardous situations; the development of tolerance; and the experience of withdrawal symptoms upon cessation of use (Panlilio et al., 2015; Simpson and Magid, 2016). Severity of the cannabis use disorder may be rated as mild, moderate, or severe depending on how many criteria are met. Besides a clinical interview, problematic cannabis use can be assessed using any of several questionnaires such as the Cannabis Problems Questionnaire, Cannabis Abuse Screening Test, Cannabis Use Disorder Identification Test, Marijuana Screening Inventory, and Marijuana Problem Scale (López-Pelayo et al., 2015).

Different approaches have been used to ascertain the prevalence of cannabis use disorder and the trajectory of developing and recovering from the disorder. According to the National Survey on Drug Use and Health, the number of people with cannabis use disorder in 2016 in the United States among people age 12 and above was approximately 4 million (Substance Abuse and Mental Health Administration, 2016). Those data are based on a combination of *DSM-IV* criteria for both substance abuse and dependence. Because the National Survey does not collect longitudinal data on individual participants, information on the onset and course of cannabis use disorder must be obtained from long-term studies on smaller cohorts of cannabis users. One such example was drawn from a community mental health study called the Oregon Adolescent Depression Project. The authors used the study to obtain longitudinal data on cannabis use disorders from childhood to 30 years of age (Farmer et al., 2015). Cannabis use disorders were diagnosed using a combination of cannabis abuse and cannabis dependence as defined in the *DSM-IV*. By these criteria, 19% of the study population was diagnosed with a cannabis use disorder at some point within the specified age range. The percentage was significantly greater for men (22.5%) than for women (16.4%). The period between 14 and 24.9 years of age represented the greatest risk for onset of the disorder, with 18.6 years being the average age of first onset. From 25 to 30 years of age, people were unlikely to develop a cannabis use disorder if onset had not already occurred. For those who did suffer from a cannabis use disorder, the average duration was approximately 32 months, and recovery had occurred over 80% of the time by the age of 30; however, relapse from initial recovery was noted for some individuals.

Additional relevant findings come from the Victorian Adolescent Health Cohort Study, a large longitudinal study conducted in the state of Victoria, Australia (Coffey and Patton, 2016). This study began in 1992 and involved almost 2000 students first surveyed at 14 to 15 years of age and followed up at 5-year intervals for the next 20 years. In this cohort, cannabis dependence according to *DSM-IV* criteria was more common in males, peaked at 20 years of age, and then declined at subsequent time points. Overall, 27% of adolescent cannabis users were diagnosed as being dependent at some time between ages 20 and 35. Together with the results from the Oregon study, the findings indicate an overall risk of cannabis users developing dependence that ranges from about 20% to 25%. This prevalence rate suggests that cannabis use disorders may be more common than previously realized, particularly among early users of the drug. On the other hand, the recovery data indicate that for most users, developing a cannabis use disorder is a temporary condition that usually resolves over time.

FACTORS CONTRIBUTING TO THE DEVELOPMENT OF CANNABIS USE DISORDER As noted above, early onset of cannabis use and progressing to daily use are two important factors in developing a cannabis use disorder. Other factors encompass a range of biological and psychosocial variables. On the biological side, researchers have determined that the risk for developing problematic cannabis use (abuse or dependence) is heritable, with a meta-analysis of various twin studies estimating that genetics accounts for 51% of this risk (Verweij et al., 2010). Some gene association studies have focused on single-nucleotide polymorphisms (SNPs) in the genes for the CB_1 receptor and for FAAH, the major anandamide-metabolizing enzyme; however, additional genes are undoubtedly important contributors to developing problematic cannabis use (Agrawal et al., 2012). Behavioral and psychosocial factors associated with risk for cannabis dependence include early adolescent onset of cannabis use, experiencing highly positive reactions to cannabis during adolescence, use of cannabis to cope with negative emotions, concurrent use of tobacco, living alone, experiencing major life stressors, and having a comorbid psychiatric disorder like attention deficit hyperactivity disorder (ADHD), major depression, or antisocial behavior (Schlossarek et al., 2016). Just as males are more likely to use cannabis than females, they are also more likely to develop a cannabis use disorder. On the other hand, females that do become cannabis-dependent show a more rapid

trajectory, and they are likely to seek treatment after fewer years of use than males (Rubino and Parolaro, 2015). Although we do not yet fully understand these sex differences, they highlight the importance of studying cannabis use patterns and vulnerability to cannabis dependence in both males and females.

TOLERANCE The literature on tolerance in human cannabis users is somewhat variable. Early reports suggested that a given dose of THC produced a "high" in heavy or frequent marijuana users that was similar to that in light or infrequent users (Lindgren et al., 1981; Kirk and de Wit, 1999). These results suggested an absence of tolerance to the intoxicating effects of THC. However, controlled laboratory studies involving 4 to 6 consecutive days of high doses of either smoked marijuana or oral THC provided evidence of tolerance to several (although not all) self-reported cannabinoid effects, including drug "high" and "good drug effect" (Haney et al., 1999a, 1999b; Gorelick et al. 2013). Whether prior use influences acute cannabis-induced cognitive impairment is also an unresolved issue. Hart and coworkers (2001) reported that the amount of prior marijuana use influenced the results on some cognitive tests, with heavier use reducing the adverse effects of acute cannabinoid exposure. This finding led the authors to hypothesize that behavioral ("cognitive") tolerance develops in heavy marijuana smokers. However, a more recent study that assessed cognitive performance in the areas of executive function, impulse control, attention, and psychomotor function found no statistically significant relationship between the amount of prior cannabis use and the degree of cognitive impairment produced by inhaled THC (Ramaekers et al., 2016). Taking all of these studies together, it appears that some tolerance to the subjective and cognitive-impairing effects of cannabis *can* occur in marijuana users, but the degree of tolerance likely depends on a number of factors, including patterns and amount of prior use as well as other factors (e.g., genetic) that have yet to be identified.

Studies in laboratory animals have been more consistent, showing that animals exposed repeatedly to THC and other CB_1 agonists develop a profound tolerance to the behavioral and physiological effects of these compounds (González et al., 2005; Panagis et al., 2008). The rate of tolerance development depends on a variety of factors, including the species, the choice of cannabinoid and dosing regimen, and which effect is being studied. Cannabinoid tolerance appears to be largely pharmacodynamic in nature, involving a combination of desensitization and down-regulation of CB_1 receptors. For example, Breivogel and coworkers (1999) found that rats given daily THC injections (10 mg/kg) over a 3-week period showed gradual reductions both in regional CB_1 receptor density and in cannabinoid agonist-mediated receptor activation. In some brain areas, the cannabinoid receptors were almost entirely desensitized following 3 weeks of THC exposure. A more recent study found a striking down-regulation of CB_1 receptors in the terminals of hippocampal GABAergic interneurons in mice treated repeatedly with THC for 6.5 days (Dudok et al., 2015). Moreover, a substantial percentage of the remaining receptors were internalized off the cell membrane, thereby dissociating those receptors from their normal signaling mechanisms (i.e., desensitizing the receptors). Finally, recovery of normal receptor levels did not occur until several weeks after cessation of treatment, suggesting that cannabinoid tolerance can be relatively long-lived.

PET imaging studies have demonstrated widespread decreases in brain CB_1 receptor binding in regular marijuana smokers (Hirvonen et al., 2012; Ceccarini et al., 2015; D'Souza et al., 2016). These findings are consistent with the abovementioned animal studies showing CB_1 receptor down-regulation in animals treated chronically with THC. The imaging studies further showed that receptor binding recovered following abstinence from cannabis, with data indicating partial recovery within a period as short as 2 days when measurements were obtained from heavy users diagnosed as being dependent on cannabis (D'Souza et al., 2016). Furthermore, in those individuals there was a significant negative correlation between the intensity of withdrawal symptoms after 2 days of abstinence and the magnitude of overall CB_1 receptor binding; that is, individuals who showed the strongest withdrawal symptoms soon after stopping marijuana use also had the lowest levels of receptor binding measured at the same time point (**FIGURE 14.18**). This correlation suggests that changes in brain CB_1 receptors due to heavy marijuana use are related to the development of cannabis dependence (see next section). Moreover, because of the significant role played by the endocannabinoid system in so many psychological and physiological processes, the ability of down-regulated cannabinoid receptors to regain normal levels following abstinence is likely to help in recovery from cannabis dependence.

WITHDRAWAL Cannabinoid withdrawal symptoms were first reported in laboratory studies in the 1970s. Later research began to recognize the existence of cannabis dependence, craving, and withdrawal in some marijuana users. Controlled studies of abstinence in long-term heavy marijuana users have reported a number of withdrawal symptoms, including irritability, increased anxiety, depressed mood, sleep disturbances, heightened aggressiveness, and decreased appetite (Cooper and Haney, 2008; 2009). These withdrawal symptoms resemble those seen with several other drugs of abuse, most notably nicotine. Overall symptomatology is greatest during the first 1 to 2 weeks

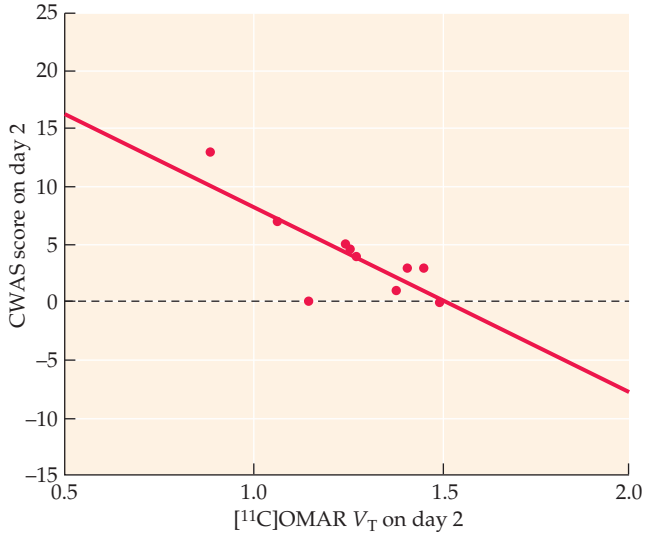


FIGURE 14.18 Withdrawal symptoms are negatively correlated with brain CB_1 receptor availability in cannabis-dependent men PET imaging using the selective CB_1 receptor ligand [^{11}C]OMAR was performed on cannabis-dependent men after 2 days of drug abstinence (the time of peak withdrawal symptoms). Severity of withdrawal symptoms in each person was assessed by means of the Cannabis Withdrawal Assessment Scale (CWAS). The composite (across brain areas) [^{11}C]OMAR volume of distribution (V_T) was negatively correlated with withdrawal severity. As V_T is an index of receptor availability, the results suggest that CB_1 receptor down-regulation is associated with greater dependence in chronic cannabis users. (After D'Souza et al., 2016.)

of withdrawal (**FIGURE 14.19**), but some symptoms may persist for a month or longer. The *DSM-5* defines a diagnostic category of cannabis withdrawal that includes all of the abovementioned symptoms but also adds physical symptoms such as abdominal pain, tremors, sweating, fever, chills, and headache (Panlilio et al., 2015; Simpson and Magid, 2016). Cannabis users in withdrawal often experience craving for the substance, and a similar kind of craving can additionally be elicited by presentation of cannabis-related cues (Norberg et al., 2016). Cannabinoid withdrawal can be alleviated by providing either smoked marijuana or oral THC, demonstrating a key role for this compound in both the development of dependence and the manifestation of withdrawal symptoms.

Early experimental studies in which animals were administered THC chronically and then were examined after the treatment was stopped found few if any signs of withdrawal. Although these results may seem to be at odds with reports of an abstinence syndrome in humans, researchers recognized that the absence of withdrawal symptoms might have been due to the long elimination half-life of THC, which causes the cannabinoid receptors to remain partially occupied for a significant time even after termination of drug treatment. Once the CB_1 receptor antagonist rimonabant was developed, it could be used to test for dependence and withdrawal, since administration of the antagonist would abruptly block the receptors despite the continued presence of THC in the animal. This approach, which is called **precipitated withdrawal**, enabled researchers to demonstrate an abstinence syndrome characterized by tremors, wet-dog shakes, increased grooming behaviors (facial rubbing, licking, and scratching), ataxia, and hunched posture (González et al., 2005; Panagis et al., 2008). As expected, no withdrawal symptoms were observed in CB_1 knockout mice given chronic THC and then challenged with rimonabant. Although the precipitated withdrawal studies have convincingly shown that chronic treatment with THC or a synthetic cannabinoid can produce physical dependence in animals, there has been some criticism that these studies used very high cannabinoid doses compared with typical human recreational use patterns. Nevertheless, the reports of human abstinence symptoms confirm that dependence and withdrawal occur under naturalistic conditions, thereby supporting at least the basic conclusions drawn from the animal research.

The neurochemical mechanisms underlying the marijuana abstinence syndrome are not yet fully understood. Some of the important findings (using the precipitated withdrawal paradigm) include decreased DA cell firing in the VTA and reduced DA release in the nucleus accumbens, increased corticotropin-releasing factor (CRF) release in the central nucleus of the amygdala, increased secretion of stress hormones such

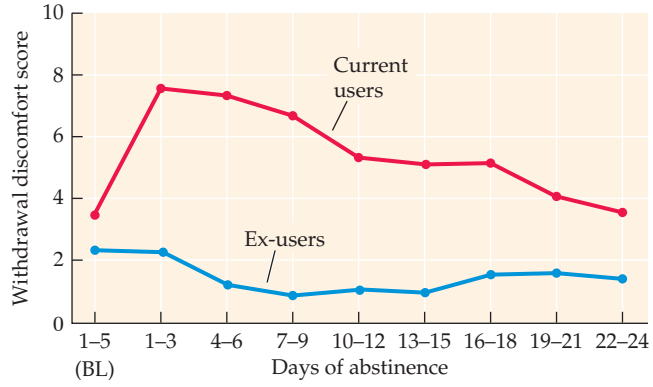


FIGURE 14.19 Time course of overall withdrawal discomfort in heavy marijuana users undergoing abstinence Current marijuana users were compared with ex-users on a battery of 15 possible self-reported withdrawal symptoms over a 5-day baseline period (BL) during which marijuana use was permitted and then during a 45-day period of abstinence. Data shown represent the mean composite withdrawal scores (up to a possible maximum of 36) during baseline and the first 24 days of abstinence. (After Budney et al., 2003.)

as corticosterone, and various changes in the endocannabinoid system (González et al., 2005; Panagis et al., 2008). Together, these alterations could contribute to the mood reduction, irritability, and stress experienced by dependent cannabis users during periods of abstinence. Moreover, at least some of the same responses have been reported to occur during withdrawal from cocaine, alcohol, and opiates, thereby linking cannabinoids with substances generally considered to have greater abuse potential.

TREATMENT OF CANNABIS USE DISORDER Although most cannabis users do not develop a cannabis use disorder and do not seek treatment, those who do become dependent on the drug report many problems (besides craving and withdrawal symptoms) that adversely influence their daily functioning. Such problems may include deteriorating social relationships, financial difficulties, and poor general satisfaction with life (Budney et al., 2007; Copeland and Swift, 2009). Some, although not all, dependent individuals eventually seek professional treatment for their problems.

Marijuana users seeking treatment are typically entered into an outpatient program that may involve cognitive behavioral therapy, relapse prevention training, and/or motivational enhancement therapy (Sherman and McRae-Clark, 2016).[3] These approaches can also be combined with a contingency management program in which participants who submit cannabinoid-negative urine samples earn vouchers redeemable for various goods and services. Although these different treatment programs have all met with some success, patients are highly vulnerable to relapse even after an initial period of abstinence. Thus, marijuana appears to be similar to other drugs of abuse with regard to the difficulty in achieving long-term treatment success in dependent individuals.

The idea of pharmacotherapy for treating cannabis use disorder is not yet widely accepted but has begun to receive more attention in recent years. Medications tested to date include various antidepressants, the antianxiety drug buspirone, the norepinephrine uptake inhibitor atomoxetine (used to treat ADHD), the mood stabilizers divalproex and lithium carbonate (used to treat bipolar disorder), the opioid receptor antagonist naltrexone, and *N*-acetylcysteine, which is hypothesized to normalize substance-induced dysregulation of the glutamatergic system (Copeland and Pokorski, 2016; Sherman and McRae-Clark, 2016). Although some of these medications seem to reduce cannabis withdrawal-related symptoms, overall none has proven to reliably improve long-term abstinence rates. An alternative approach is to treat patients with a cannabinoid agonist, which follows the same agonist substitution approach as seen in the use of nicotine-based medications for cigarette smokers or methadone for opiate addicts. A recent study found that nabiximols, the spray formulation that contains both THC and CBD, significantly reduced overall cannabis withdrawal symptoms, including craving, in a double-blind placebo-controlled inpatient trial of cannabis-dependent patients in Australia (Allsop et al., 2014; 2015). On the other hand, the treatment had no effect on long-term reductions in cannabis use. Thus, current findings indicate that existing medications of any kind are valuable primarily as a treatment adjunct to help patients deal with their withdrawal symptoms. Long-term abstinence seems to require intensive psychotherapy using the methods described above.

[3]Motivational enhancement therapy is a type of psychotherapy that seeks to elicit a desire for behavioral change on the part of the patient.

Chronic cannabis use can lead to adverse behavioral, neurobiological, and health effects

Given that dedicated cannabis users may consume the drug on a regular, even daily, basis for many years, concern has arisen over whether such lengthy periods of chronic drug exposure might lead to adverse psychological, neuropsychiatric, or physiological effects. Evidence for such effects is discussed in this final section of the chapter.

EDUCATIONAL PERFORMANCE AND IQ Survey studies indicate that the amount of cannabis use by young people is inversely related to educational performance. That is, greater use is associated with poorer grades, more negative attitudes about school, and increased absenteeism (Lynskey and Hall, 2000). However, prospective longitudinal studies have yielded a more complex picture. Early research suggested that regular cannabis use beginning relatively early in life is a significant risk factor for poor performance in school and even dropping out (Townsend et al., 2007; Brook et al., 2008). In contrast, a recent longitudinal study found that low to moderate use in early adolescence has relatively minor effects on academic performance that appear to dissipate after a period of abstinence (Pardini et al., 2015). Yet another study found that daily tobacco use assessed at the age of 15 had a stronger impact on academic test scores 1 year later than weekly cannabis use (Stilby et al., 2015). These findings must be viewed cautiously, however, because of the possibility that heavier use of cannabis (e.g., daily use) would have produced greater impairment than seen in more moderate users.

At the present time, we do not know whether there is a causal relationship between amount of cannabis use and educational achievement. Even if there is, the direction of causation would still need to be established. Does early cannabis use cause a lack of success in school, or does a lack of success early in one's academic career cause an increase in cannabis use?

One hypothesis is that heavy cannabis use leads to persistent cognitive deficits, thereby impairing school performance (Jacobus et al., 2009). It is possible that students who use cannabis heavily over a long period of time could perform poorly in school because of the cognitive impairment. In support of this hypothesis are findings of Meier and colleagues (2012), who conducted a prospective study of cognitive function in approximately 1000 people in New Zealand. The participants were recruited into the study at 3 years of age and were subjected to neuropsychological testing at 13, before the onset of cannabis use, and again at 38 years of age. The amount of cannabis use and the appearance of symptoms of cannabis dependence were significantly associated with neuropsychological impairment (including lower IQ) at 38 years, even after controlling for level of education. These were among the first data to suggest a causal relationship between long-term cannabis use and cognitive deficits. Despite these findings, it is important to consider alternate hypotheses. One hypothesis is that the social context surrounding heavy cannabis use at a relatively early age promotes the rejection of mainstream social values such as educational achievement in favor of a more unconventional lifestyle (Fergusson et al., 2003a; Lynskey et al., 2003). Jackson and coworkers (2016) additionally reported that lower IQ in adolescent marijuana users was influenced significantly by an early family environment that promoted both initiation of marijuana use and poor intellectual attainment. Both approaches attempt to account for lower IQ and poor school performance in marijuana users without postulating a direct effect of cannabis on these variables.

Another possibility involves drug-related motivational changes that would have a negative impact on performance in the classroom. Indeed, research going back more than 40 years has found evidence for apathy, aimlessness, loss of achievement motivation, lack of long-range planning, and decreased productivity in chronic marijuana users. For example, a 1968 review by McGlothlin and West states, "Clinical observations indicate that regular marihuana use may contribute to the development of more passive, inward-turning, amotivational personality characteristics. For numerous middle-class students, the subtly progressive change from conforming, achievement-oriented behavior to a state of relaxed and careless drifting has followed their use of significant amounts of marihuana" (p. 372). This so-called **amotivational syndrome** has continued to be discussed in the cannabis literature (e.g., see Lynskey and Hall, 2000). We cannot rule out the possibility that some users experience a loss of drive and achievement motivation as a result of chronic, heavy exposure to cannabis. However, one could argue just as plausibly that such personality characteristics are a cause, rather than a consequence, of adopting a marijuana-centered lifestyle. For example, the Victorian Adolescent Health Cohort Study found evidence that disengagement with school, culminating in some cases with completely dropping out, was concomitant with or even preceded the onset of daily cannabis use (Coffey and Patton, 2016). Thus, it seems that current evidence is still insufficient to determine the potential direction of causality with respect to marijuana use, motivation, and educational achievement.

COGNITIVE EFFECTS In the earlier section on acute effects of cannabis use, we saw that memory and other cognitive functions are impaired shortly after smoking marijuana. However, most people do not engage in this behavior while they're at work or in class or at other times when a high level of functioning is required. If marijuana is used only during recreational times (e.g., evenings and weekends), and if drug-related cognitive deficits do not outlast the period of use, then one could argue that such deficits are harmless. On the other hand, it is possible that heavy recreational use over a long period of time somehow compromises brain function such that cognitive problems persist even after drug use is stopped. The question of residual cognitive deficits from marijuana use has been controversial, but some reasonably consistent findings have emerged. There is substantial evidence that long-term cannabis use leads to impairment in many cognitive domains when assessed following 2 weeks of abstinence, a time period sufficient for withdrawal symptoms to subside (Crean et al., 2011; Broyd et al., 2016; Ganzer et al., 2016). The affected functions include attention, the ability to concentrate, executive functions, verbal learning and memory, and psychomotor performance. **TABLE 14.1** compares the effects of acute versus chronic cannabinoid exposure on cognitive function, noting that acute effects are more reliably observed. Of additional importance is the question of whether partial or full recovery of function occurs following longer periods of abstinence from the drug. As shown in Table 14.1, some kinds of cognitive deficits may be persistent, although the data are not entirely consistent and more research is needed (Crean et al., 2011; Schreiner and Dunn, 2012; Broyd et al., 2016). Indeed, persistent deficits are most likely to occur in individuals who begin regular, heavy cannabis use early in adolescence.

NEUROPSYCHIATRIC EFFECTS The recognition that long-term cannabis use is associated with impaired cognitive function and motivation has generated much interest in using structural neuroimaging techniques such as magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI, a technique that assesses white matter structure) to identify potential neural correlates of these effects. As has been the case for much

TABLE 14.1 Summary of Acute and Chronic Effects of Cannabinoid Exposure on Cognitive Processes

Acute effects
Impaired verbal learning and memory
Impaired working memory
Impaired attention (task- and dose-dependent)
Impaired inhibitory control and (to a lesser extent) other executive functions
Impaired psychomotor function
Chronic effects
Impaired verbal learning and memory
Impaired attention; attentional bias
Possibly impaired psychomotor function
Possibly impaired executive function (depending on frequency of use and age of onset)
Recovery of function with abstinence
Likely persistent effects on attention and psychomotor function
Possibly persistent effects on verbal learning and memory (insufficient and mixed evidence)

Source: After Broyd et al., 2016.
Note: Some acute cannabinoid effects are dependent on the specific task and dose, whereas some chronic effects depend on frequency of use and age of onset of cannabis use. Lesser reliability of findings is denoted by terms such as *possibly* and *likely*.

of the other research discussed in this chapter, MRI findings have been somewhat inconsistent across studies. This is not surprising considering between-study variability in amounts and patterns of cannabis usage, age of onset of use, length of time during which use has occurred, and length of abstinence interval if participants were required to maintain abstinence prior to undergoing scanning. Despite this variability, several findings have been sufficiently consistent to mention here. Compared with nonusers, regular cannabis users show a lower volume of several brain areas, notably the hippocampus and parts of the PFC (reviewed in Lorenzetti et al., 2014; Lubman et al., 2015). Some studies have associated reductions in regional brain volume to early adolescent cannabis use. A few DTI studies have additionally found evidence for deficits in white matter integrity in heavy cannabis users (Jacobus and Tapert, 2014; Lubman et al., 2015). These findings raise the possibility that regular cannabis use, especially in large amounts, may cause damage to brain areas that play important roles in learning, memory, and other cognitive functions. We need to keep in mind, however, that almost all of the studies reviewed were cross-sectional, thus allowing for the possibility that brain differences preceded rather than followed use of cannabis. Moreover, cannabis users typically consume other substances as well, such as alcohol and nicotine, and many studies do not adequately control for such polydrug use.

Neurochemical differences between cannabis users and nonusers have been examined using PET imaging. Considerable research has focused on the dopaminergic system, because abnormalities in this system could cause changes in mood and motivation, alter the risk of consuming other substances of abuse, and contribute to craving for cannabis and other symptoms of dependence. Thus, two recent studies found reduced striatal DA synthesis in regular cannabis users compared with nonusers (Bloomfield et al., 2014a; 2014b). DA synthesis capacity in the cannabis group was correlated with scores on a self-rated apathy scale (i.e., lower DA synthesis was related to greater apathy), amount of cannabis use (i.e., lower DA synthesis was related to greater use), and age of onset of use (i.e., lower DA synthesis was related to earlier onset of use). Two other studies used PET to examine striatal responses to amphetamine, which releases DA, or methylphenidate, which blocks DA reuptake. Individuals who met criteria for marijuana abuse or dependence exhibited decreased striatal DA responses to these drugs. Moreover, the dopaminergic deficits in the amphetamine study were correlated with poorer attention, poorer working memory, and negative symptoms (van de Giessen et al., 2016), whereas the deficits in the other study were related to blunted subjective and physiological responses to methylphenidate, negative emotionality, severity of cannabis dependence, and craving (Volkow et al., 2014b). Together, these four PET imaging studies provide compelling evidence that chronic use of cannabis, including use patterns that result in abuse and dependence on the drug, are associated with (and may have caused) substantially impaired dopaminergic

function in the striatum. Importantly, such impairment likely plays a role in long-term disturbances in mood, motivation, and cognition, as well as contributing to the development of cannabis use disorders. The effects of heavy cannabis use on the brain could also manifest over time in other serious neuropsychiatric disorders. Indeed, a number of large-scale epidemiologic studies have found a significant relationship between early heavy marijuana smoking and increased risk for the later development of psychotic disorders such as schizophrenia. This important area of research is discussed in greater detail in **Web Box 14.2**.

Rodent studies have played a significant role in modeling the neurochemical and behavioral effects of chronic cannabinoid exposure. Because heavy cannabis users usually begin early in adolescence and because the adolescent brain is still undergoing important developmental processes (Bava and Tapert, 2010; Colver and Longwell, 2013; Houston et al., 2014), many of the rodent studies have focused on chronic adolescent dosing paradigms using either THC or synthetic cannabinoids such as WIN 55,212-2 or CP-55,940. Although a detailed account of these studies is not possible because of space limitations, it is clear that repeated exposure of rodents to cannabinoids during adolescence results in numerous changes in the glutamatergic, GABAergic, and dopaminergic systems (reviewed in Renard et al., 2016a; Rubino and Parolaro, 2016). These neurochemical changes are accompanied by altered synaptic plasticity (i.e., LTP and LTD), dysregulated emotional (i.e., anxiety-like and depressive-like) and social behaviors, and impaired cognitive function. Structural studies of the PFC, hippocampus, and nucleus accumbens additionally found evidence for cannabinoid-induced developmental abnormalities of dendritic arbors and spines, both of which are critical for synapse formation and synaptic function. One such example involves a study in which adolescent rats received daily doses of CP-55,940 from postnatal day 29 through day 50 (Renard et al., 2016b). Drug doses were increased at the beginning of the second and third weeks of treatment to model escalation of use and to account for potential cannabinoid-induced tolerance. No additional drug treatments were given after day 50. Some of the cannabinoid-exposed and vehicle-treated rats were killed in adulthood at 92 days of age for examination of dendritic arborization in pyramidal neurons within the PFC. Other animals were tested at about the same age for LTP in the pathway from the hippocampal CA1 area to the PFC. The results from these measurements are presented in **FIGURE 14.20**. The average total dendritic length was significantly reduced in the cannabinoid-treated animals, and this effect was almost entirely due to a difference in the basal dendrites, which are dendrites that emanate from the base of the cell (Figure 14.20A). The cannabinoid-treated rats also exhibited a deficiency in LTP induced at the synaptic pathway from the hippocampus to the PFC (Figure 14.20B). Thus, an animal model of daily cannabis use during adolescence demonstrated deficits in prefrontal pyramidal cell dendritic development

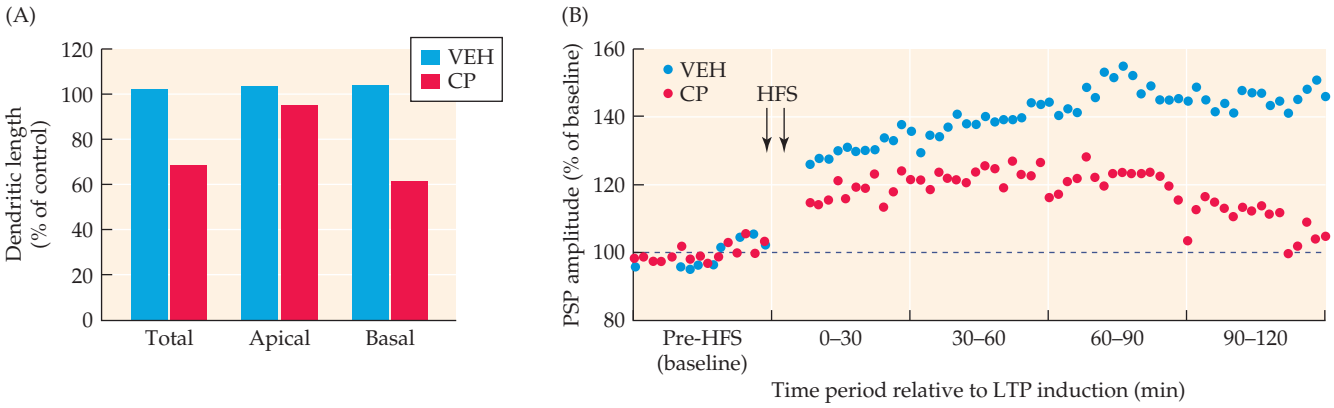


FIGURE 14.20 Chronic treatment of adolescent rats with CP-55,940 reduced PFC dendritic length and impaired LTP at hippocampal–PFC synapses Male rats were given daily IP injections of CP-55,940 (CP) or drug vehicle (VEH) from postnatal day 29 through day 50. Doses were 0.15 mg/kg for the first 7 days, 0.20 mg/kg for the next 7 days, and 0.30 mg/kg for the last 7 days. No further treatments were given after day 50. (A) On postnatal day 92, some of the CP-55,940–treated and control rats were killed and their brains were stained to visualize dendritic arbors using a modification of the Golgi technique (see Chapter 2). Measurement of dendritic length of cortical layer 2/3 pyramidal neurons in the PFC revealed a significant reduction in basal dendrites and in total dendritic length (basal + apical). (B) Separate groups of rats at day 92 or later were anesthetized for electrophysiological analysis of long-term potentiation (LTP) at hippocampal–PFC synapses that was induced by high-frequency stimulation (HFS). LTP in the drug-treated rats (measured as postsynaptic potential [PSP] amplitude) was impaired when compared with vehicle-treated animals. (After Renard et al., 2016b.)

and a concomitant impairment of synaptic plasticity in the hippocampal–PFC circuitry. Although not proven by the existing data, it is reasonable to speculate that these structural and functional changes contribute to the well-known cognitive deficits associated with chronic adolescent cannabis exposure.

HEALTH EFFECTS In considering the potential health consequences of cannabis use, there is both good and bad news. The good news is that there is no published report of anyone dying as a result of cannabis overdose. This means that the use of this substance has a margin of safety that is lacking with many other substances of abuse such as heroin, cocaine, and sedative–hypnotic drugs. The bad news is that the lack of fatal overdosing does not mean that cannabis use, particularly in large amounts or for long periods of time, is without risk for adverse health outcomes beyond those already described (Volkow et al., 2014a; Hall, 2015). This section will, therefore, review some of the major health consequences of cannabis use. While some of these adverse health consequences are, as with tobacco, due to inhaling toxic substances and particulate matter from burning plant material, others stem from the disruption of endocannabinoid signaling in various peripheral organ systems (reviewed in Maccarrone et al., 2015).

Because cannabis is usually consumed by smoking, the possibility of lung damage is one obvious area of concern. Although marijuana joints and tobacco cigarettes contain different psychoactive ingredients (i.e., cannabinoids versus nicotine), the smoke they produce has the same kinds of irritants and carcinogens. Tar from cannabis smoke actually contains higher concentrations of certain carcinogens known as benzanthracenes and benzpyrenes. Even so, one might think that marijuana smoking is not harmful because users typically smoke only one or a few joints a day, compared with the one or more packs of cigarettes smoked by regular tobacco users. Unfortunately, it appears that the amounts of tar and carbon monoxide taken in *per cigarette* are much greater for marijuana joints than for tobacco cigarettes (Wu et al., 1988). It is not surprising, therefore, that regular marijuana smoking is associated with various respiratory symptoms, including chronic cough, increased phlegm production, wheezing, and emphysema (Martinasek et al., 2016). There is even some preliminary evidence for an increased risk for developing lung cancer in heavy or long-term marijuana smokers. Of particular concern is the co-use of marijuana and tobacco, which may pose a greater health risk than either substance alone (Meier and Hatsukami, 2016).

Marijuana smoking also appears to adversely affect the cardiovascular system. Accumulated evidence indicates that the risk of experiencing a myocardial infarction (heart attack) is significantly elevated during the hour after smoking marijuana (Thomas et al., 2014; Franz and Frishman, 2016). Note that this increased risk is not limited to older users who may have atherosclerosis or coronary artery disease but also occurs in users who are in their 20s or 30s. Furthermore, a recent animal study suggests that even exposure to secondhand marijuana smoke can impair vascular function (Wang et al., 2016).

Another system susceptible to interference by cannabis use is the reproductive system. It is clear that the endocannabinoid system plays an important role in both the male and female reproductive systems (du Plessis et al., 2015; Brents, 2016), raising the possibility that marijuana smoking could have an adverse influence on reproduction. In support of this possibility, animal studies have consistently shown that THC suppresses the release of hypothalamic gonadotropin-releasing hormone (GnRH) in both males and females. In turn, this reduction in GnRH leads to decreased secretion of luteinizing hormone (LH) from the pituitary gland (Maccarone and Wenger, 2005). Additional research on women and on female rhesus monkeys, which constitute an excellent animal model for women's reproductive physiology, suggests that marijuana smoking (in women) or THC exposure (in monkeys) can cause menstrual cycle irregularities (Brents, 2016). Such irregularities are likely related, at least in part, to the inhibitory effects of THC on GnRH and LH. The comparable reproductive effects in males consist of reduced circulating testosterone levels and deficits in sperm concentrations, viability, and motility (du Plessis et al., 2015). Note, however, that despite these findings there is still relatively scant evidence for a deleterious effect of marijuana on fertility in male users.

Since the discovery of CB_2 receptor expression in cells of the immune system, this system has been closely associated with endocannabinoid modulation. Both CB_1 and particularly CB_2 receptors are expressed by various immune cells, and administration of THC or other cannabinoid receptor agonists generally suppresses immune function (Tanasescu and Constantinescu, 2010). This immunosuppressive action is mediated, in part, by activation of CB_2 receptors on T lymphocytes. Interestingly, however, this same effect could potentially be exploited therapeutically by administering a selective CB_2 receptor agonist to patients with autoimmune disorders or who have received tissue grafts (Eisenstein and Messler, 2015). In both instances, benefit is obtained from dampening native immune responses. Importantly, THC has been found to impair an organism's resistance to bacterial and viral infections in laboratory animals under controlled experimental conditions (Eisenstein and Meissler, 2015). We don't yet know, however, whether marijuana use leads to an increased incidence of infectious disease under real-life conditions.

Legalization of marijuana, whether generally or for specific medical use, is expected to increase the number of individuals exposed to this substance beyond preexisting recreational users. Among those affected in this way will be pregnant women. As recently noted in an opinion piece by Volkow and colleagues (2017), marijuana is the most frequently used illicit drug by pregnant women, and the authors raise concern that marijuana is currently being touted as a remedy for pregnancy-related nausea. This raises the important question of whether use of cannabis during pregnancy poses risks to the offspring. Because THC is a lipophilic compound, it readily passes through the placenta into the fetal circulation. The fetal brain expresses cannabinoid receptors and a developing endocannabinoid system that could be disrupted by repeated exposure to phytocannabinoids such as THC (Calvigioni et al., 2014). Recent meta-analysis and reviews of studies on maternal and neonatal outcomes found a significant association between maternal cannabis use and increased risk for maternal anemia, low birth weight of the infant, smaller head circumference at birth (which indicates reduced brain size and predicts lower IQ later in life), and greater likelihood that the newborn will require placement in the neonatal intensive care unit (Calvigioni et al., 2014; Gunn et al., 2016). These effects were substantiated even when the data were controlled for maternal tobacco use, which itself is a major cause of intrauterine growth restriction. Growth restriction during fetal development is thought to be related, at least in part, to activation of placental CB_1 receptors and resulting decreases in nutrient and oxygen availability to the fetus (Jaques et al., 2014). Additionally, there are some reports that infants born of cannabis-using women show behavioral irritability and EEG disturbances compared with infants of nonusers.

Several longitudinal studies have investigated the possible impact of prenatal cannabis exposure on behavior later in infancy and childhood. Depending on the age of offspring assessment, amount of maternal cannabis use, and the period of prenatal exposure (i.e., trimester of pregnancy), deficits in a number of outcome measures have been reported. These measures include visual perceptual function, language skills, attention, memory, and educational achievement (Calvigioni et al., 2014; Jaques et al., 2014; see **TABLE 14.2** for a summary of these effects). Developmental researchers have theorized that adverse outcomes that appear later in life may require multiple "hits" to the organism, in which early exposure to a drug or toxicant exposure is the first hit and later events such as major life stressors constitute the additional hits needed to produce the observed deficit. Indeed, Richardson and coworkers (2016) have hypothesized this kind of mechanism to account for the adverse effects of prenatal cannabis exposure. It is important to acknowledge the difficulty of attributing the effects summarized here specifically to cannabis, as pregnant women who persist in using cannabis may differ from nonusers in other important ways, including tobacco use, nutrition during pregnancy, amount of prenatal care they receive, socioeconomic status, and their levels of stress exposure (Jaques et al., 2014). For the longitudinal studies, we additionally need to be concerned about the potential impact of differing postnatal environments under which the children are reared. Nevertheless, sufficient concerns exist to highlight the importance of educating women about the dangers of continuing to smoke marijuana during pregnancy and encouraging the development of treatment programs to serve marijuana-dependent women who become pregnant.

The final topic of this chapter brings us back to the chapter opener, which gave a published account of a man severely intoxicated from using a potent synthetic cannabinoid. Use of these substances has increased in recent years because of their easy access and the powerful "high" they produce. However, synthetic cannabinoids pose even greater health risks than cannabis. The history, mechanism of action, and toxicity of synthetic cannabinoids are discussed in **BOX 14.1**.

TABLE **14.2** Summary of Reported Effects of Maternal Cannabis Use on the Offspring

Time of assessment			
Birth/neonatal	**Infancy/early childhood**	**Mid–late childhood**	**Adulthood**
↓ Body weight and head circumference	Impaired cognitive development	Externalizing behaviors	Altered visuospatial functioning
↑ Startles and tremors	↑ Aggression and inattention (girls)	Impaired cognitive/ executive function	
↓ Habituation to light	Impaired memory function	Deficits in educational achievement	
Altered sleep EEG	↓ Verbal performance	↑ Depressive symptoms and behavior problems	

Source: After Calvigioni et al., 2014.

BOX 14.1 Of Special Interest

Beyond Cannabis: The Rise of Synthetic Cannabinoids

Several synthetic compounds with cannabinoid activity, such as CP-55,940 and WIN 55,212-2, have been widely used in research for several decades. However, recreational use of synthetic cannabinoids was unknown until 2004, when advertisements began to appear on the internet for new herbal preparations containing synthetic designer cannabinoids that were legal at the time (Debruyne and Le Boisselier, 2015; Karila et al., 2016a). These substances were typically sold under the names "K2" or "Spice," and although these names are still in use, many additional names like "Black Mamba," "Bliss," and "Chill" have since been added to the street lexicon. The first synthetic cannabinoid constituent of Spice was identified as JWH-018[1] in 2008 by a German forensic laboratory. Since then, more and more distinct drugs have become available as illicit laboratories that synthesize these compounds attempt to stay ahead of the legal system. Indeed, testing of herbal mixtures has identified at least 200 different synthetic cannabinoids that could be obtained for recreational use (van Amsterdam et al., 2015a). It is not unusual for an advertised product to contain more than one type of cannabinoid drug, thereby providing the user with more options in seeking the desired reaction. Moreover, the increasing distribution of these drugs can be seen in the fact that European law enforcement agencies made over 21,000 synthetic cannabinoid seizures in 2013 alone (Karila et al., 2016a).

Synthetic cannabinoids are produced in a powdered form that can be solubilized and then sprayed onto herbs or other plant materials. The resulting material is then sold with labels like "herbal incense," often with the disclaimer "not for human use." The spiked herbs can be rolled into a joint or mixed with tobacco or regular marijuana. In other cases, the drugs are vaporized and inhaled using a bong or an e-cigarette cartridge. Users of synthetic cannabinoids are typically adolescents and young adults, including high school and college students (Hu et al., 2011; Johnston et al., 2016). Some individuals prefer synthetic cannabinoids over marijuana because of perceived or actual legality, if the particular cannabinoid has not yet been added to the list of illicit substances; to avoid detection, as these substances are not usually tested for in routine drug screens; because of easy availability through the internet or other sources; to obtain a stronger "high"; or simply out of curiosity. Surveyed cannabis users reported that compared with natural cannabis, the peak effects of synthetic cannabinoids occur more rapidly but also dissipate more quickly. Importantly, the majority of these cannabis users displayed a preference for natural cannabis over synthetic cannabinoids (Winstock and Barratt, 2013; Cooper, 2016), which presumably helps account for why the prevalence of cannabis use continues to be much greater than that of synthetic cannabinoids.

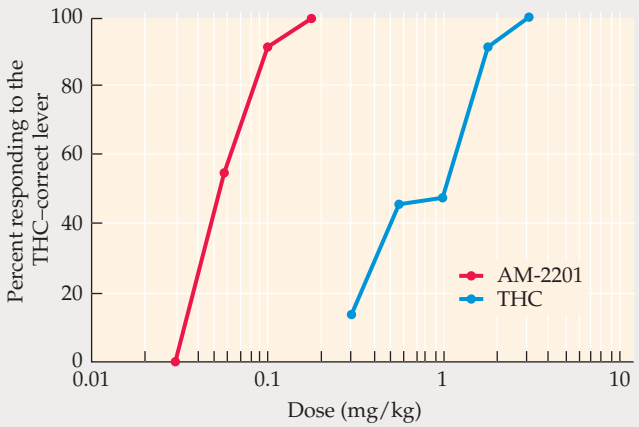
Recreational synthetic cannabinoids are similar to THC in that they typically activate both CB_1 and CB_2 receptors. However, they are more potent on a per-dose basis, are often full rather than partial agonists at the receptors, and are metabolized by different biochemical pathways (Fantegrossi et al., 2014). Together, these factors help explain the greater incidence of toxic reactions to synthetic cannabinoids compared with cannabis. An example of potency difference for the compound AM-2201 is illustrated in the **Figure**. Like JWH-018, AM-2201 has been used and abused recreationally. Järbe and Raghav (2016) provided the following description of the powerful effects of AM-2201 intoxication: "My heart starts pounding so fast and hard and doesn't feel real. As of that moment, I no longer know who I am, where I am and what is real" (p. 265). As noted by Järbe and Raghav, such a description is similar to the accounts of Parisian hashish users almost 200 years earlier!

Over time since the onset of recreational synthetic cannabinoid use, it has become clear that regular users can become dependent and exhibit withdrawal symptoms upon cessation of use (Macfarlane and Christie, 2015). Unlike the situation with cannabis, we don't yet have information on whether these substances can cause long-term health problems. However, there is ample information on the subjective and physiological effects of acute intoxication with synthetic cannabinoids, as well as reports of more serious health-related consequences for some users. Castaneto and colleagues (2014) summarized the features of synthetic cannabinoid intoxication documented in published case reports. Major psychological symptoms consist of agitation or restlessness,

(Continued)

[1]Compounds with the designation "JWH" belong to a class of cannabinoids first synthesized and characterized by the organic chemist John W. Huffman. Although Huffman's work was intended for potential therapeutic applications, publication of the chemical structures and synthetic pathways has enabled others to make use of the information to synthesize and sell many of these same cannabinoids for recreational purposes. A different group of synthetic cannabinoids is designated "AM," which stands for Alexander Makriyannis. Makriyannis is another pioneer in the discovery and characterization of synthetic cannabinoid drugs.

BOX 14.1 Of Special Interest (*continued*)



Potency of AM-2201 compared with THC in a rat drug discrimination paradigm Rats were trained on a two-lever drug discrimination paradigm (see Chapter 4) to discriminate THC (3 mg/kg IP) from vehicle. When different doses of THC were administered to previously trained rats (blue circles), the resulting dose–response curve yielded an ED_{50} of 0.76 mg/kg (ED_{50} in this task is defined as the dose resulting in 50% of responses on the drug-correct lever). When AM-2201 was substituted for THC and a similar dose–response function was determined (red circles), the curve was shifted far to the left and the calculated ED_{50} was 0.06 mg/kg. The leftward shift and lower ED_{50} are indicative of greater potency of the synthetic cannabinoid. (After Järbe and Raghav, 2016, and Järbe et al., 2016.)

feelings of anxiety, confusion, and cognitive deficits such as memory loss. Intoxicated users also exhibit somatic symptoms such as elevated heart rate and blood pressure, nausea and vomiting, chest pain, breathlessness, sweating, twitching, and dilated pupils. Although a similar symptom profile can occur as a consequence of excessive cannabis use, the risk of needing emergency medical treatment is significantly greater with synthetic cannabinoids (Winstock et al., 2015). Synthetic cannabinoid toxicity may be related not only to the greater potency of these compounds but also to a phenomenon called "superconcentrations" or "hot spots" (Papanti et al., 2014). These terms refer to instances in which the synthetic cannabinoid is not mixed with the herbal material in a uniform manner, thereby allowing the possibility of consuming excessive amounts of the drug.

Most cases of acute intoxication resolve uneventfully, and at most, the hospital emergency department can release the patient after observation and perhaps provision of supportive care consisting of IV fluids, oxygen, and (if needed) treatment with an anxiolytic medication. A small percentage of cases, however, involve more serious consequences. Among the reported adverse events that require hospitalization are myocardial infarction, hemorrhage, several kinds of kidney injury, seizures, and panic attacks (Tait et al., 2016; White, 2016). A number of cases of first-onset psychotic reactions have also occurred (van Amsterdam et al., 2015a; Fattore, 2016). Some researchers have hypothesized that CBD may serve a protective role against acute psychotic reactions to the THC constituent of cannabis and that the lack of CBD in synthetic cannabinoid preparations increases the risk of experiencing such a reaction. Most disturbingly, there are documented reports of fatal reactions to synthetic cannabinoids (Trecki et al., 2015), which contrasts with natural cannabis, for which fatalities are virtually unknown. Some of these cases seem to involve cannabinoid overdose alone, whereas others may involve reactions to polydrug use involving synthetic cannabinoids taken together with other substances.

In summary, a large variety of different synthetic cannabinoids have appeared on the street. Unlike natural cannabis material that contains known cannabinoid compounds, K2 and Spice preparations contain unknown mixtures of synthetic drugs. Use of these substances can produce highly toxic reactions due to their potency, their ability to function as full agonists at cannabinoid receptors, and the possibility of superconcentrations or hot spots in an herbal preparation. Prospective users of synthetic cannabinoids should be aware of the risks to their health, even if they believe the material to be legal at the time of use.

Section Summary

- Marijuana is the most heavily used illicit drug in the United States.
- Early behavioral (e.g., conduct) problems have been associated with an increased likelihood of early marijuana use.
- Initial exposure to marijuana usually occurs during adolescence, after the individual has already had experience with alcohol and/or cigarettes. Some investigators have hypothesized that alcohol and tobacco are "gateway" drugs to marijuana, which then serves as a potential gateway to other illicit drugs. However, it is difficult to determine whether marijuana actually facilitates the progression to these more dangerous substances.
- Other factors such as family issues, poor school performance, and a strong positive response

to early marijuana experience are risk factors for the transition to regular use and possibly dependence.

- *DSM-5* contains diagnostic criteria for cannabis intoxication, withdrawal, and use disorder. Criteria for cannabis use disorder are the same *DSM-5* criteria for any substance use disorder but applied specifically to cannabis. Severity may be mild, moderate, or severe depending on the number of criteria met by the patient. Longitudinal studies indicate that about 20% to 25% of cannabis users will develop a cannabis use disorder at some point in time, but the disorder resolves over time in most individuals.
- Many factors contribute to the risk of developing a cannabis use disorder, including early onset of use, progressing to daily use, experiencing highly positive reactions to cannabis, use of cannabis to cope with negative emotions, concurrent use of tobacco, living alone, experiencing major life stressors, having a comorbid psychiatric disorder, possessing specific SNPs for the CB_1 and FAAH genes, and being male.
- Controlled laboratory studies have demonstrated tolerance to repeated THC exposure in both humans and experimental animals. Such tolerance is related to a desensitization and down-regulation of central CB_1 receptors, including CB_1 receptors in the cerebral cortex of regular marijuana smokers.
- Heavy (e.g., daily) marijuana users are at significant risk for developing dependence on the drug and for undergoing withdrawal symptoms upon becoming abstinent. Withdrawal symptoms include heightened irritability, anxiety, aggressiveness, depressed mood state, sleep disturbances, reduced appetite, craving for marijuana, and a cluster of physical symptoms consisting of abdominal pain, tremors, sweating, fever, chills, and headache.
- Chronic THC exposure in laboratory rodents also causes the development of dependence that can be demonstrated using the procedure of precipitated withdrawal with rimonabant. Neurochemical studies of cannabinoid-dependent animals undergoing withdrawal have found reduced DA cell firing, increased CRF release, and endocannabinoid system changes that could contribute to some of the symptoms of cannabis withdrawal in human users.
- Individuals who have developed cannabis dependence report a number of life problems, which leads some of these individuals to seek treatment.
- Some success has been achieved with various kinds of psychotherapeutic interventions, and there has been additional improvement in outcome from adding a voucher-based incentive program to the standard treatment approach. Nevertheless, most dependent individuals find it difficult to maintain long-term abstinence.
- Pharmacotherapeutic approaches to the treatment of cannabis dependence are now being investigated. Approaches using cannabinoid agonists such as nabiximols spray are also being tested. Although such treatments can reduce withdrawal symptoms in cannabis-dependent patients, they have not yet demonstrated efficacy in improving long-term recovery from the disorder.
- Concerns have been raised over possible adverse consequences of chronic cannabis consumption. There is a negative association between the amount of cannabis use by young people and their educational performance, although it is not yet known whether this association is causal. It is possible that heavy cannabis use can produce persistent cognitive deficits and/or an amotivational syndrome characterized by apathy, loss of achievement motivation, and decreased productivity. Alternatively, early cannabis use may be linked to the adoption of an unconventional lifestyle that devalues educational striving and achievement.
- Testing of long-term cannabis users after (possible) withdrawal symptoms have subsided has revealed deficits in several cognitive domains, including attention, ability to concentrate, executive functions, verbal learning and memory, and psychomotor performance. Some, though not all, of these deficits may persist over a long time period following abstinence.
- Neuroimaging studies of regular cannabis users have reported structural and biochemical changes in certain brain regions, including reduced volume of the hippocampus and parts of the PFC, and deficits in white matter integrity. Chronic use has also been associated with decreased striatal DA synthesis and decreased dopaminergic responses to challenge with psychostimulant drugs. These neurochemical differences were correlated with some of the psychological (cognitive and mood) differences between users and nonusers.
- Rodent studies have investigated the neurobiological and behavioral consequences of repeated cannabinoid exposure during adolescence. This research has shown cannabinoid-mediated changes in several neurotransmitter systems, altered synaptic plasticity, dysregulated emotional and social behaviors, and impaired cognitive function. In addition, abnormal growth of dendritic arbors and

spines has been observed in the PFC, hippocampus, and nucleus accumbens.

- Health consequences of heavy marijuana smoking include respiratory problems, increased risk of a myocardial infarction, interference with the reproductive system in both men and women, suppression of immune function, and adverse effects on offspring development when used by pregnant women.
- Synthetic designer cannabinoids marketed as "K2" or "Spice" began to be sold over the internet in 2004. Synthetic cannabinoids are typically highly potent full agonists at both CB_1 and CB_2 receptors. Because of their potency, these substances can produce a severe state of intoxication and have caused a range of adverse health effects including kidney damage, seizures, panic attacks, first-onset psychosis, and even a small number of fatalities. Taken together, these consequences indicate the unusually high degree of toxicity of synthetic cannabinoids, and they highlight the danger of using these substances.

STUDY QUESTIONS

1. What is the difference between phytocannabinoids and endocannabinoids? List two examples from each category.
2. What was the name of the first federal legislation aimed at regulating the sale of marijuana? Is that law still in effect now?
3. (a) Describe the principal routes of cannabis administration. Which of these routes provides the greatest cannabinoid bioavailability? (b) Name two major metabolites of THC.
4. Discuss the main characteristics of cannabinoid receptors, including receptor subtypes, receptor distribution within and outside of the brain, and whether the receptors are metabotropic or ionotropic.
5. Discuss the subcellular localization and signaling mechanisms of the CB_1 receptor.
6. Describe the features of cannabinoid receptor pharmacology presented in the text. Your answer should include a description of both receptor agonists (natural versus synthetic, full versus partial agonism) and receptor antagonists.
7. Discuss the functional roles of CB_1 and CB_2 receptors as revealed by pharmacological and genetic approaches.
8. Describe what is currently known about endocannabinoid synthesis, uptake, and metabolism.
9. Discuss the three mechanisms of endocannabinoid signaling presented in the text.
10. What are the functional effects of the endocannabinoid system on mood, learning/memory, eating behavior/energy metabolism, and pain regulation? Support your answer with relevant experimental findings.
11. Describe the features of cannabis intoxication in humans, including the four stages characterized by Iversen and discussed in the Acute Behavioral and Physiological Effects of Cannabinoids section.
12. What is the evidence that cannabis use acutely compromises memory and other cognitive processes?
13. Describe the evidence for cannabinoid-mediated reward and reinforcement. How does the strength of cannabinoid reinforcement compare with the reinforcing effects of, for example, psychostimulant or opiate compounds?
14. Discuss the features of long-term cannabis use, including information on the progression from occasional to chronic use.
15. (a) How is cannabis use disorder defined in *DSM-5*? (b) Do most recreational cannabis users ultimately develop such a disorder? (c) What factors contribute to the development of cannabis use disorder?
16. What is known about the molecular mechanisms that underlie the development of tolerance to repeated cannabis exposure?

STUDY QUESTIONS *(continued)*

17. (a) Describe the symptoms of withdrawal from chronic cannabis use. (b) How is cannabis withdrawal studied in experimental animal models?
18. Discuss the current treatments for cannabis use disorder, including new information on the potential efficacy of nabiximols as an adjunct therapy for reducing withdrawal symptoms.
19. Chronic cannabis use has been associated with a variety of adverse effects. Summarize these effects in the realms of (a) educational performance and IQ, (b) cognitive effects, (c) neuropsychiatric effects, and (d) overall health effects.
20. Discuss both the currently accepted and novel therapeutic applications of cannabinoid medications. In light of our current knowledge, present the arguments both in favor of and against the legalization of medical marijuana.
21. (a) What is meant by the street names "Spice" and "K2"? (b) How do these substances differ from THC, and how are they typically consumed recreationally? (c) What is currently known about the dangers of consuming these substances?

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CHAPTER 15

The surreal visual experiences produced by hallucinogenic drugs are reflected in works such as this of "psychedelic art." (BSIP/Diomedia.)

Hallucinogens, PCP, and Ketamine

"I PUT MY LIGHTER TO THE BOWL AND INHALED.... After about twenty seconds I started to feel extremely light headed kind of like after I stand up suddenly after reclining for extended periods of time. With my eyes still closed I exhaled. It's hard to describe how I arrived at my destination. All I can remember is that the reality that I had previously known had ceased to exist. I was face to face with what looked like an entity composed of hundreds of my faces. They were all leering at me in the sort of way that made me feel like they were happy in the knowledge I had finally gone insane. I immediately winced in my mind and tried to tell myself everything was going to be all right but at the same time I was terrified and had no idea why I was seeing what I saw. As if my subconscious was answering my fears, a voice said from behind me 'This is a bad trip (my name). You are going to have to deal with it.' I tried screaming 'how' but as the monster made up of all my faces moved closer, my body seemed to crumble into multiple interlocking puzzle pieces which made it impossible to run or defend myself. Eventually these puzzle pieces became so distorted and strung out, I could no longer identify them as my body. I could vaguely remember something called 'salvia' but I made no connection from the drug to where I was. All my hope of ever returning to reality was beginning to fade."

The above is a segment from a self-reported account of a user's first experience with the hallucinogenic plant *Salvia divinorum*.[1] As we will see in the first section of this chapter, hallucinogenic substances like *Salvia*, LSD, and others have powerful effects on perceptual and conscious processes. Where do these substances come from, and how do they produce their effects? We will also cover phencyclidine (PCP), ketamine, and related compounds. These drugs are also known for their mind-altering properties, but they act through a different neurochemical mechanism than typical hallucinogens. ■

[1]This passage was taken from "The End of All Existence: An Experience with *Salvia divinorum*" on the Erowid.org website.

Hallucinogenic Drugs

Some substances are valued primarily for the unusual perceptual and cognitive distortions they produce. Users may find such distortions novel, stimulating, or even spiritually uplifting. Among the substances categorized in this way are **lysergic acid diethylamide** (**LSD**), **mescaline**, **psilocybin**, **bufotenine**, **dimethyltryptamine** (**DMT**), **5-methoxy-dimethyltryptamine** (**5-MeO-DMT**), and **salvinorin A** (the main psychoactive ingredient of *Salvia*). Over the years, many different names have been given to this drug class, including **psychotomimetic** (psychosis-mimicking), **psychedelic** (mind-opening), and **hallucinogenic** (hallucination-producing). The term *psychotomimetic* is now rarely used in this context, because most researchers no longer consider these compounds to be useful models of psychosis. Of the two remaining alternatives, the term *psychedelic* is often preferred by recreational users and by those who take such drugs in a quest for spiritual or mystical experiences. The modern pharmacological literature, however, strongly favors the term *hallucinogenic*, and we will follow that practice in most parts of the chapter. Specifically, we will define hallucinogens as substances whose primary effect is to cause perceptual and cognitive distortions without producing a state of toxic delirium.

Mescaline

Many hallucinogenic drugs either are synthesized by plants or are based on plant-derived compounds. Mescaline, for example, is found in several species of cactus, such as the **peyote cactus** (*Lophophora williamsii*) (**FIGURE 15.1**). When the crown (top part) of this small spineless cactus is cut off and dried, it is known as a **mescal button** or **peyote button**. These buttons can be chewed raw or cooked and then eaten to obtain their psychoactive effects. Alternatively, the mescaline can be extracted from the cactus and consumed as a relatively pure powder. The peyote cactus is native to the southwestern United States and northern parts of Mexico, and archeological evidence suggests that inhabitants of these regions used peyote for at least a few thousand years before invasion by the Spanish. Indeed, a radiocarbon dating of mescaline-containing peyote buttons from Mexico found them to be 5700 years old (Bruhn et al., 2002). Peyote was used by Native Americans for religious and healing rituals, and such rituals continue to take place under the auspices of the Native American Church of North America, which was founded in 1918 and has chapters in many states as well as Canada and Mexico.

FIGURE 15.1 Peyote cactus (Photo courtesy of Gerhard Köhres.)

Pure mescaline was first isolated from peyote in 1896 by Arthur Heffter and was synthesized in 1919 by Ernst Spath. However, the drug did not enter mainstream American culture until the famous novelist Aldous Huxley tried mescaline in 1953 and subsequently described his experience in a book entitled *The Doors of Perception*. Publication of this book and its sequel, *Heaven and Hell*, were among the seminal events that spawned a major rise in hallucinogenic drug use in the United States in the 1960s. At present, however, mescaline is not as readily available as various other hallucinogens because of the relatively high cost of synthesis and the lack of a large market for the drug.

Psilocybin

Numerous species of mushrooms manufacture alkaloids with hallucinogenic properties. These fungi, which are sometimes called "magic mushrooms" or simply "shrooms," include members of the genera *Conocybe*, *Copelandia*, *Panaeolus*, *Psilocybe*, and *Stropharia*, which are found in many places around the world (**FIGURE 15.2**). Depending on the species, users take 1 to 5 g of dried mushrooms to obtain the desired effects. The dried material may be eaten raw, boiled in water to make tea, or cooked with other foods to cover its bitter flavor. The major ingredients of these mushrooms are psilocybin and the related compound **psilocin**. After ingestion, the psilocybin is enzymatically converted to psilocin, which is the actual psychoactive agent. A different species of mushroom, *Amanita muscaria* (fly agaric; see Figure 8.21), produces a state of delirium that also includes hallucinations, but its primary active agents are muscimol and ibotenic acid.

The use of hallucinogenic mushrooms probably goes back at least as far historically as peyote use. There are two spectacular rock cave paintings in Algeria, dated at least to 3500 BCE, depicting people holding mushrooms in their hands and dancing. The more famous of the two paintings shows a single man (possibly

FIGURE 15.2 ***Psilocybe*** **mushrooms** (© Nigel Bean/ Naturepl.com.)

a shaman[2]) with a beelike head and mushrooms sprouting from his entire body. In Mexico and Central America, the Aztec, Mayan, Mazatec, and other peoples developed religious rituals around the eating of psilocybin-containing mushrooms (Carod-Artal, 2015).

After defeating the Aztecs, the Spaniards soon learned of their use of hallucinogenic mushrooms, which they called *teonanácatl*, meaning "flesh of the gods." The conquerors brutally suppressed mushroom eating along with other aspects of the Aztec religion, but they were unable to completely wipe it out. Nevertheless, the existence of hallucinogenic mushrooms in the New World was largely ignored until 1938, when Richard Schultes of the Harvard Botanical Museum traveled to Oaxaca, Mexico, and collected specimens of several different types of mushrooms being used in sacred rituals by the Mazatec people of that region. The publication of Schultes's findings ultimately led Gordon Wasson, a wealthy investment banker and amateur mycologist (someone who studies fungi), to visit Oaxaca in 1953 and again in 1955. During the second visit, Wasson and a photographer friend became the first known Westerners to participate in a Native American mushroom-eating ritual, which was led by a Mazatec *curandera*, or shaman, named María Sabina (**FIGURE 15.3**). In a 1957 *Life* magazine article entitled "Seeking the Magic Mushroom,"[3] Wasson described his reaction as follows:

> *We lay down on the mat that had been spread for us, but no one had any wish to sleep except the children, to whom mushrooms are not served. We were never more wide awake, and the visions came whether our eyes were opened or closed. They emerged from the center of the field of vision, opening up as they came, now rushing, now slowly, at the pace that our will chose. They were in vivid color, always harmonious. They began with art motifs…. Then they evolved into palaces with courts, arcades, gardens… Then I saw a mythological beast drawing a regal chariot. Later it was as though the walls of our house had dissolved, and my spirit had flown forth, and I was suspended in mid-air viewing landscapes of mountains, with camel caravans advancing slowly across the slopes, the mountains rising tier above tier to the very heavens…. For the first time the word ecstasy took on real meaning. For the first time it did not mean someone else's state of mind.*
>
> *Wasson, 1957, pp. 102, 103, 109*

FIGURE 15.3 María Sabina engaged in the mushroom eating ritual (The Tina & R. Gordon Wasson Ethnomycological Collection Archives, Harvard University.)

Among those who read Wasson's account was Timothy Leary, a young clinical psychologist pursuing a mainstream academic career. But after gaining a lectureship at Harvard in late 1959, Leary began to have reservations about his chosen career path. Then while vacationing in Mexico the following summer, Leary ate a handful of "magic mushrooms" and underwent the same kind of transformative experience reported

[2]In ancient cultures, shamans were people thought to possess special abilities to contact the spirit world.

[3]The title of this article is generally considered to be the first use of the term *magic mushroom*.

by Huxley several years earlier with mescaline. Leary returned to work, where he founded the Harvard Psilocybin Project. In his own words, the purpose of this project was "to teach individuals how to self-administer psychoactive drugs in order to free their psyches without reliance upon doctors or institutions" (Leary, 1984, p. 35). Over the next few years, Leary and his colleague Richard Alpert (later known as Ram Dass) gave psilocybin to many graduate students and faculty members, as well as to notable artists, writers, and musicians. He also began experimenting with LSD, having taken the drug for the first time in 1962. Leary and Alpert's work became increasingly controversial, and they were dismissed from Harvard in 1963, but they continued their activities privately and went on to become leaders of the psychedelic movement.

Dimethyltryptamine and Related Tryptamines

DMT and the related tryptamines 5-MeO-DMT and bufotenine are found in a number of plants that are indigenous to South America. Native tribes in Brazil, Colombia, Peru, and Venezuela make hallucinogenic snuffs from plants containing these compounds (Araújo et al., 2015). Bufotenine and 5-MeO-DMT are additionally present in toxic secretions of an American desert toad, *Bufo alvarius*, which accounts for the origin of the name "bufotenine." Of these three substances, DMT is most often used recreationally. DMT lacks bioactivity when ingested orally, because of liver metabolism (i.e., first-pass metabolism) by monoamine oxidase (MAO); however, when smoked or taken by insufflation (snorting), it can produce a brief (about 30 minutes) but intense hallucinatory experience.

Several orally active synthetic DMT analogs, such as α-methyltryptamine (AMT) and 5-methoxy-diisopropyltryptamine, have become popular in recent years. The latter compound is known on the street as "Foxy Methoxy," or simply "Foxy." Foxy is typically taken orally in tablet form, although the pills can also be crushed and then either snorted or smoked.

Finally, it is important to mention **ayahuasca**, which is a Quechua Indian word meaning "vine of the soul." Ayahuasca is a strong reddish-brown drink originally created by peoples of the Amazonian rain forest. This potent hallucinogenic brew requires at least two different kinds of plants, typically stalks from the *Banisteriopsis caapi* vine as well as leaves from *Psychotria viridis* and/or *Diplopteris cabrerena*. *Psychotria* and *Diplopteris* provide DMT, whereas the vines contribute several alkaloids called β-carbolines, which are known to inhibit MAO activity. The β-carbolines block DMT breakdown by liver MAO, thereby permitting the substance to reach the brain and exert its hallucinogenic effects. Because of the drug's oral route of administration, the onset of hallucinations can be delayed for up to an hour after ingestion, but on the other hand these effects may persist for about 4 hours (Araújo et al., 2015).

Use of ayahuasca by native South American peoples dates back to the pre-Columbian era (the period before European influence on the indigenous peoples). Like peyote and *Psilocybe* mushrooms, ayahuasca was used in spiritual/religious ceremonies in order to "enter into contact with the unseen side of reality" (Luna, 2011, p. 9). It continues to be used in the same manner not only in South America but elsewhere in the world by practitioners of religions such as *Santo Daime*, *União do Vegetal*, and *Barquinha* that merge elements of Catholicism, shamanism, and various folk traditions (dos Santos et al., 2016a). Possession and use of ayahuasca is illegal in the United States except in sacred ceremonies by members of the abovementioned religions. Whether or not the user formally belongs to one of these groups, ayahuasca is most often consumed for the purpose of enhancing one's personal or spiritual growth (Harris and Gurel, 2012).

LSD

Unlike mescaline, psilocybin, and DMT, LSD is a synthetic compound, although its structure is based on a family of fungal alkaloids. The famous story about the synthesis of LSD and the discovery of its astonishing psychoactive potency is presented in **BOX 15.1**. Once LSD was made available to psychiatrists and medical researchers in the late 1940s and early 1950s, the drug began to be intensively studied. Indeed, there were only six published papers on LSD before 1951, but from 1951 to 1962 more than 1000 LSD-related articles appeared in the scientific literature (U.S. Department of Health, Education, and Welfare, 1968). During this period, researchers were first beginning to appreciate that nerve cells in the brain communicate with each other chemically by means of neurotransmitters like serotonin (5-HT). When LSD was reported to alter serotonergic activity (see the Pharmacology of Hallucinogenic Drugs section below), the finding generated tremendous excitement about the possibility of understanding human mental activity and behavior at a chemical and physiological level.

Some researchers approached LSD as a psychotomimetic drug that would help reveal the biochemical underpinnings of schizophrenia. However, the LSD model proved to be inadequate in a number of ways, and it subsequently gave way to a PCP/ketamine model that is discussed later in this chapter. Others believed that LSD could be a valuable tool in psychotherapy or psychoanalysis. One way of using LSD was in **psycholytic therapy**, which was mainly practiced in continental Europe. This therapeutic method was based on the concept of drug-induced "psycholysis,"

BOX 15.1 History of Pharmacology

The Discovery of LSD

LSD was first synthesized in 1938 by Albert Hofmann, a chemist working for the Sandoz pharmaceutical company in Switzerland. Sandoz was interested in alkaloids obtained from **ergot**, a substance produced by the parasitic fungus *Claviceps purpurea*, which can infest rye and wheat (see **Figure**). Ergot is an extremely toxic material, and consumption of ergot-contaminated grain can cause a serious illness known as **ergotism**.

Although no outbreak of ergotism has occurred in recent years, the disease was quite common in the Middle Ages and is thought to have caused the death of as many as 40,000 people in the year 944. Nevertheless, ergot came to have medicinal value because it produces powerful contractions of the uterus that can help trigger labor and reduce postbirth uterine hemorrhage.

Hofmann began to combine **lysergic acid**, which is the core structure in all ergot alkaloids, with other compounds to see what would emerge. The twenty-fifth different substance synthesized in the course of this research was D-lysergic acid diethylamide, which Hofmann abbreviated LSD-25 (from the German name L*ysergSäure-Diäthylamid*). Hofmann's purpose in making this compound was to generate a new circulatory and respiratory stimulant (such drugs are sometimes called **analeptics**). This expectation was based on the structural similarity of LSD to nicotinic acid diethylamide, a known analeptic drug. However, LSD failed to show any analeptic activity, so the compound was temporarily abandoned.

Five years later, Hofmann decided to reexamine LSD, thinking that it might have useful pharmacological properties not recognized during initial testing. In the final stages of synthesizing a new batch of the compound, he was overcome by a series of strange sensations that prevented him from continuing in the lab. The following famous passage is taken from Hofmann's report to Sandoz, which describes the world's first LSD "trip":

> *Last Friday, April 16, 1943, I was forced to interrupt my work in the laboratory in the middle of the afternoon and proceed home, being affected by a remarkable restlessness, combined with a slight dizziness. At home I lay down and sank into a not unpleasant intoxicatedlike condition, characterized by an extremely stimulated imagination. In a dreamlike state, with eyes closed (I found the daylight to be unpleasantly glaring), I perceived an uninterrupted stream of fantastic pictures, extraordinary shapes with intense, kaleidoscopic play of colors. After some two hours this condition faded away.*
>
> *Hofmann, 1979, p. 58.*

© Ken Wagner/Visuals Unlimited, Inc.

Hofmann suspected that this amazing experience had come from accidentally ingesting a small amount of the newly synthesized LSD. Therefore, the following Monday, he carefully measured out a minute amount of the drug, 250 μg (1/4000 of a gram), dissolved it in a small volume of water, and drank it. Hofmann soon underwent an even more intense experience than before. He somehow managed to ride his bicycle home with the help of a lab assistant (that day came to be known as "Bicycle Day"), and his hallucinations took a threatening form that later passed, leaving him the next day with a profound sense of well-being and a temporarily heightened perceptual awareness.

Hofmann's colleagues at Sandoz initially did not believe that LSD could be as potent as he claimed, but when they took minute quantities themselves, they were able to confirm Hofmann's result. Sandoz first marketed LSD in 1947 under the name Delysid for the purpose of helping patients with neuroses to uncover repressed thoughts and feelings. The company also suggested that psychiatrists self-administer the drug in order to better understand the perceptual distortions and hallucinations suffered by patients with schizophrenia.

Albert Hofmann continued to write and lecture about the positive effects of LSD and other hallucinogens, even after retiring from Sandoz in 1971. He passed away in 2008 at the age of 102.

meaning psychic loosening or opening. It involved giving LSD in low but gradually increasing doses to promote the release of repressed memories and to enhance communication with the analyst. British, Canadian, and American psychiatrists, on the other hand, tended to prefer **psychedelic therapy**, in which the patient was typically given a single high dose of LSD with the hope of gaining insight into his problems through a drug-induced spiritual experience. During the 1950s and 1960s, a number of studies were performed using this technique to treat patients who were alcoholics (Dyck, 2005). Unfortunately, these studies were marred by poor experimental control and inconsistent findings, leading to cessation of this work by the early 1970s.

Interestingly, at the same time that LSD was being investigated as a possible aid to psychotherapy, this and other hallucinogenic agents were also being considered by the U.S. government as potential weapons. Over a period of many years, the U.S. Army conducted secret experiments at the Edgewood Arsenal in Maryland during which they exposed thousands of military volunteers to numerous compounds, including LSD and other psychoactive drugs (Ketchum, 2006; Smith et al., 2014). One of the goals of these experiments was to find a chemical agent that could be dispersed in the battlefield to incapacitate enemy soldiers without killing them (which is the common result of exposure to nerve gases) or destroying their property and weapons. The program was eventually terminated when no such agent was identified. Another top secret program, called MK-ULTRA, was instituted in the early 1950s by the Central Intelligence Agency (CIA). The purpose of MK-ULTRA was to investigate the possible use of LSD as a mind control agent (Lee and Shlain, 1992). In one particularly disgraceful part of this program, CIA operatives administered LSD to unsuspecting members of the public in order to observe their behavioral reactions. According to Lee and Shlain (1992), Fidel Castro and then Egyptian president Gamal Abdel Nasser were among the foreign leaders targeted for LSD "attacks," although it appears that no such attacks were actually carried out before the program was eventually disbanded.

LSD's popularity exploded with the hippie culture of the 1960s. As part of their nonconformist, anti-Establishment attitudes, hippies openly sought mind expansion through the use of psychedelic drugs, especially LSD. However, the inevitable backlash soon occurred amid growing anecdotal accounts as well as scientific reports of LSD-related problems. A 1965 federal law greatly restricted new research on LSD, and soon thereafter Sandoz stopped distributing LSD for research purposes and recalled all of the existing drug that had previously been supplied to investigators. After a long period of inactivity, however, clinical research on LSD has begun to make a slow comeback. An organization called MAPS (Multidisciplinary Association for Psychedelic Studies) has been promoting new research on the potential psychotherapeutic applications of hallucinogens (see the MAPS website at www.maps.org). Nevertheless, given the general cultural and governmental attitudes toward LSD and other hallucinogenic drugs, it seems unlikely that these compounds will enter mainstream psychiatric practice any time soon.

Recreational use of LSD was banned nationwide in 1967. Of course, LSD didn't disappear; it merely went underground. Indeed, in recent years, hallucinogenic drug use has increased as a new generation of young people has rediscovered these substances. LSD is active orally, and that is the standard mode of administration. The drug is so potent that a single dose in crystalline form is barely visible to the naked eye (see Box 15.1). Consequently, larger amounts of LSD representing many doses are usually dissolved in water, and then droplets containing single-dose units are applied to a sheet of paper (a "blotter") and dried. The paper is subsequently divided into individual squares, often decorated with fanciful designs, and sold as single-dose "tabs" to be swallowed by the user.

NBOMes

In 2010, a new class of synthetic hallucinogens appeared on the scene. These substances belong to a class of compounds called ***N*-benzylphenethylamines**, which is typically abbreviated **NBOMes** (Halberstadt, 2017). The first member of the class, which continues to be widely distributed, is designated **25I-NBOMe**. Its chemical name is *N*-(2-methoxybenzyl)-2,5-dimethoxy-4-iodophenethylamine. The *I* in 25I-NBOMe and the corresponding *iodo* in the chemical name both designate the presence of an iodine atom on the phenyl ring. Other NBOMes include 25B-NBOMe, which contains a bromine atom in place of the iodine, and 25C-NBOMe, which contains a chlorine atom. Like LSD, NBOMes may be distributed as single doses on blotter paper, or alternatively larger amounts can be obtained in powdered form. Because NBOMes are subject to first-pass metabolism when consumed orally, they are usually taken by the sublingual or buccal routes of administration. To accomplish this, a piece of drug-infused blotter paper is placed under the tongue or on the surface of the gum until the drug has been absorbed through the tissue membranes. Powdered drug can be taken by insufflation.

NBOMes are very potent, which makes it easy for the user to overdose. Numerous cases of NBOMe toxicity have been reported, often with severe, even fatal, consequences. Toxic effects of NBOMes are described later in the section on adverse reactions to hallucinogenic drugs.

Salvinorin A

Salvia divinorum is a member of the mint family native to Oaxaca, the same region of Mexico mentioned earlier with respect to hallucinogenic mushrooms (**FIGURE 15.4**). Indeed, the plant's psychoactive properties were first reported by Albert Hofmann, the discoverer of LSD, and Gordon Wasson, of "magic mushroom" fame (Casselman et al., 2014). Like psilocybin-containing mushrooms, *Salvia* was historically used in religious rituals by Mazatec shamans and then later attained the status of a recreational substance in Western countries such as the United States. Despite knowledge of *Salvia*'s properties and its recreational use, the Drug Enforcement Agency (DEA) has surprisingly not listed the herb in its Schedule of Controlled Substances. However, it is worth noting that 31 states have enacted legislation prohibiting human consumption (and in most cases even possession) of this substance (Siebert, 2015).

An unusual compound called salvinorin A is the principal psychoactive ingredient in *Salvia* (Zawilska and Wojcieszak, 2013). Recreational users may chew fresh *Salvia* leaves, smoke dried and crushed leaves, or consume a concentrated salvinorin A-containing extract either through sublingual and buccal absorption of a liquid preparation or by smoking a dried extract. Chewing *Salvia* leaves or placing a liquid extract under the tongue or into the cheek permits absorption of the compound through the oral mucosa. Few, if any, psychoactive effects are produced by swallowing *Salvia*, because the salvinorin A is inactivated in the gastrointestinal tract. On the other hand, the drug is quickly and effectively absorbed through the lungs when smoked, thereby yielding the most rapid and intense hallucinogenic experience. As with DMT, the effects of smoking *Salvia* dissipate rapidly, typically within 15 minutes.

FIGURE 15.4 ***Salvia divinorum*** (© Ted Kinsman/Science Source.)

There are several published studies of the subjective effects produced by *Salvia* use. In general, the substance produces vivid hallucinations, out-of-body experiences, and other feelings resembling but not identical to those produced by other hallucinogens (see the chapter opener). In an early study by Siebert (1994), the following themes were most commonly reported by users:

- Becoming objects (yellow plaid French fries, fresh paint, a drawer, a pant leg, a Ferris wheel, etc.)
- Visions of various two-dimensional surfaces, films, and membranes
- Revisiting places from the past, especially childhood
- Loss of the body and/or identity
- Various sensations of motion, or being pulled or twisted by forces of some kind
- Uncontrollable hysterical laughter
- Overlapping realities; the perception that one is in several locations at once (Siebert, 1994)

Subsequent studies have reported that the subjective effects of *Salvia* overlap with, but are not identical to, those produced by LSD (González et al., 2006), DMT and psilocybin (Johnson et al., 2011), marijuana (Albertson and Grubbs, 2009), or the dissociative anesthetic ketamine (Dalgarno, 2007) (see the PCP and Ketamine section below). Because of the diversity of these effects, *Salvia* is considered to have a unique psychoactive profile (MacLean et al., 2013).

Pharmacology of Hallucinogenic Drugs

The chemical structures of hallucinogenic drugs, their potency and time course of action, and the psychological and physiological responses to hallucinogen administration are well characterized. Researchers have also obtained considerable information about the mechanisms of action of these substances, although the story is not yet complete.

Different hallucinogenic drugs vary in potency and in their time course of action

One way of comparing the potency of various hallucinogenic drugs is to consider the typical doses taken by recreational users. Common dose ranges for LSD, 25I-NBOMe, psilocybin, mescaline, DMT, and salvinorin A are presented in **TABLE 15.1**. You can see that these compounds vary widely in their potency, ranging from LSD as the most potent to mescaline as the least potent.

TABLE 15.1 Route of Administration and Potency of Various Hallucinogenic Drugs

Drug	Usual route of administration	Typical dose range
LSD	Oral	50–100 µg (0.05–0.10 mg)
25I-NBOMe	Sublingual/buccal	250–800 µg (0.25–0.80 mg)
Psilocybin	Oral	10–20 mg
Mescaline	Oral	200–500 mg
DMT	Smoking	20–50 mg
Salvinorin A	Smoking	200–1000 µg (0.2–1.0 mg)

All of the hallucinogens that are taken orally have a fairly similar time course of action. Depending on the dose and when the user last ate, the psychedelic effects of these substances generally begin within 30 to 90 minutes following ingestion. An LSD or mescaline "trip" typically lasts for 6 to 12 hours or even longer, whereas the effects of psilocybin-containing mushrooms may dissipate a bit sooner. The effects of NBOMes persist for 3 to 10 hours, depending on the dose and route of administration. In contrast to these long time periods, the time courses of smoked DMT and *Salvia* present a very different picture. The effects of those substances are felt within seconds, reach a peak over the next few minutes, and are over within an hour or less.

Hallucinogens produce a complex set of psychological and physiological responses

We have already given a description of some of the subjective experiences reported by users of hallucinogenic mushrooms as well as *Salvia*. In this section, we will focus on the psychological and physiological responses associated with the ingestion of LSD and psilocybin, two prototypical hallucinogens. As we noted earlier, other hallucinogens may have somewhat different response profiles, but the core effects are similar across most of these drugs. The state of intoxication produced by LSD and other hallucinogens is usually called a "trip," presumably because the user is taking a mental journey to a place different from his normal conscious awareness. The LSD trip can be divided into four phases: (1) onset, (2) plateau, (3) peak, and (4) "comedown." Trip onset occurs about 30 minutes to an hour after one takes LSD. Visual effects begin to occur, with intensification of colors and the appearance of geometric patterns or strange objects that can be seen with one's eyes closed. The next 2 hours of the trip represent the plateau phase. The subjective sense of time begins to slow and the visual effects become more intense during this period. The peak phase generally begins after about 3 hours and lasts for another 2 or 3 hours. During this phase, the user feels as if she's in another world in which time has been suspended. She sees a continuous stream of bizarre, distorted images that may be either beautiful or menacing. The user may experience **synesthesia**, a crossing-over of sensations in which, for example, colors are "heard" and sounds are "felt." The peak is followed by the comedown, a phase lasting 2 hours or longer, depending on the dose. Most of the drug effects are gone by the end of the comedown, although the user may still not feel completely normal until the following day. In addition to the sensory–perceptual effects just described, hallucinogenic drugs produce a wide variety of other psychological changes. These include feelings of depersonalization, emotional shifts to a euphoric or to an anxious and fearful state, and disruption of logical thought.

A hallucinogenic trip as a whole may be experienced either as mystical and spiritually enlightening (a "good trip") or as disturbing and frightening (a "bad trip"). Whether the user has a good or a bad trip depends in part on the dose; the individual's personality, expectations, and previous drug experiences; and the physical and social setting (Preller and Vollenweider, 2016). But even in the best of circumstances, one cannot predict in advance the outcome of an LSD trip.

Scientific studies investigating the influence of hallucinogens on mental state often rely on psychometric instruments developed to quantify the effects of these compounds: the **altered states of consciousness** (**ASC**) **rating scale**, which was introduced by Dittrich (1998), or Strassman's **hallucinogen rating scale** (Strassman et al., 1994). The following five primary dimensions make up the ASC scale: oceanic boundlessness, ego-disintegration anxiety, visionary restructuralization, reduced vigilance, and auditory alterations (Dittrich, 1998; Vollenweider and Kometer, 2010). *Oceanic boundlessness* refers to derealization and loss of ego boundaries (i.e., feeling one with the world), which corresponds closely to the positive mystical experiences often reported by users of hallucinogenic drugs. In contrast, the ego-disintegration anxiety dimension includes drug-induced thought disorder as well as negative emotional responses to the loss of ego boundaries. Dittrich (1998) likened this dimension to the experience of a "bad trip" on the part of the user. *Visionary restructuralization* refers to distortions of visual perception, including visual illusions, hallucinations, and visual synesthesias. The last two dimensions, reduced vigilance and auditory alterations (including auditory hallucinations), do not occur with all hallucinogenic drugs and are considered less important items in the ASC scale.

The five primary dimensions of the ASC rating scale can be further divided into subdimensions to provide more detailed information on drug-induced reactions. **FIGURE 15.5** shows the subjective responses to a moderate dose of psilocybin compared with

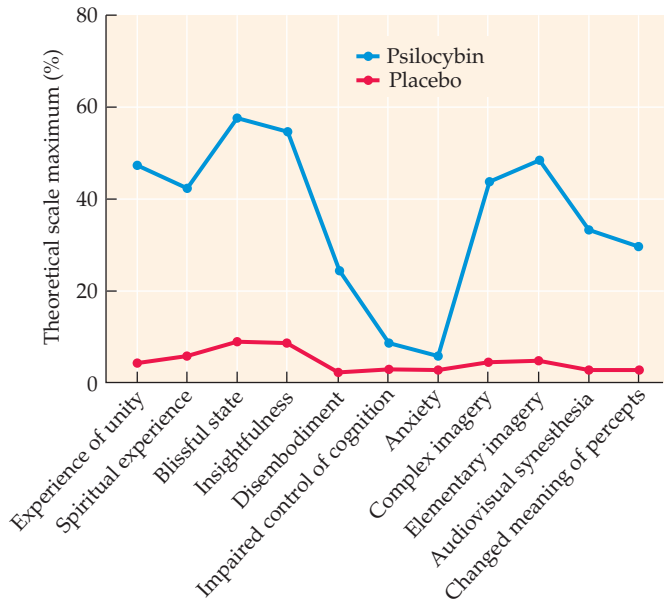


FIGURE 15.5 Dose-dependent subjective effects of psilocybin using the 11 subdimensions of the 5-dimension altered states of consciousness (ASC) rating scale The graph shows that psilocybin exerted significant subjective effects on all subdimensions of this psychological instrument except for "impaired control of cognition" and "anxiety." (After Halberstadt, 2015.)

placebo, measured using 11 subdimensions of the 5-dimension ASC (Halberstadt, 2015). At this dose, the drug produced particularly strong effects on the categories "blissful state" and "insightfulness" but little or no effect on "impaired control of cognition" or "anxiety."

Besides their psychological effects, hallucinogens also give rise to various physiological responses. In the case of LSD, these responses reflect activation of the sympathetic nervous system and include pupil dilation and small increases in heart rate, blood pressure, and body temperature. LSD use can also lead to dizziness, nausea, and vomiting, although such reactions are more likely to occur after consumption of peyote or psilocybin-containing mushrooms.

Most hallucinogenic drugs share a common indoleamine or phenethylamine structure

Most hallucinogenic drugs have either a serotonin-like or a catecholamine-like structure. The serotonin-like, or **indoleamine**, hallucinogens include LSD, psilocybin, psilocin, DMT, 5-MeO-DMT, and the synthetic tryptamines mentioned earlier. When the 5-HT molecule is oriented in the proper manner, it is easy to see how its basic structure is incorporated into the structures of these hallucinogenic compounds (**FIGURE 15.6**). Important studies in the early 1950s by John Gaddum in Scotland and by Edward Wooley and David Shaw in the United States led these investigators to conclude that LSD works by antagonizing the action of 5-HT in

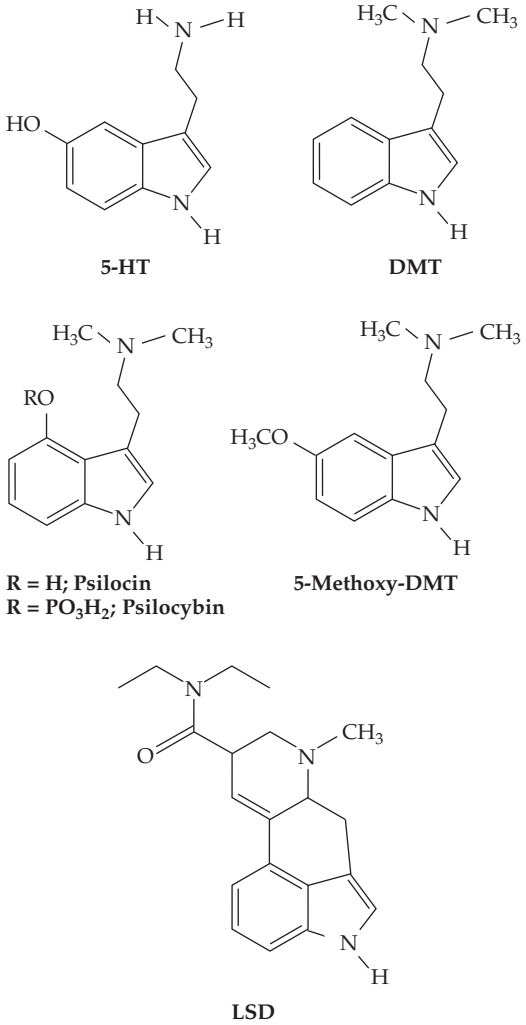
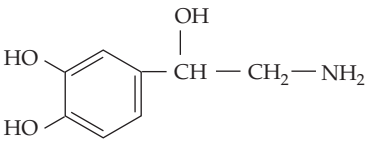


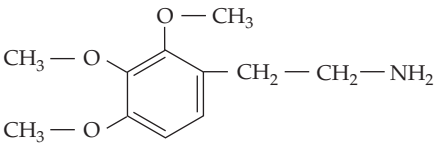
FIGURE 15.6 Structures of 5-HT and the indoleamine hallucinogens

the brain. We shall see in the next section that LSD can be understood more as an agonist than as an antagonist in the serotonergic system. Nevertheless, the linking of 5-HT with such a powerful psychoactive drug as LSD brought this recently discovered neurotransmitter into the forefront of behavioral and psychiatric research—a place that it continues to hold to the present day.

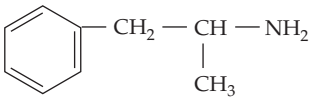
Of the hallucinogens covered in this chapter, only mescaline and the NBOMes have a catecholamine-like structure. As shown in **FIGURE 15.7**, mescaline and 25I-NBOMe are structurally related to the neurotransmitter norepinephrine (NE) as well as to the psychostimulant amphetamine. Indeed, amphetamine can produce hallucinogenic effects with prolonged administration of high doses, and several amphetamine analogs such as 2,5-dimethoxy-4-methylamphetamine (DOM, also known as "STP") and 3,4,5-trimethoxyamphetamine (TMA) possess even greater hallucinogenic properties. Mescaline, the NBOMes, DOM, and TMA are known as **phenethylamine** hallucinogens.



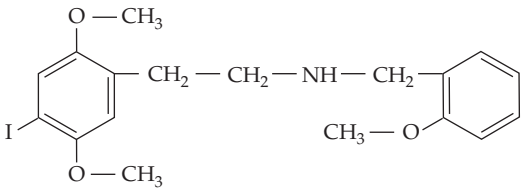
NE



Mescaline



Amphetamine



25I-NBOMe

FIGURE 15.7 Structures of NE and the phenethylamine hallucinogens

The most unusual hallucinogen structurally is salvinorin A. **FIGURE 15.8** presents the structure of this compound, which chemically is known as a neoclerodane diterpene. Also shown is a related drug called ketocyclazocine, which has a similar mechanism of action as salvinorin A and can also induce hallucinations.[4] The shared mechanism of action of these compounds is discussed in a later section.

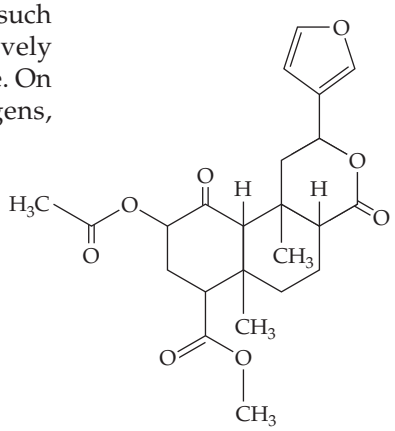
Indoleamine and phenethylamine hallucinogens are 5-HT$_{2A}$ receptor agonists

Although we still don't completely understand how hallucinogens produce their dramatic perceptual and cognitive effects, some progress has been made. Over time it has become clear that the serotonergic system is intimately involved in this process, at least in the case of the indoleamine and phenethylamine hallucinogens. Beginning our exploration of hallucinogenic action with LSD, we can immediately see that this is a very complicated substance with respect to its potential effects on the serotonergic system. LSD binds with relatively high affinity to at least eight different serotonergic receptor subtypes: 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{2A}$, 5-HT$_{2C}$, 5-HT$_{5A}$, 5-HT$_{6}$, and 5-HT$_{7}$ (Nichols, 2004). Other classical indoleamine hallucinogens such as psilocybin and DMT similarly possess a relatively nonselective serotonergic receptor binding profile. On the other hand, the phenethylamine hallucinogens, including mescaline, DOM, and the NBOMes, only bind with high affinity to the class of 5-HT$_2$ receptors. Moreover, an abundance of research has convincingly demonstrated that both indoleamine and phenethylamine hallucinogens are 5-HT$_{2A}$ receptor agonists, and the hallucinogenic properties of these compounds require activation of this receptor subtype (Halberstadt, 2015; Nichols, 2016).

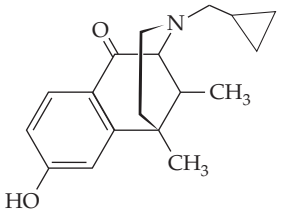
[4]Although ketocyclazocine is hallucinogenic, it also produces extreme dysphoria when administered and, therefore, is not used recreationally.

Since the discovery of LSD's profound subjective effects, researchers have been puzzled about why the drug is so potent and why an LSD "trip" lasts so long. Drug molecules typically bind rather loosely to their receptors, thereby requiring more drug molecules to produce a biological effect as well as curtailing the length of time during which the effect is manifested. This mystery was finally solved by researchers at the University of North Carolina who, in a heroic series of experiments, were able to visualize the structure of LSD bound to the human 5-HT$_{2A}$ receptor. What the researchers discovered is that when the drug binds to its target, a kind of "lid" formed by part of the receptor protein closes over the binding pocket, thus temporarily trapping the drug in place (Wacker et al., 2017; also see commentary by Chen and Tesmer, 2017). This type of discovery attests to the power of contemporary pharmacological methods for addressing complex questions about the mechanisms of drug action on the brain.

Other information about the mechanisms of hallucinogenic drugs has been obtained from behavioral tests in laboratory animals. Two widely used approaches are the drug-induced head twitch response and drug discrimination tests (see Chapter 4), both of which are thought to screen for hallucinogenic-like effects in humans (Hanks and González-Maeso, 2013). **FIGURE 15.9** depicts a dose–response function for 25I-NBOMe to elicit the head twitch response in mice, a 5-HT$_{2A}$ receptor-mediated behavior that is elicited by all known hallucinogens acting through this mechanism.



Salvinorin A



Ketocyclazocine

FIGURE 15.8 Structures of salvinorin A and ketocyclazocine

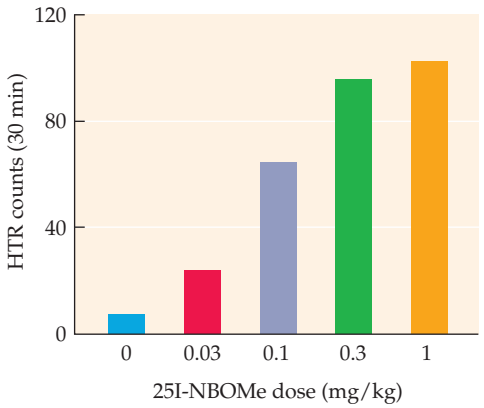


FIGURE 15.9 Dose-dependent stimulation of the head twitch response in mice Male C57BL/6J mice were injected SC with varying doses of 25I-NBOMe or saline (0 dose). The total number of head twitch responses (HTR) was recorded over a 30-minute period. (After Halberstadt, 2017; Halberstadt and Geyer, 2014.)

Note that a dose of 0.3 mg/kg elicited nearly a peak response, thus demonstrating the high potency of this synthetic hallucinogen.

Salvinorin A is a κ-opioid receptor agonist

When salvinorin A was discovered to be the primary psychoactive agent in *Salvia divinorum*, researchers discovered that its pharmacology was unlike that of the indoleamine and phenethylamine hallucinogens. In particular, salvinorin A has little effect on 5-HT_{2A} or any other 5-HT receptors. Instead, this compound, along with the previously mentioned ketocyclazocine, is a potent agonist at the κ-opioid receptor (Cunningham et al., 2011). Positron emission tomographic (PET) imaging of rats given salvinorin A confirmed significant occupancy by that drug of brain κ-opioid receptors (Placzek et al., 2015). Moreover, in humans the subjective and physiological effects produced by inhalation of vaporized salvinorin A were blocked by the general opioid receptor antagonist naltrexone but not by the 5-HT_{2A} antagonist ketanserin (Maqueda et al., 2016). These results confirm that the responses to salvinorin A are mediated by κ-opioid receptor activation, and they further account for the dissimilarities in the drug's subjective properties compared with those associated with typical indoleamine and phenethylamine hallucinogens. The sites in the brain responsible for salvinorin A's hallucinatory and other effects are not known; however, Maqueda and colleagues (2015) have proposed involvement of the thalamus, temporal cortex, parietal cortex, and claustrum (a thin sheet of neurons beneath the cortex), all of which express high levels of κ-opioid receptors.

The neural mechanisms underlying hallucinogenesis are not yet fully understood

We have discussed that 5-HT_{2A} receptor activation is necessary for the hallucinogenic effects of indoleamine and phenethylamine compounds; however, this information alone does not tell us *where* the critical receptors are located or *how* activation of these receptors produces the sensory and cognitive distortions experienced during a "trip." Researchers have begun to address these questions by using electroencephalography (EEG) and functional magnetic resonance imaging (fMRI). EEG studies have shown that hallucinogens disrupt the normal rhythmic oscillations measured in the cerebral cortex, probably because of excitation of 5-HT_{2A} receptor–expressing pyramidal neurons in layer 5 of the prefrontal cortex (PFC) and other cortical areas (Muthukumaraswamy et al., 2013; Carhart-Harris et al., 2016). This oscillatory activity depends on a glutamatergic network within the cortex, and its disruption helps account for some of the consciousness-altering aspects of a "trip" (**FIGURE 15.10**). An fMRI study with ayahuasca further suggested an association of visual perceptual changes with neural activation in the primary visual cortex (de Araujo et al., 2012). However, the results from brain imaging studies of hallucinogenic drugs must be interpreted cautiously, as these results are not always consistent across studies (dos Santos et al., 2016b).

Hallucinogenic drug use leads to adverse effects in some users

Hallucinogens lack the high degree of abuse potential seen with most other recreational drugs, such as opoids, psychostimulants, cannabis, alcohol, and nicotine. They do not produce physical withdrawal symptoms after chronic use, and they are not effective reinforcers in animal tests such as the self-administration paradigm. However, a hallucinogen dependence syndrome has been identified in a small percentage of users, particularly in individuals with early exposure to such compounds (Stone et al., 2006; 2007). The *Diagnostic and Statistical Manual of Mental Disorders*, 5th edition, (*DSM-5*) contains a category called **other hallucinogen use disorder**, which covers the substances mentioned above along with 3,4-methylenedioxymethamphetamine (MDMA) but not PCP (which has its own diagnostic categories) (Hardaway et al., 2016). Most hallucinogenic drugs, with the possible exceptions of DMT and salvinorin A, also produce rapid tolerance with repeated use. Early studies involving LSD administration to human volunteers found that over a 4-day period of daily dosing, nearly complete tolerance was observed by the fourth day (Nichols, 1997). In most cases, hallucinogen tolerance has been linked to 5-HT_{2A} receptor down-regulation (Halberstadt, 2015). One exception is mescaline, which has not been found to produce 5-HT_{2A} receptor down-regulation despite the fact that this compound can produce behavioral tolerance similar to that seen with the indoleamine hallucinogens. Therefore, there may be multiple mechanisms that can give rise to hallucinogenic drug tolerance.

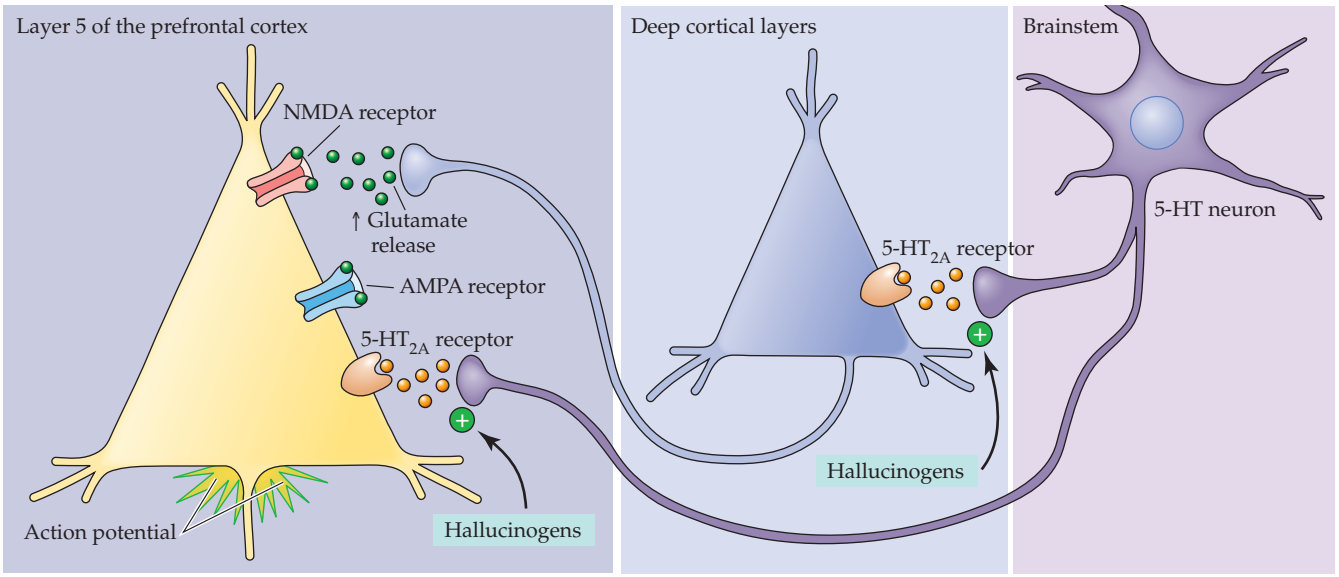


FIGURE 15.10 Proposed mechanism of action of indoleamine and phenethylamine hallucinogens Activation of 5-HT_{2A} receptors is believed to be necessary for the subjective effects of indoleamine and phenethylamine hallucinogens. Among the neurons affected by these drugs are pyramidal cells in cortical layer 5 that are directly stimulated as the result of activation of 5-HT_{2A} receptors on the cells themselves and are secondarily stimulated by glutamate released from other 5-HT_{2A} receptor–bearing pyramidal cells in the deep cortical layers that project to layer 5. According to the model shown here, such activation disrupts the functioning of glutamatergic networks involved in normal electrical oscillatory activity within the cortex. (After Vollenweider and Kometer, 2010.)

Despite the relative lack of addictive potential, hallucinogens can still cause serious problems for some users. As mentioned earlier, the user may have a "bad trip" in which he experiences an acute anxiety or panic reaction in response to the drug's effects (**FIGURE 15.11**). Although we don't understand the exact cause of these reactions, they are probably related to an interaction between the drug, the individual's emotional state going into the trip, and the external environment. In most cases, friends talk the person through the ordeal. However, if this is unsuccessful, then it may be necessary to take the person to the hospital emergency room for treatment. The incidence of bad trips is not known, but existing data from older clinical studies of LSD administration suggest that they are rare when users are prescreened for emotional stability and the environmental conditions are carefully controlled.

Other potential complications of hallucinogen use are the occurrence of occasional **flashbacks** and a more serious problem called **hallucinogen persisting perception disorder** (**HPPD**) (Lerner et al., 2014; Halpern et al., 2016). Flashbacks are transient, usually innocuous, re-experiences of some of the visual distortions that occurred during previous episodes of hallucinogen consumption. Users typically do not consider occasional flashbacks to be distressing, and some users even refer to them as "free trips." In contrast, HPPD is a more serious disturbance in which severe perceptual symptoms persist for a long period of time following drug use and are experienced sufficiently frequently to cause significant distress or impairment to the individual. Recent studies have identified cases of HPPD in some users of the 5-HT–releasing drug MDMA (see Chapter 6), which is consistent with a hypothesis that the serotonergic system plays a role in the development of this disorder (Litjens et al., 2014).

The most severe adverse reactions to hallucinogen drug use are toxic reactions that may involve psychiatric and/or somatic symptoms, depending on the drug and dose. For example, psychotic episodes have occurred

FIGURE 15.11 LSD users sometimes experience acute panic or anxiety reactions to the drug (a "bad trip") (© Maciej Toporowicz/Glasshouse Images/aegfotostock.)

following use of LSD or tryptamine hallucinogens (e.g., DMT) in vulnerable individuals either suffering from a current psychiatric disorder or possessing a preexisting risk for developing psychosis (Araújo et al., 2015; De Gregorio et al., 2016). Acute psychotic symptoms have additionally been seen in some users of *Psilocybe* mushrooms and *Salvia* (Zawilski and Wojcieszak, 2013; Vallersnes et al., 2016). However, the NBOMes are the most dangerous substances among the hallucinogens discussed above. Numerous reports have appeared in the recent literature of NBOMe users suffering from delusions, severe agitation and aggressive behavior, seizures, tachycardia, hypertension, extreme hyperthermia, rhabdomyolysis (muscle breakdown), and kidney damage (Kyriakou et al., 2015; Nikolaou et al., 2015; Suzuki et al., 2015). Severe NBOMe toxicity can lead to hospitalization in an intensive care unit and even death. Based on these findings, it's clear that people seeking a psychedelic experience should avoid using NBOMes because of the risk of serious adverse reactions.

Can hallucinogenic drugs be used therapeutically?

In describing the early history of LSD, we mentioned that the drug was used for a number of years in the treatment of psychiatric disorders. In their 1979 book entitled *Psychedelic Drugs Reconsidered*, Grinspoon and Bakalar state that over 1000 clinical articles were published between 1950 and the mid-1960s discussing the treatment of roughly 40,000 patients with psychedelic drug therapy. Early claims touted the efficacy of LSD in treating alcohol dependence along with many other disorders; however, careful scrutiny of the literature revealed that almost all of the evidence came either from case reports or from clinical studies that were poorly controlled, if at all (Grinspoon and Bakalar, 1979). Therapeutic use of hallucinogenic drugs was quickly abandoned when LSD and other hallucinogens were given a Schedule I designation in the 1970 Schedule of Controlled Substances (see Chapter 9). However, the recent upsurge of research on these compounds has given rise to renewed interest in their potential as psychotherapeutic agents.

Contemporary research investigating the therapeutic use of hallucinogenic drugs is performed with careful attention to patient selection and the therapeutic setting (Tupper et al., 2015). Sessions are conducted in quiet treatment facilities designed to have a pleasant and relaxing ambience. At least one, if not two, therapists are present at all times during the period of drug action. However, instead of interacting with the therapist(s) as in a typical psychotherapy session, the patient is asked to turn her attention inward during the session. These measures are designed to maximize the likelihood of therapeutic benefit while minimizing the possibility of an adverse reaction to the treatment.

The theoretical underpinnings of psychedelic drug therapy can be related to the time course of the drug's subjective effects (**FIGURE 15.12**). The first important component of the therapeutic process is the **peak experience**, which is the desired subjective state during the period of drug action. Pahnke and coworkers (1970) describe six elements of the peak psychedelic experience: "(1) sense unity or oneness…(2) transcendence of time and space, (3) deeply felt positive mood (joy, peace, and love), (4) sense of awesomeness, reverence, and wonder, (5) meaningfulness of psychological or philosophical insight or both; and (6) ineffability (sense of difficulty in communicating the experience by verbal description)" (p. 1857). The goal of the therapist(s) is to assist the patient in reaching and maintaining a peak experience. If this is accomplished, then the session may be followed by a period of days to weeks that is termed the **afterglow**. The features of this second therapeutic component are described as follows: "Mood is elevated and energetic; there is a relative freedom

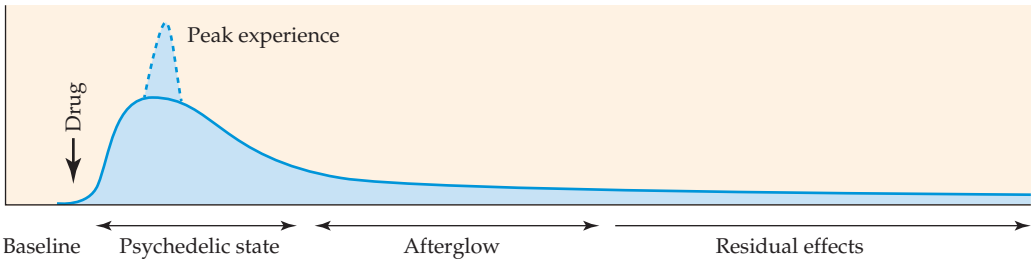


FIGURE 15.12 Hypothesized time course of the therapeutic effects elicited by psychedelic drugs According to Majić and coworkers, the potential therapeutic actions of psychedelic (hallucinogenic) drugs encompass several different stages of subjective experience, beginning with an acute "psychedelic state" that lasts from minutes to hours, followed by an "afterglow" that lasts from days to weeks, and, if successful, a long-term stage of "residual effects" on the patient's mood and cognitive set. The greatest likelihood of success is thought to be associated with elicitation of an intense "peak experience" during the acute stage. (After Majić et al., 2015.)

from concerns of the past and from guilt and anxiety, and the disposition and capacity to enter into close relationships is enhanced" (Pahnke et al., 1970, p. 1858).

Hallucinogenic agents as adjuncts to psychotherapy are currently being studied for the treatment of drug addiction, major depression, and obsessive-compulsive disorder and for helping terminally ill (e.g., cancer) patients cope with the fear and anxiety associated with impending death (Bogenschutz and Johnson, 2016; Garcia-Romeu et al., 2016; Rucker et al., 2016). Given the previous history of unfounded claims regarding the therapeutic benefits of LSD, it is important to remain cautious until much more evidence has accumulated. Nevertheless, as with the potential therapeutic applications of MDMA reviewed in Chapter 6, the situation regarding the classical hallucinogens bears close watching as time goes on.

Section Summary

- Hallucinogens are substances that cause perceptual and cognitive distortions in the absence of delirium. Many hallucinogens, such as mescaline, psilocybin, DMT, 5-MeO-DMT, and salvinorin A, are plant compounds that were used for hundreds or thousands of years in spiritual or religious ceremonies before their discovery by Western culture. In contrast, LSD is a synthetic drug, although it is based on a series of alkaloids found in ergot fungus. NBOMes constitute an additional class of synthetic hallucinogens that have appeared on the street more recently.
- Recognition of the powerful mind-altering properties of hallucinogenic drugs led to both clinical and recreational use beginning in the late 1950s. Some psychiatrists gave patients LSD in the course of psycholytic or psychedelic therapy. At the same time, secret government programs were testing the feasibility of using LSD or other potent hallucinogens as weapons. LSD became readily available on the street despite a federal ban on recreational use in 1967.
- Many hallucinogenic drugs are orally active, with a slow onset of action and a long time course of action. Two exceptions are DMT and *Salvia*, which are usually smoked, thereby leading to rapid drug effects and a much shorter duration of action. NBOMes can be taken orally but are more typically administered by the sublingual or buccal routes. Of the commonly used hallucinogens, LSD is the most potent and mescaline is the least potent, based on the range of doses taken by users.
- An LSD "trip" can be divided into four phases: onset, plateau, peak, and comedown. During the trip, the user experiences vivid visual hallucinations, a slowing of the subjective sense of time, feelings of depersonalization, strong emotional reactions, and a disruption of logical thought. Research on hallucinogens has made use of the altered states of consciousness rating scale, which consists of the following five primary dimensions: oceanic boundlessness, ego-disintegration anxiety, visionary restructuralization, reduced vigilance, and auditory alterations. These primary dimensions can be divided into subdimensions to provide more detailed information on drug-induced reactions.
- Most hallucinogenic drugs are classified chemically as either indoleamines or phenethylamines. The indoleamines are related structurally to 5-HT, whereas the phenethylamines instead share a common structure with NE. Of the hallucinogens discussed in this chapter, only mescaline and the NBOMes belong to the phenethylamine class. Both the indoleamine and phenethylamine hallucinogens are 5-HT_{2A} receptor agonists. Of particular interest is a recent discovery that the binding of LSD to the receptor protein causes the drug to be trapped temporarily within the binding pocket by a lid-like structure. This accounts for the extended length of an LSD trip.
- Salvinorin A, the active compound in *Salvia divinorum*, does not interact with the 5-HT_{2A} receptor but instead is a κ-opioid receptor agonist. This difference accounts for why the subjective effects produced by salvinorin A are somewhat different from the effects associated with the other compounds that act through the serotonergic system.
- The specific brain areas and mechanisms responsible for the production of hallucinogenic drug effects are not yet fully understood. However, EEG studies have shown that hallucinogens disrupt the normal electrical oscillations measured in the cerebral cortex. This effect is thought to be mediated by activation of 5-HT_{2A} receptors on glutamatergic pyramidal neurons in layer 5 of the PFC and other cortical areas.
- Hallucinogens are not dependence forming or addictive for most users; however, a small percentage of users meet the *DSM-5* diagnostic criteria for other hallucinogen use disorder. Most hallucinogenic drugs, except possibly DMT and salvinorin A, produce rapid tolerance with repeated use. This phenomenon has been linked primarily to 5-HT_{2A} receptor down-regulation.
- Use of hallucinogens can also lead to other adverse effects such as "bad trips" and flashbacks. People who suffer from severe, recurring flashbacks long after discontinuing hallucinogenic

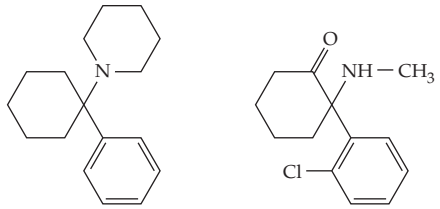
drug use are diagnosed as having hallucinogen persisting perception disorder. At the present time, little is known about the causes or treatment of HPPD, except for a possible involvement of the serotonergic system. Most seriously, dangerous toxic reactions involving psychiatric and/or somatic symptoms have been known to occur following ingestion of a hallucinogenic agent. Psychiatric symptoms resemble those seen in acutely psychotic patients and are most likely to occur in users who either are suffering from a current psychiatric disorder or possess a preexisting vulnerability for developing psychosis. The most severe reactions, which consist of both behavioral and dangerous somatic symptoms, are produced by NBOMe use. Users may experience delusions, severe agitation and aggressive behavior, and numerous physiological reactions that can, in extreme cases, be fatal.

- In recent years there has been renewed interest in the potential use of hallucinogenic drugs as adjuncts to psychotherapy. Contemporary research of this kind is much more carefully and safely conducted than in the early days of LSD-based therapy. Psychedelic drug therapy is theoretically based on the ability of the drug to produce a peak experience in the patient, followed over succeeding days or weeks by an afterglow period characterized by elevated mood, reduced guilt and anxiety, and an enhanced ability to form close social relationships. Current studies involving hallucinogenic drug therapy are focused on the treatment of drug addiction, major depression, and obsessive-compulsive disorder and on helping terminally ill patients cope with their fear of dying.

PCP and Ketamine

Background and History

This section of the chapter deals with two closely related compounds: **phencyclidine** and **ketamine**. Phencyclidine is usually abbreviated **PCP**, which comes from the drug's full chemical name, 1-(1-*p*henyl*c*yclohexyl) *p*iperidine (**FIGURE 15.13**). PCP (trade name Sernyl) was first tested in the mid-1950s by Parke-Davis and Company as a potential anesthetic agent. However, early studies revealed that the drug produced an unusual kind of anesthesia. Although individuals given PCP showed no responsiveness to nociceptive (painful) stimuli, they were not in the typical state of relaxed unconsciousness seen with traditional anesthetics like barbiturates. Instead, they exhibited a trancelike or catatonic-like state characterized by a vacant facial expression, fixed and staring eyes, and maintenance of



Phencyclidine (PCP) Ketamine

FIGURE 15.13 Chemical structures of PCP and ketamine

muscle tone. Indeed, it was not unusual for individuals to develop either rigidity or waxy flexibility—motor symptoms often observed in catatonic patients with schizophrenia (Li and Vlisides, 2016).

At first, PCP was thought to be clinically promising because it did not produce the respiratory depression associated with barbiturate anesthesia and thus possessed a high therapeutic index (see Chapter 1). However, early enthusiasm was soon tempered by reports of problematic reactions in many patients. In a few cases, this took the form of marked agitation rather than quieting during the drug-induced state. In other instances, PCP induced postoperative reactions ranging from blurred vision, dizziness, and mild disorientation to much more serious reactions involving hallucinations, severe agitation, and even violence. These problems caused the clinical use of PCP to be terminated in 1965.

Of course, the abandonment of PCP as a medication did not prevent it from coming into illicit use. In 1967, PCP found its way onto the streets of several cities, including San Francisco, where it was dubbed the "PeaCe Pill" by the drug culture protesting the Vietnam War. By the mid-1970s, PCP use and abuse under new street names such as "angel dust" and "hog" had become much more widespread across the country. Yet the popularity of this drug never rivaled that of marijuana or even cocaine or heroin, and the incidence of PCP use subsequently declined to a rather low level.

Ketamine came into being as a safer alternative to PCP. Even before PCP was withdrawn, Parke-Davis had begun to screen related compounds in the hope of finding one that would be less toxic in its behavioral effects. One such compound, designated CI-581, was synthesized in 1962 and was first tested in humans 2 years later. CI-581, later renamed ketamine, was less potent and shorter acting than PCP. Ketamine was soon found to be a valuable anesthetic for certain medical procedures, particularly in children, and it is also widely used by veterinarians as a general sedating and immobilizing agent. It is currently marketed legally as a prescription medication under the trade names Ketalar, Ketaset, and Vetalar. Despite its lower potency, ketamine can still cause adverse emergence reactions in human patients similar to those seen with

PCP. Fortunately, the frequency of such reactions is low in infants and children—the groups for which ketamine is most commonly used.

Pharmacology of PCP and Ketamine

Although PCP and ketamine can be considered anesthetic agents, the subjective experience produced by these compounds is unlike that produced by more typical anesthetic drugs. In the sections below, we discuss the characteristics of PCP- and ketamine-induced dissociation, the mechanism of action of these compounds, abuse and dependence of PCP, ketamine, and a ketamine analog called **methoxetamine**, and both current and possible future therapeutic applications of ketamine.

PCP and ketamine produce a state of dissociation

PCP is generally obtained in powdered or pill form, and the drug can be ingested by virtually any common route. It can be taken orally, administered intranasally (i.e., snorted), or injected intravenously or intramuscularly. Many PCP users apply the drug to tobacco, marijuana, or parsley cigarettes for purposes of smoking. Illicitly used ketamine typically comes from the diversion or theft of medical- or veterinary-grade material. Ketamine is marketed commercially as an injectable liquid, but street sellers commonly evaporate the liquid to yield a powder that is either snorted directly or compressed into pill form (**FIGURE 15.14**). It is sold on the street under such names as "K," "special K," or "cat Valium." Users who don't wish to become too intoxicated often snort small lines or piles of ketamine called "bumps." However, initial snorting of ketamine can escalate over time to intramuscular or even intravenous (IV) injection of the liquid solution as the user becomes tolerant to the drug or seeks a more powerful effect.

FIGURE 15.14 Ketamine crystals (Courtesy of Coaster420/Wikipedia.)

The first studies on the subjective effects of PCP were conducted in the late 1950s and early 1960s. When given a subanesthetic dose of PCP, study participants reported feeling detached from their bodies, sensations of vertigo or of floating, numbness, and sometimes a dreamlike state. They also experienced a variety of affective reactions including drowsiness and apathy, loneliness, negativism or hostility toward the experimenters, or, alternatively, feelings of euphoria and inebriation. Finally, all of the treated individuals exhibited a marked cognitive disorganization manifested by difficulty in maintaining concentration or focus, deficiencies in abstract thinking, and halting speech. These effects of PCP, which have been compared to the symptoms of schizophrenia, presumably account for the waning of the drug's popularity and its current low incidence of use. In fact, Edward Domino and Elliott Luby, two of the early researchers studying the influence of PCP on humans, noted several years ago, "It was astounding to us that phencyclidine [initially] became a major drug of abuse. Few of our volunteer subjects were ever willing to take the drug a second time" (Domino and Luby, 2012).

Domino and colleagues at the University of Michigan also published the first study on the pharmacological effects of ketamine in 1965. Low doses of ketamine yielded reactions similar to those mentioned in the previous paragraph for low-dose PCP administration. However, when study participants received doses in the anesthetic range (at least 1 mg/kg IV), the investigators observed a remarkable phenomenon. The individuals appeared to lose all mental contact with their environment for up to 10 minutes or longer, despite the fact that their eyes remained open and they retained significant muscle tone. When Domino described to his wife how the ketamine-treated individuals seemed to be disconnected from their environment, she proposed the term **dissociative anesthesia** to describe this unique state of detachment (E. F. Domino, personal communication). This term was subsequently applied to both ketamine and PCP.

More recent studies have documented the subjective experiences reported by ketamine users while in the dissociated state (**TABLE 15.2**). As noted in the table, the individual may feel separated from his body, perhaps floating above and looking down at himself. Some have described this as a "near-death" experience (Jansen, 2000; 2001), even though the person is not actually dying. This state of being, which is called the "K-hole," can be either spiritually uplifting or terrifying. As one user put it, "A K-hole can be anything from going to hell and meeting Satan to going to heaven and meeting God" (quote from *Time Out*, 2000, p. 20, cited in Kelly, 2001).

Researchers have used EEG and fMRI to investigate ketamine-induced changes in network connectivity within the cerebral cortex. When administered at an anesthetic dose, the drug disrupts cortical information

TABLE 15.2 Subjective Experiences Reported by Ketamine Users

Sensations of light coming through the body and/or of colorful visions
Complete loss of time sense
Bizarre distortions of body shape or size
Altered perception of body consistency (e.g., feeling as though one is made of a strange material such as rubber, plastic, or wood)
Sensations of floating or hovering weightlessly in space
Feelings of leaving one's body
Sudden insights into the mysteries of existence or of the self
Experiences of being "at one" with the universe
Visions of spiritual or supernatural beings

Source: From Dalgarno and Shewan, 1996.

transfer, especially between frontal and posterior cortical regions (Li and Vlisides, 2016). This effect is believed to play an important role in the dissociative anesthetic properties of both ketamine and PCP.

PCP and ketamine are noncompetitive antagonists of NMDA receptors

The principal molecular target for both PCP and ketamine is the NMDA (*N*-methyl-D-aspartate) receptor. To review briefly, the NMDA receptor is an important ionotropic receptor for the excitatory amino acid neurotransmitter glutamate. PCP and ketamine are both noncompetitive antagonists at the NMDA receptor complex; that is, they block the receptor at a site different from the site at which glutamate or NMDA binds. In fact, as we saw in Chapter 8, the PCP/ketamine binding site is found inside the receptor's ion channel (see Figure 8.5). NMDA receptors are widely distributed in the brain and play a key role in glutamate signaling. The cerebral cortex and hippocampus contain significant numbers of NMDA receptors, and blockade of the receptors in these areas likely contributes to the cognitive deficits produced by PCP and ketamine. Another potential mechanism of PCP and ketamine action is increased *presynaptic* glutamate release (and therefore overactive glutamate transmission via non-NMDA receptors) within the cortex, which is a secondary consequence of NMDA receptor antagonism (Deakin et al., 2008; Gunduz-Bruce, 2009). Consistent with this hypothesis, a type of brain imaging technique known as proton magnetic resonance spectroscopy[5] showed a significant increase in glutamate levels in the anterior cingulate cortex after a single IV infusion of ketamine (Stone et al., 2012). Moreover, this increase was significantly correlated with the positive psychotic symptoms induced by the drug treatment.

PCP/KETAMINE MODELS OF SCHIZOPHRENIA In the early years following discovery of the hallucinogenic and cognition-impairing features of acute LSD administration, researchers argued that these symptoms were similar to those observed in patients with schizophrenia. This is why LSD and other hallucinogens are sometimes called psychotomimetic agents, as mentioned in the chapter introduction. However, there are important differences between the features of LSD intoxication and the symptoms of schizophrenia. For example, LSD and other hallucinogenic drugs produce mainly visual distortions, whereas hallucinations in patients with schizophrenia are typically auditory, such as hearing voices that aren't real. Furthermore, hallucinogenic drugs fail to reproduce the so-called negative symptoms of schizophrenia, which include reduced speech, flat affect, and social withdrawal (see Chapter 19).

The LSD model of schizophrenia was later replaced by a model involving the subjective and behavioral effects of PCP or ketamine (Frohlich and Van Horn, 2014). Healthy people given a high dose of either of these drugs display a variety of symptoms consisting of disordered thought, delusions, and motor disturbances ranging from extreme agitation to catatonia. Some negative symptoms may also be observed. Moreover, several studies have demonstrated an exacerbation of symptoms in patients with schizophrenia following ketamine administration. These findings led to a glutamate hypothesis of schizophrenia, which proposed that hypoactivity of the glutamatergic system is a key factor in the pathogenesis of the disorder (Moghaddam and Javitt, 2012). This hypothesis is supported not only by the ability of NMDA receptor antagonists to model many of the symptoms of schizophrenia, but also by the finding of reduced NMDA receptor subunit expression in postmortem brain tissues from patients with schizophrenia.

Several animal models have been proposed to test the ability of PCP treatment to produce schizophrenia-like symptoms. Some researchers treat adult animals with multiple doses of PCP over several days (subchronic PCP model), whereas others use a model involving early postnatal PCP administration that is designed to invoke the kind of neurodevelopmental insult thought to set the stage for later onset of schizophrenic symptoms. Interested readers are referred to recent reviews (Grayson et al., 2015; Janhunen et al., 2015) for more information about these models.

PCP and ketamine have significant abuse potential

Both PCP and ketamine are highly reinforcing in several different species of animals, as shown by drug

[5]Magnetic resonance spectroscopy (MRS) is an imaging method that uses equipment similar to that used for magnetic resonance imaging (MRI), but instead of yielding information on brain structure or regional activation, it measures the levels of various brain chemicals.

self-administration. Indeed, both compounds are subject to abuse and dependence, although the prevalence of ketamine use and abuse is currently much greater than that of PCP.

REINFORCING EFFECTS Many PCP and, to a lesser extent, ketamine self-administration studies have been conducted using rhesus monkeys. Interestingly, early studies on monkeys that self-administered high doses of PCP found that the animals took in sufficient quantities of the drug to be intoxicated almost continuously (Balster and Woolverton, 1980; 1981). Under the influence of PCP, the subjects could not support themselves on four legs, but instead were typically found near the response lever either in an awkward sitting position or lying on the cage floor. The ability to elicit self-intoxication in animals is not unique to PCP; it has also been observed with cocaine, amphetamine, opioids, and in some cases alcohol.

PCP and ketamine activate midbrain dopamine (DA) cell firing and stimulate DA release, particularly in the PFC. This enhancement of dopaminergic neurotransmission could contribute to PCP's and ketamine's reinforcing effects. On the other hand, rats will also self-administer PCP directly into the nucleus accumbens, and this local reinforcing effect appears to be DA-independent (Carlezon and Wise, 1996). Thus it seems likely that both dopaminergic and nondopaminergic mechanisms contribute to PCP and ketamine reinforcement.

The pleasurable effects of IV ketamine were studied in healthy volunteers without prior exposure to this compound (Morgan et al., 2004). As illustrated in **FIGURE 15.15**, the investigators found dose-dependent increases in drug liking and in the desire for more drug, confirming that ketamine is rewarding not only in laboratory animals but also in humans.

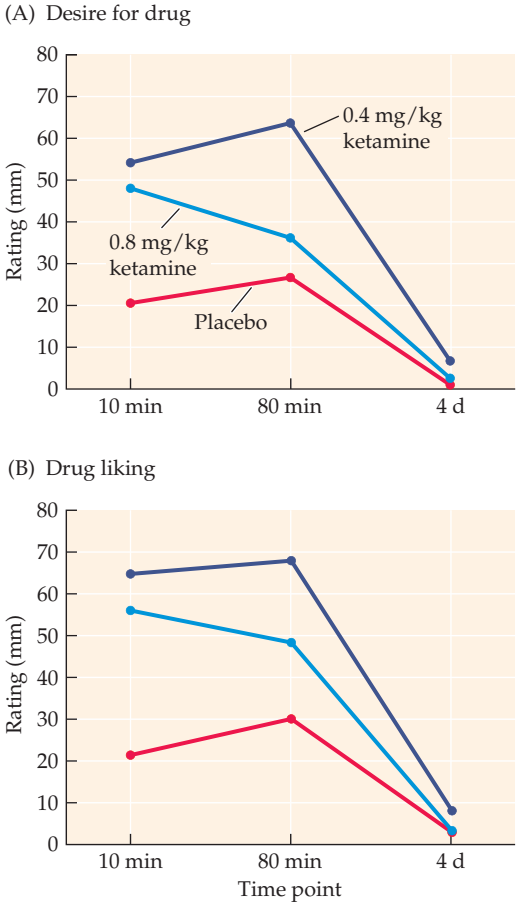


FIGURE 15.15 IV ketamine administration produces dose-dependent rewarding effects in humans Study volunteers who had never used ketamine before were given an IV infusion of placebo or 0.4 or 0.8 mg/kg ketamine over a period of 80 minutes. At the 10- and 80-minute time points, as well as 4 days later (follow-up), the volunteers were tested for their subjective responses to the drug, using a visual analog scale (see Web Box 12.1, Figure C caption for a description of this method). Both doses produced increased desire for the drug (A) and increased drug liking (B) compared with placebo. (After Morgan et al., 2004.)

USE AND ABUSE As mentioned earlier, PCP is not widely used compared with many other substances of abuse. The 2016 National Survey on Drug Use and Health estimated that approximately 21,000 people age 12 or older in the United States were current users of PCP, compared with 374,000 LSD users and over 1.9 million users of cocaine (Substance Abuse and Mental Health Services Administration, 2017). Despite the low prevalence of PCP use, the *DSM-5* lists diagnostic categories of phencyclidine use disorder and phencyclidine intoxication. The latter category is important for differentiating some of the symptoms of PCP intoxication, such as analgesia, ataxia, and muscle rigidity, from the symptoms associated with intoxication produced by hallucinogens such as LSD or psilocybin (which are covered separately in a diagnostic category called other hallucinogen intoxication). Individuals who use PCP have found some novel (and dangerous) drug combinations, including a combination variously referred to as "fry," "wet," or "illy," in which tobacco or marijuana cigarettes are dipped in a liquid containing PCP and embalming fluid (!) and then smoked (Peters Jr. et al., 2008).

In contrast to PCP, the use of ketamine has grown because of the drug's popularity within the dance scene (Wolff and Winstock, 2006). Although widespread recreational use of ketamine is a relatively recent phenomenon, illicit use and abuse of this substance actually date back many years. Some abusers were, and continue to be, medical or veterinary practitioners who have easy access to ketamine in the course of their work. Ketamine was also favored by some intellectuals as a mind-expanding drug in the tradition of LSD. Two of the most famous ketamine users were Marcia Moore,

a well-known astrologer and author in the 1970s, and Dr. John Lilly, a physician and researcher known for his groundbreaking studies on interspecies communication (e.g., with dolphins) and on the psychological effects of sensory isolation. Both Moore and Lilly became heavily dependent on ketamine, and both developed psychotic reactions as a result.[6]

Development of ketamine tolerance and dependence can be seen not only in the extreme cases of Marcia Moore and John Lilly but also in the self-reports of other heavy users. Accounts of dose escalation and compulsive use are presented by Karl Jansen, a British psychiatrist who has investigated ketamine use for many years (Jansen, 2001; Jansen and Darracot-Cankovic, 2001). Interestingly, many of the ketamine-dependent individuals studied by Jansen are described as being highly intelligent, even PhD students. One straight-A PhD candidate said that overcoming his ketamine problem was "harder than heroin" (Jansen, 2001, p. 167).

Recreational ketamine use typically occurs outside the home at parties, raves, or clubs (De Luca et al., 2012). Ketamine is sometimes snorted as part of a drug combination that may include methamphetamine, heroin, cocaine (called the "Calvin Klein"), or other compounds. Oral ketamine can be combined with MDMA, whereas smoked ketamine may be taken in conjunction with marijuana.

Several other noncompetitive NMDA receptor antagonists are also used recreationally for their psychoactive properties (Morris and Wallach, 2014). One of these is **dextromethorphan**, a common ingredient in over-the-counter cough and cold medications. This compound is discussed in **BOX 15.2**. Whereas dextromethorphan has been available for many years, a more recent development has been the appearance on the street of methoxetamine, a potent analog of ketamine. Methoxetamine (street names "Mexxy," "MXE," or "special M") first appeared in 2010 and continues to be sold on the internet despite an increasing number of countries declaring the drug to be illegal for nonresearch use. Methoxetamine can be taken orally, by insufflation, and by intramuscular injection. It produces a dissociative reaction like that of ketamine, but the effect is much longer lasting (Corazza et al., 2013; Kjellgren and Jonsson, 2013). Methoxetamine stimulates mesolimbic dopaminergic activity and exhibits both rewarding (conditioned place preference) and reinforcing (self-administration) properties in animals (Botanas et al., 2015; Mutti et al., 2016). Taken together, these properties predict abuse potential of methoxetamine. As with ketamine, users report a variety of experiences that may be euphoric, spiritual, or terrifying (Kjellgren and Jonsson, 2013).

[6]In Moore's case, the consequences were especially tragic when she left her home on a cold wintry night in 1979, climbed a tree, gave herself a ketamine injection, and froze to death while in a state of drug intoxication.

Use of PCP, ketamine, or related drugs can cause a variety of adverse consequences

Chronic use of ketamine or PCP can produce many different negative effects. Some ketamine users develop distressing urological symptoms including cystitis (bladder inflammation), painful urination, and incontinence (Chu et al., 2008; Wood et al., 2011). Severe cases may even involve damage to the kidneys. Some ketamine users develop gastrointestinal disturbances such as upper abdominal pain, gallbladder problems, and signs of liver toxicity (Bokor and Anderson, 2014). Furthermore, deficits in memory and other cognitive functions involving the frontal and medial temporal lobes have been reported in chronic recreational ketamine users (Morgan and Curran, 2006; Chan et al., 2013). Some of these deficits seem to persist even following a significant reduction in drug consumption. Ketamine users have also exhibited much greater delusional thinking than nonusers (Morgan et al., 2009), although it is impossible to know from this study whether such abnormal ideation was caused by or preceded ketamine exposure. The neurobiological mechanisms underlying these psychological effects are not yet known, although brain imaging studies have found evidence for both gray and white matter abnormalities in chronic ketamine users (Liao et al., 2010; 2011). There is also a report of increased D_1 dopamine receptor binding in the prefrontal cortex of ketamine users, which may be a result of decreased presynaptic dopaminergic transmission in this brain area (Narendran et al., 2005).

Experimental animal studies have demonstrated additional effects of repeated PCP or ketamine exposure on the brain. Adolescent cynomolgus monkeys given 1 mg/kg ketamine intravenously every day for 6 months displayed a significant reduction in tyrosine hydroxylase immunoreactivity (used as a marker of DA axons and terminals) in the prefrontal cortex (Yu et al., 2012). This finding is consistent with the results of Narendran and coworkers (2005) in human ketamine users. The same ketamine-treated monkeys also showed substantial regional changes in neural activity compared with control animals when subjected to fMRI. Moreover, researchers have found altered NMDA receptor levels (Newell et al., 2007; Xu and Lipsky, 2015) and a reduced number of asymmetrical spine synapses (usually considered to be excitatory glutamatergic synapses) in the prefrontal cortex in both rats (Elsworth et al., 2011b) and monkeys (Elsworth et al., 2011a) following repeated PCP or ketamine administration. Even more striking, repeated treatment with high doses of ketamine provokes apoptotic cell death in the developing brain of both rats and monkeys (Yan and Jiang, 2014)

BOX 15.2 Pharmacology In Action

Getting High on Cough Syrup

One of the most annoying features of a bad cold is the persistent cough that it may bring on. That is why **antitussives**, medications that suppress the cough reflex, are big sellers in pharmacies across the country. The main ingredient in most of these over-the-counter products is an opioid-like compound called dextromethorphan. For example, dextromethorphan is the antitussive agent in Robitussin DM cough syrup and in Coricidin HBP cough and cold tablets.

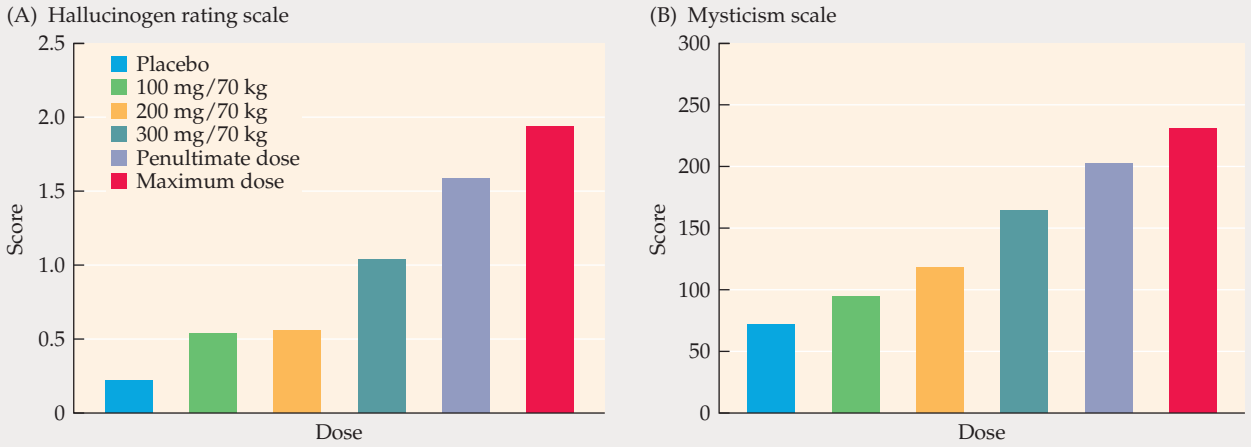
Cough medications based on dextromethorphan have been on the market for many years. The first one was Romilar, a dextromethorphan-containing tablet that was introduced in the 1960s. Romilar was meant to be a replacement for codeine-containing medications, since the latter were already being abused. However, it did not take long before users discovered the psychoactive properties of Romilar and began abusing it as well. The drug was eventually withdrawn from the market and was later replaced with a codeine-containing, prescription-only version. Pharmaceutical companies subsequently decided to put dextromethorphan into a cough syrup, presumably to discourage recreational use by requiring the ingestion of large amounts of the syrup to obtain a psychoactive effect. However, this has not prevented users, typically adolescents or young adults, from continuing to experiment with this substance. Indeed, data from the National Poison Data System show that the prevalence of dextromethorphan abuse more than doubled from 2004 to 2013 (Sheridan et al., 2016).

Following ingestion, dextromethorphan is metabolized to the active substance **dextrorphan**, which is thought to be responsible for most of the psychoactive effects produced by high doses of dextromethorphan-containing cough medicines. Both dextromethorphan and dextrorphan have a complex pharmacology, with significant affinities for NMDA receptors and the serotonin transporter, along with lower affinities for the norepinephrine transporter and several monoamine receptor subtypes (C. P. Taylor et al., 2016). As in the case of PCP and ketamine, dextromethorphan and its metabolite can produce a dissociative reaction (see below) through noncompetitive antagonism of NMDA receptors.

When used recreationally, dextromethorphan is typically taken orally in the form of cough syrup or tablet. On the street, it goes by names like "Red Devils," "Triple C's," "Skittles," or "Robo." Consuming high doses of dextromethorphan-containing products to become intoxicated is commonly referred to as "Robotripping" (Stanciu et al., 2016). The standard dose of dextromethorphan for cough suppression is 20 mg (found in 0.33 ounces of cough syrup), but recreational users take single doses that are 8 to 50 times higher (Banken and Foster, 2008). This requires drinking anywhere from a quarter of a typical 8-ounce bottle of cough syrup to as much as two whole bottles. There are even reports of heavy users ingesting three or four bottles in one day. Drinking this much cough syrup usually causes nausea and vomiting as a result of the effects of guaifenesin, an expectorant (agent that facilitates expulsion of phlegm from the throat or airways) found in most cough syrups. Some users have tried to avoid this unpleasant side effect by taking large amounts of dextromethorphan-containing Coricidin tablets. However, this is a very dangerous, even potentially fatal, practice because of the presence of chlorpheniramine in these tablets. Chorpheniramine is an antihistamine/anticholinergic agent that not only produces serious reactions by itself at high doses but also intensifies the effects of dextromethorphan by inhibiting its metabolism by the liver. Given the limitations associated with dextromethorphan use via standard cough medications, enterprising users have discovered methods for extracting the substance from cough syrup (Hendrickson and Cloutier, 2007). As a result, dextromethorphan has become available on the street or via the internet in repackaged pills or capsules for oral administration, and even in powdered form for intranasal use (snorting). Indeed, tablets sold as "ecstasy" occasionally contain dextromethorphan instead of the expected MDMA.

Users report that the subjective effects of dextromethorphan occur as a series of four dose-related "plateaus" (Romanelli and Smith, 2009; Burns and Boyer, 2013). Low doses (100–200 mg dextromethorphan) produce the first plateau, during which the user feels a mild euphoria and intoxication and may also experience slight perceptual effects. This stage is said to be similar to the effects of MDMA consumption. The second plateau is the most commonly sought after and requires a dose of 200 to 400 mg dextromethorphan. At this stage, the user suffers from impaired balance, experiences visual hallucinations when he closes his eyes, and is significantly more intoxicated than at the first plateau. The third plateau occurs at doses ranging from 300 to 600 mg and yields more intense hallucinations, cognitive impairment, and partial dissociation. At the fourth plateau, produced by more than 600 mg dextromethorphan, the user becomes severely ataxic, experiences complete personality dissociation, and may become delusional. These effects resemble those produced by increasing doses of PCP or ketamine. Some evidence exists that the doses needed to reach each plateau may increase because

BOX 15.2 Pharmacology In Action *(continued)*



High doses of dextromethorphan produce subjective effects similar to those observed with classical hallucinogens Individuals who were experienced users of hallucinogenic drugs (including dextromethorphan in some cases) were given varying oral doses of dextromethorphan or placebo under relaxing conditions that facilitated the possibility of experiencing hallucinogenic drug–like effects. The highest possible drug dose was 800 mg/70 kg body weight, but not all individuals achieved that dose because of adverse effects in some cases. The graphs show scores on the hallucinogen rating scale (perception scale), which reflects hallucinations, illusions, and synesthesias (A), and on a mysticism scale that has been used to assess both natural and drug-induced mystical experiences (B). The maximum and penultimate doses are the highest and next-to-highest doses tolerated by each individual. (After Reissig et al., 2012.)

of a rapid development of tolerance in chronic users (Stanciu et al., 2016). Withdrawal symptoms have also been reported, though this phenomenon has not yet been systematically investigated.

Sufficient doses of dextromethorphan produce not only visual hallucinations but other subjective changes typically seen with hallucinogenic drugs such as psilocybin and LSD. Reissig and coworkers (2012) investigated the subjective effects of oral dextromethorphan using several questionnaires, including the standard hallucinogen rating scale as well as a mysticism scale to assess the intensity of drug-induced mystical experiences. The results showed that dextromethorphan dose-dependently produced a range of subjective effects similar to those reported following the use of classical hallucinogens. The **Figure** illustrates two such effects, namely, increases in perceptual distortions (illusions and hallucinations) and increases in overall mystical experience.

Some users report positive experiences with dextromethorphan, but others have serious negative reactions. Acute toxicity can occur not only in users who take extremely high doses of dextromethorphan but also in a small percentage of individuals who are genetically deficient in their metabolism of the drug (Schwartz, 2005). Extreme dextromethorphan intoxication has been linked to psychotic reactions, violent behavior, and even death (Amaladoss and O'Brien, 2011; Logan et al., 2009; Logan et al., 2012; Miller, 2005).

Although the present discussion has centered mainly around dextromethorphan abuse, it's worth noting that this drug has an interesting therapeutic application beyond its common use as an antitussive agent. Specifically, a formulation marketed as **Nuedexta** is licensed for the treatment of a rare neurological condition called **pseudobulbar affect** (C. P. Taylor et al., 2016). This condition, which is seen in a small percentage of patients with brain injury or disease, is characterized by frequent, uncontrollable episodes of laughing or crying that are incongruent with the person's emotional state. Each Nuedexta capsule contains 20 mg of dextromethorphan along with 10 mg of quinidine sulfate, which prolongs dextromethorphan bioavailability by inhibiting its metabolism. Researchers do not yet understand how dextromethorphan works to reduce the symptoms of pseudobulbar affect, although the mechanism may involve some combination of the drug's actions on its various molecular targets.

In summary, dextromethorphan is a powerful substance that, at high doses, exhibits psychoactive properties similar to those of the other drugs discussed in this chapter. It has substantial abuse potential and can produce severe adverse reactions in users who are poor metabolizers of the compound and/or who take extremely large amounts of the drug. Dextromethorphan is not currently included in the Schedule of Controlled Substances, which explains why medications containing the drug can be sold without a prescription. Nevertheless, the DEA has listed it as a drug of concern, and it may be added to the schedule at some future time.

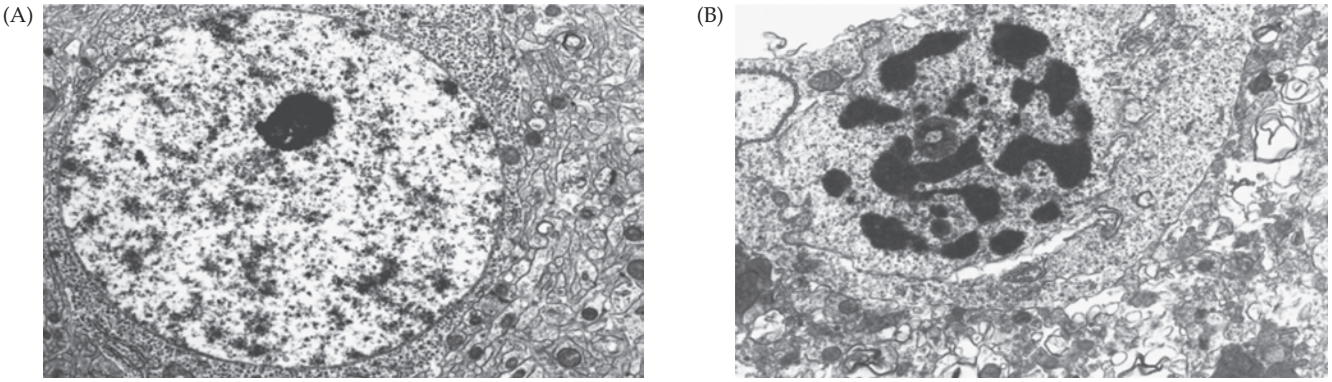


FIGURE 15.16 Electron micrographs depicting ketamine-induced neurodegeneration in the frontal cortex of an infant rat Seven-day-old rats were given 5, 10, or 20 mg/kg ketamine subcutaneously once, three times, or six times at 2-hour intervals. Animals were killed at 6 hours after the last injection, and their brains were prepared for histological examination using electron microscopy. The micrographs show normal neuronal morphology in the frontal cortex of a saline vehicle control rat (A) but fragmentation of the cell nucleus indicative of apoptotic cell death in the same brain area of a rat given six 20 mg/kg doses of ketamine (B). Lower doses or fewer treatments did not produce this neurodegenerative effect. (From Zou et al., 2009.)

(**FIGURE 15.16**). Dong and Anand (2013) have additionally reviewed evidence for ketamine-induced disruption of neurogenesis (formation of new nerve cells) during early brain development. Although lower drug doses do not produce such effects, researchers have nonetheless raised concerns about these findings given that ketamine is a recommended anesthetic agent for pediatric procedures that involve significant pain and/or require immobilization of the patient (Krauss and Green, 2000). A number of other anesthetics that work by enhancing $GABA_A$ receptor activity can also induce apoptosis when given to experimental animals during early development (Hays and Deshpande, 2011). Because of a lack of direct histological evidence, there is currently no way to know whether routine anesthetic administration during development in humans leads to apoptotic cell death in the brain. Some preliminary findings suggest that exposure to general anesthesia in infancy or early childhood, particularly on multiple occasions, may be associated with later cognitive deficits (Wilder, 2010). However, various confounding factors make this research difficult to interpret, and further studies are needed to clarify whether young humans are at risk when they undergo procedures that require general anesthesia.

Before closing this section on adverse effects, it's important to take note of recent reports of severe, even fatal, reactions to methoxetamine. This substance is reportedly preferred to ketamine by some users because it is advertised online as being "bladder friendly" (i.e., not producing the urinary tract problems associated with chronic ketamine use) (Zawilska, 2014). Initial animal studies suggest that methoxetamine can produce bladder inflammation and kidney toxicity just like ketamine. However, these effects have not yet been confirmed in human users of the drug. More importantly, numerous reports have appeared showing severe toxic reactions to methoxetamine characterized by tachycardia, hypertension, hyperthermia, and an array of neuropsychiatric symptoms that can include hallucinations, confusion, ataxia, catatonia, and coma (Zawilska, 2014; Chiappini et al., 2015; Zanda et al., 2016). Fatal reactions have occurred, sometimes with only methoxetamine detected in the person's body but often as a result of using methoxetamine along with other psychoactive drugs such as alcohol, marijuana, psychostimulants, MDMA, or opiates. Health authorities are accordingly concerned about the increased availability and use of this dangerous compound.

Novel therapeutic applications have been proposed for ketamine

We have seen that ketamine is an approved anesthetic for veterinary use as well as for some procedures in human infants and children. Recent research suggests that this drug may have additional therapeutic applications in the treatment of major depression and in pain relief. Practitioners have known for many years that selective serotonin reuptake inhibitors (SSRIs), the first-line drugs prescribed for patients with major depression, require several weeks of administration before significant therapeutic benefit occurs (see Chapter 19). This so-called "therapeutic lag" is of significant concern not only because of the continued suffering of the patient, but also because a depressed patient who is suicidal could take her life before her mood improves. For reasons that are not yet understood, a single IV dose of ketamine has been demonstrated to produce rapid, although temporary, symptom improvement in clinically depressed patients (Strasburger et al., 2017). Clinical trials are under way to determine whether ketamine or a similar NMDA receptor antagonist could be developed as an approved treatment for major depression. Ketamine is also being tested for use as a non-opioid analgesic agent for a variety of chronic

pain conditions and for peri- and postoperative analgesia (Gorlin et al., 2016; Vadivelu et al., 2016). Because tolerance to repeated opiate drug treatment is partially mediated by the NMDA receptor, an additional benefit of ketamine administration is the ability to reverse opioid tolerance. Although these novel applications of ketamine are intriguing, caution is warranted because of the abuse potential of this compound and the narrow therapeutic window in affording pain relief without putting the patient to sleep.

Section Summary

- PCP and ketamine belong to the class of drugs known as dissociative anesthetics.
- PCP was withdrawn from clinical use because of its prominent adverse side effects, but ketamine, which is less potent than PCP, has significant applications in both human and veterinary medicine.
- The acute effects of PCP and ketamine include sensory distortions and altered body image, cognitive disorganization, and various affective changes. High doses of ketamine give rise to a state called the "K-hole," in which the user feels separated from his body, perhaps in the manner of a near-death experience.
- PCP and ketamine bind to a site within the NMDA receptor channel, thereby acting as noncompetitive receptor antagonists. A secondary consequence of NMDA receptor blockade is increased presynaptic glutamate release, which may contribute to the behavioral and subjective effects of PCP and ketamine.
- Models of schizophrenia in both humans and experimental animals have been developed based on the hallucinogenic and cognition-impairing effects of PCP and ketamine.
- Both PCP and ketamine are reinforcing to animals, as indicated by drug self-administration. These reinforcing effects may be mediated by both dopaminergic and nondopaminergic mechanisms. Ketamine also produces pleasurable effects in humans.
- Although illicit use of ketamine has occurred for many years, the popularity of this compound is on the rise. Heavy ketamine users show dose escalation and compulsive use, which indicate the development of tolerance and dependence on the drug.
- Abuse potential has been shown for a few other noncompetitive NMDA receptor antagonists. These include dextromethorphan, an opioid-like compound found in cough medications, and the potent ketamine analog methoxetamine. These compounds produce dissociative subjective effects and, in the case of dextromethorphan, other effects like those of the classical hallucinogens.
- Chronic ketamine or PCP exposure leads to a variety of adverse effects including urological symptoms, cognitive deficits, gray and white matter abnormalities, altered dopaminergic function, and a reduced number of asymmetrical spine synapses in the prefrontal cortex. Repeated high ketamine doses also cause apoptotic cell death in the developing brains of laboratory animals, which raises concern for the use of this compound as a pediatric anesthetic agent. The acute toxic effects of methoxetamine are even more severe than those observed with ketamine or PCP. These effects have, in some cases, led to death of the user.
- Despite its potential for abuse, ketamine may have novel therapeutic applications in the treatment of major depression and for pain relief. Caution must be observed, however, because of the drug's narrow therapeutic window.

STUDY QUESTIONS

1. The substances discussed in the first section of this chapter have variously been termed "hallucinogenic," "psychedelic," and "psychotomimetic." (a) What do each of these terms mean, (b) why have the authors chosen to use the term "hallucinogenic" in most of the chapter, and (c) what is the definition of a hallucinogenic drug?
2. Mescaline and psilocybin are two plant-derived hallucinogenic compounds. (a) In what plants are these substances found? (b) How were the effects of psilocybin discovered by Westerners?
3. Discuss the characteristics of the tryptamine hallucinogens and their use in ayahuasca.
4. Describe the circumstances around the discovery of LSD, its subsequent use in psycholytic and psychedelic therapy, and the features of its recreational use.

(*Continued*)

STUDY QUESTIONS (*continued*)

5. What are NBOMEs, and how are they administered by recreational users?
6. What is *Salvia divinorum*, what is its active ingredient, and how is it consumed recreationally?
7. Discuss the differences in potency and duration of activity of various hallucinogenic drugs when consumed by their typical routes of administration.
8. (a) Describe the four phases of a typical LSD trip and the typical user's experiences in each phase. (b) Define synesthesia, a phenomenon that is sometimes experienced by LSD users.
9. Discuss the use of the altered states of consciousness (ASC) rating scale in research, and describe the five primary dimensions of this scale.
10. What is the difference between indoleamine and phenethylamine hallucinogens? List a few compounds belonging to each category.
11. (a) What is the primary molecular target of both the indoleamine and phenethylamine compounds that mediates their hallucinogenic effects? (b) What behavioral test procedures are used to screen for hallucinogenic-like effects in laboratory animals?
12. How does the mechanism of action of salvinorin A differ from that of the other hallucinogenic drugs discussed in the Hallucinogenic Drugs section of the chapter?
13. Discuss the interactions between the serotonergic and glutamatergic systems in mediating the consciousness-altering effects of hallucinogenic drugs.
14. Describe the hallucinogenic drug-related disorders mentioned in the chapter.
15. Discuss current ideas regarding the potential therapeutic uses of hallucinogenic drugs.
16. Discuss the history of the development, therapeutic use, and recreational use of PCP and ketamine.
17. What are the features of being in a state of "dissociative anesthesia"?
18. Describe the primary mechanism of action of PCP and ketamine. How has this mechanism been related theoretically to the neurochemical basis of schizophrenia?
19. Summarize both human and experimental animal research demonstrating the abuse potential of PCP and ketamine.
20. Describe the adverse consequences of heavy PCP or ketamine use on the brain and on peripheral organ systems.
21. Discuss the pharmacology and recreational use of dextromethorphan.

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CHAPTER 16

Young people are particularly attracted to inhalants because of their low cost and easy availability and the rapid "high" they produce. (Jerry Redfern/LightRocket/Getty Images.)

Inhalants, GHB, and Anabolic–Androgenic Steroids

AIR CONDITIONING IS ONE OF THE MARVELS OF MODERN CIVILIZATION, especially for those living in areas with hot and humid summer weather. We usually think of air conditioners as safe appliances; however, intentional exposure to the refrigerant inside the air conditioner by inhalation can be very dangerous. Why would someone purposely inhale air conditioning refrigerant? The answer, as you may already know, is because the fumes produce a brief "high" in the user. The same can be said for numerous other volatile liquids and gases, including glues, aerosol propellants, cigarette lighter fluids, and so-called "laughing gas."

These and related chemicals all belong to a broad class of abused substances known as **inhalants**. Most inhalants are perfectly legal, can easily be purchased at retail stores by people of any age, and are readily accessible in the home by anyone who can reach into a medicine cabinet or a kitchen drawer, or who can walk down the stairs to the basement or open the door to the garage. Moreover, in recent years these substances have been responsible for the abrupt death of a number of children and teenagers in many countries, including the United States. Indeed, the parents of children who have died from abusing inhalants invariably say that they never knew how dangerous these substances could be.

The first part of this chapter discusses where inhalants come from, how they affect behavioral and neural functioning, and what health risks they pose. Later sections of the chapter cover γ-hydroxybutyrate (GHB) and anabolic–androgenic steroids. Although inhalants, GHB, and steroids differ in their mechanisms of action, they share the fact that they are all relative newcomers to the drug abuse scene compared with many other substances, such as alcohol, cannabis, tobacco, opioids, and the plant-derived hallucinogens. ■

Inhalants

Background

Inhalants represent a novel and diverse group of abused substances. They are also unusual in that users are typically children and adolescents.

Inhalants comprise a range of substances including volatile solvents, fuels, halogenated hydrocarbons, anesthetics, and nitrites

Abused inhalants often come from everyday household items. These substances have the following characteristics:

- They are either volatile (easily vaporized) liquids or gases at room temperature.
- They are used by inhaling fumes from a rag saturated with the substance ("huffing"),[1] sniffing fumes from a container of the substance ("sniffing" or "snorting"), inhaling fumes of the substance inside a plastic or paper bag ("bagging"), inhaling the substance from a balloon (a common method of nitrous oxide inhalation), or spraying an aerosol of the substance directly into one's nose or mouth (e.g., "glading" from a can of air freshener or "dusting" from a dust removal spray can).
- They do not belong to another defined class of abused substances that can be inhaled through smoking, which includes nicotine, Δ^9-tetrahydrocannabinol (THC), and cocaine.

Inhalants that are susceptible to recreational use mostly come from four categories: volatile solvents, fuels, halogenated hydrocarbons, and anesthetics (**TABLE 16.1**). **Volatile solvents** are chemicals that are liquid at room temperature but give off fumes that can be inhaled. Solvents are found in numerous household and industrial products, including adhesives, the ink used in felt-tip marking pens, spray paints, paint thinners and removers, nail polish remover, air fresheners, moth balls, and industrial degreasing agents. **Fuels** are volatile liquids or gases that serve many purposes, including automobile fuel, fuels for lamps and heating appliances, lighter fluids, and propellants used in many kinds of spray cans. The third category, **halogenated hydrocarbons**, is defined by the chemical structures of its members. These substances are all hydrocarbon molecules possessing one or more chlorine or fluorine atoms. Halogenated hydrocarbons have numerous commercial uses as refrigerants, solvents, degreasers, and adhesives. They are also present as propellants in some compressed air dusters for computers and other devices and as the solvent in bottled typewriter or ink correction fluids. A small category of **anesthetics** includes nitrous oxide (also known as laughing gas), which is used in dentistry and in whipped cream dispensers, and two liquids (chloroform and ether) that were formerly used as small-animal anesthetic agents in veterinary medicine and laboratory research. Although these two compounds have been phased out as anesthetics in the United States, they are still produced and sold for specific chemical applications.

The fifth group of inhalants, called **nitrites**, is often placed in a separate category apart from other inhaled substances. Whereas most inhalants are taken to obtain a euphoric effect, or "high," nitrites are typically used to heighten sexual arousal and pleasure. Furthermore, unlike other inhalants, which are thought to act directly on nerve cells, nitrites produce their subjective effects primarily by dilating blood vessels and causing muscle relaxation. The most common members of this group are amyl nitrite ("poppers") and butyl nitrite. In the remainder of this section, we will focus on the first four classes of inhalants.

Abused inhalants are rapidly absorbed and readily enter the brain

We saw in previous chapters that drugs taken by vaporization and smoking, for example, nicotine, crack cocaine, or THC, are rapidly absorbed through the lining of the lungs. The same is true for abused inhalant drugs. Once absorbed into the bloodstream, inhaled compounds rapidly reach the brain and readily cross the blood–brain barrier. For this reason, the acute effects of inhalants occur very quickly and may dissipate just as quickly once brain concentrations decline to low levels after a single use.

Most of us are occasionally exposed to low concentrations of abused inhalants because of their presence in glues, household cleaners, spray cans, paints and paint removers, and gasoline. One example is toluene, which not only has several common uses (see Table 16.1) but has also been studied extensively as a representative inhalant. Unlike other drugs discussed in previous chapters, inhalant doses are calculated with respect to the amount of the chemical in the air. The typical units used are **parts per million** (**ppm**), which correspond to the number of molecules of inhalant (e.g., toluene) per million molecules of other gases in the air, which are mostly nitrogen and oxygen. Health agencies in various countries consider a "safe" exposure to toluene to be within the range of 10 to 100 ppm (Cruz et al., 2014). Exposures greater than 500 ppm are believed to pose a potential danger to the person's health. Consider, then, that misuse of toluene-based products can cause exposure to thousands of parts per million each time the product is inhaled.

[1] Although the term huffing has the specific meaning given here, it is often used more generally to denote the breathing in of an inhalant.

TABLE **16.1** Some Commonly Abused Inhalants

Compound	Principal uses
Volatile solvents	
Acetone	Adhesives, nail polish remover, paint thinner
n-Hexane	Adhesives, degreasers
Methanol	Paint remover, degreasers
Methylene chloride	Paint remover
Methyl butyl ketone	Denaturant, adhesive thinner, degreasers
Naphtha	Spray paint, adhesives, marker pens, lighter fluid
Naphthalene	Moth balls, toilet fresheners
Paradichlorobenzene	Moth balls, toilet fresheners
Toluene	Spray paint, adhesives, paint remover
Xylene	Spray paint, adhesives
Fuels	
Butane	Cigarette lighter refill, propellant
Gasoline (petrol)	Portable containers
Kerosene	Lighter fluid, lamp oil
Naphtha	Lighter fluid, propellant
Propane	Portable heating appliances
Halogenated hydrocarbons	
1,1-Difluoroethane (R-152a)	Compressed gas computer duster, propellant
Tetrachloroethane (R-130 and R-130a)	Refrigerant
Tetrafluoroethane (R-134a)	Refrigerant, computer duster
Tetrachloromethane (CCl_4)	Solvent, refrigerant
Trichloromethane	Adhesives, degreaser, spot remover
Trichloroethane	Adhesives, degreaser, spot remover, typewriter or ink correction fluid
Tetrachloroethylene ("PERC")	Dry cleaning solvent
Trichloroethylene	Degreaser
Anesthetics	
Chloroform	Laboratory anesthetic
Ether	Laboratory anesthetic
Nitrous oxide	Dental anesthetic, whipped cream dispensers
Nitrites	
Amyl nitrite	Poppers
Butyl nitrite	Video head cleaner, poppers

Source: After Ford et al., 2014.

These substances are particularly favored by children and adolescents

Statistics from the 2016 National Survey on Drug Use and Health indicate that more than 600,000 Americans 12 years of age or older were current users of inhalants at the time of the survey (Substance Abuse and Mental Health Services Administration, 2017). Compared with other categories of abused drugs covered in previous chapters, inhalant abuse is much less common. What is troubling, however, is that inhalants are most commonly used by children and adolescents, with first use typically occurring between the ages of 12 and 16 (Nonnemaker et al., 2011). Indeed, it is not uncommon for inhalants to be the first substances tried by children, used even earlier than alcohol, tobacco, or marijuana. Solvents and aerosols in particular can be obtained legally and inexpensively. In fact, there are almost certainly plenty of them at home already in the kitchen, basement, or garage, and it may be difficult for parents and teachers to detect the use of inhalants if the child is careful. This is not to say that adults don't use inhalants, because obviously some do. Nevertheless, this class of substances is unusual in its special attractiveness to young people.

Inhalant abuse by young people is a global problem. Indeed, because these substances are so easy to obtain and relatively inexpensive, they are widely used by "street children" in various countries throughout the world (Embleton et al., 2013). Roy Gigengack, a Dutch anthropologist and ethnographer, studied the cultural practices of inhalant use by street youth in Mexico City and in Delhi, India (Gigengack, 2014a; 2014b). Inhalation of glues, typewriter correction fluids, and/or turpentine was quite common and was considered to be a socially acceptable practice by street youth in both cities. Gigengack noted in his first paper, "Throughout I argue that Mexico City's young street people acquire *gusto*, that is, an appetite for inhalants. These young people have learned to recognize, deal with, and like the psychoactive effects induced by solvents and glues. They call the inhalants *su vicio*, and this 'vice' of theirs means that inhalants are always present in their lives" (Gigengack, 2014a, pp. 61–62). Unfortunately,

significant harm can be produced by chronic inhalant use, particularly when the brain and body are still developing. This topic is discussed in a later section.

Behavioral and Neural Effects

The sections below will discuss the subjective effects of inhalants, their mechanism of action, and their propensity for abuse and dependence.

Many inhalant effects are similar to alcohol intoxication

The acute effects of volatile and gaseous inhalants are often compared to those seen with alcohol intoxication. The user initially experiences euphoria, stimulation, and disinhibition, which are followed by drowsiness and lightheadedness (Duncan and Lawrence, 2013). Heavier exposure causes stronger depressant effects, characterized by slurred speech, poor coordination, ataxia, and lethargy. Sensory distortions, even hallucinations, may occur. Indeed, a study of Mexican teenagers conducted several years ago found that some users took inhalants specifically for the resulting illusions and hallucinations (Cruz and Domínguez, 2011). Very high doses can lead to anesthesia, loss of consciousness, and coma.

The pattern of inhalant effects varies not only with dose but also with frequency of use. Garland and Howard (2010) found that low-frequency inhalant users mainly reported positive effects of use (e.g., euphoria), whereas high-frequency users reported a mixture of positive and aversive effects (e.g., depressed mood, irritability, or suicidal thoughts). Some inhalant users experience delusional ideas, including the delusion that one can fly. In an early study by Evans and Raistrick (1987), users who thought they could fly actually jumped out of windows or trees, leading to at least one broken bone and various minor injuries, but fortunately no fatalities.

Chronic inhalant use can lead to tolerance and dependence

Although some individuals who try inhalants discontinue their use relatively quickly, others escalate their usage and subsequently develop tolerance and dependence on these substances (Perron et al., 2009a; Dhawan et al., 2015). Similar to users of other abused substances, tolerant inhalant users need to take higher doses to obtain the expected euphoric effect. There is also evidence for an inhalant withdrawal syndrome, with symptoms such as nausea, fatigue, irritability, anxiety, sleep disturbances, and intense craving (Perron et al., 2009b; 2011). Not surprisingly, withdrawal symptoms are most commonly experienced by users who meet the criteria for inhalant dependence.

Very little information has been published on the availability and efficacy of specific treatment programs for inhalant abuse and dependence. Inhalant users suffering from symptoms of acute intoxication are monitored closely and given supportive care until the symptoms subside. For longer-term treatment of individuals seeking to recover from chronic inhalant use, treatment providers typically employ standard approaches used with other abused substances, such as 12-step programs, cognitive behavioral therapy, motivational enhancement, and family therapy (Nguyen et al., 2016). Remarkably, an exhaustive review conducted several years ago found *no* published randomized controlled trials of inhalant treatment outcomes (Konghom et al., 2010). This is clearly an area in need of study (Howard and Garland, 2013).

Rewarding and reinforcing effects have been demonstrated in animals

There are relatively few studies of inhalant reward and reinforcement using animal models, partly because of the difficulty of controlling airborne delivery of these substances by investigators who wish to model the typical route of human exposure. Nonetheless, procedures have been developed that demonstrate the ability of toluene vapors to support place conditioning in rats and mice (Funada et al., 2002; Lee et al., 2006). In the study by Funada and colleagues, place conditioning was conducted using an airtight inhalation shuttle box in which the two compartments of the apparatus differed in the sensory cues they presented to the animals. Mice were given 10 conditioning sessions over 5 days, one session each day in the toluene-containing compartment, and an additional session in the compartment that contained only air. As shown in **FIGURE 16.1**, exposure to 700 ppm or more of toluene led to a significant preference for the toluene-associated side of the apparatus. Reinforcing and rewarding effects of toluene have additionally been demonstrated by means of IV self-administration and by the compound's ability to enhance intracranial self-stimulation (Cruz et al., 2014).

The neural mechanisms underlying inhalant reward and reinforcement are likewise not well understood. In previous chapters, we saw that dopamine (DA) plays a role in the reinforcing properties of many abused drugs. Both in vivo and in vitro studies have shown that toluene can stimulate the firing of a subpopulation of dopaminergic neurons in the ventral tegmental area (VTA) (Woodward and Beckley, 2014). This effect may result from an interaction of the compound with ion channels in the membrane of the VTA neurons. In accordance with its electrophysiological effects, toluene also increases extracellular DA levels in forebrain dopaminergic projection areas such as the dorsal striatum, nucleus accumbens, and prefrontal cortex (Woodward and Beckley, 2014). Although such findings raise the possibility that DA is involved in inhalant reward and

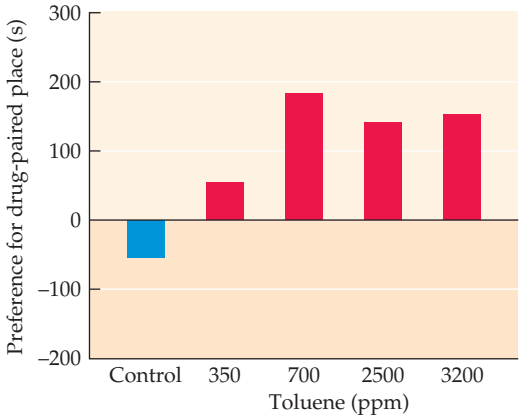


FIGURE 16.1 Rewarding effects of toluene in mice Mice were given two training sessions per day for 5 days. Each session lasted 20 minutes and involved pairing one compartment of an airtight chamber with toluene vapor and pairing the other compartment, which differed from the first compartment in its sensory characteristics, with air. Toluene concentrations ranged from 350 to 3200 parts per million (ppm) as shown. On the test day, no toluene was administered and the mice were allowed to move freely between the two compartments. Toluene concentrations of at least 700 ppm resulted in a significant conditioned place preference for the toluene-paired side, as indicated by the amount of time spent on that side. Control mice not given any toluene showed no significant preference for either side. (After Funada et al., 2002.)

reinforcement, it will be important to test this hypothesis by determining whether DA receptor antagonists can block the effects of toluene in self-administration or place conditioning paradigms.

Inhalants have complex effects on central nervous system (CNS) function and behavioral activity

Because of their more recent arrival on the scene, less is known about the mechanism of action of inhalants than of other abused substances. Furthermore, all inhalants may not work the same way, because of their chemical diversity. Nevertheless, our understanding of these substances is increasing, and several important findings have been reported.

As mentioned earlier, inhalants are rapidly absorbed from the lungs into the bloodstream, and they quickly enter the brain because of their high lipid solubility. Madina Gerasimov and colleagues at the Brookhaven National Laboratory used positron emission tomography (PET) to investigate the localization of [^{11}C]toluene that had been administered to baboons (Gerasimov et al., 2002). The radiolabeled toluene reached all parts of the brain, but its distribution was not uniform (**FIGURE 16.2**). Quantitative measurements of the striatum, frontal cortex, thalamus, cerebellum, and white matter showed particularly high uptake in the striatum, thalamus, and deep cerebellar nuclei. These findings indicate that localization of inhalants within the brain needs to be taken into account by researchers who are trying to understand how these substances affect brain function and behavior.

Rodent studies have examined the influence of toluene on locomotor activity as well as the compound's interoceptive properties, using the drug discrimination paradigm. The effects of toluene on locomotor activity are biphasic: concentrations up to 1000 ppm produce locomotor activation, whereas higher concentrations produce increasing degrees of sedation (Beckley and Woodward, 2013). This inverted U-shaped dose–response curve is seen with ethanol and many other sedative drugs, and it also fits well with the initial euphoric and disinhibitory effects of toluene intoxication in humans that are later followed by sedation as brain inhalant concentrations continue to increase. Drug discrimination studies have shown that high concentrations of toluene and related volatile solvents fully substitute for ethanol and for the barbiturate sedative pentobarbital. These findings indicate that at least some inhalants produce interoceptive cues like those of other sedative–hypnotic drugs.

The CNS-depressant actions of high concentrations of inhalants can be explained, at least in part, by their

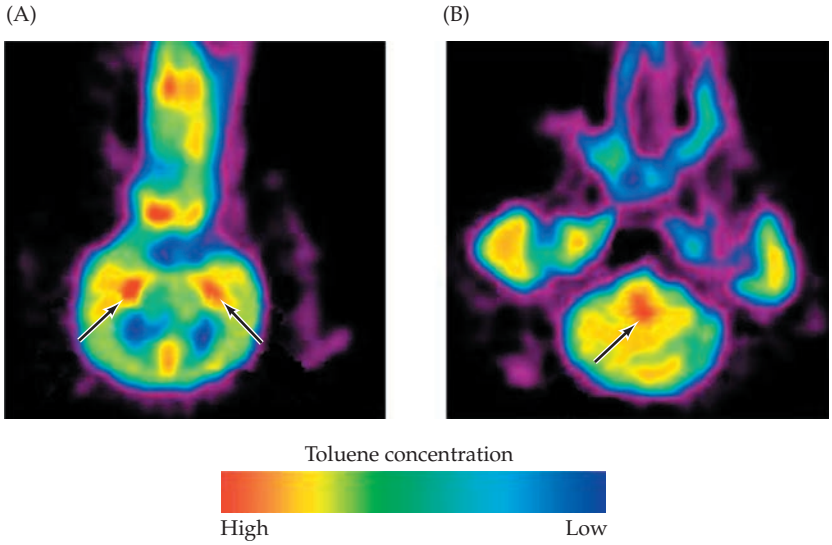


FIGURE 16.2 PET images of brain uptake and distribution of radiolabeled toluene in a baboon The animal was injected intravenously with [^{11}C]toluene and then was imaged 2 minutes later. The arrows show toluene being concentrated in the striatum (A) and in the deep cerebellar nuclei (B). (After Gerasimov et al., 2002; images courtesy of Madina Gerasimov.)

effects on various ionotropic receptors. For example, toluene acutely enhances the function of inhibitory $GABA_A$ and glycine receptors, whereas it inhibits the activity of excitatory *N*-methyl-D-aspartate (NMDA) glutamate and nicotinic cholinergic receptors (Beckley and Woodward, 2013). Several voltage-gated ion channels are also influenced by inhalants. A similar profile of ionotropic receptor and voltage-gated ion channel effects has been demonstrated for other depressant drugs, including ethanol and various anesthetic compounds. Combining these data with the behavioral findings discussed above, we may hypothesize that the sedating and discriminative properties of inhalants involve brain mechanisms that are shared with many other CNS depressant agents.

It is important to note that prolonged exposure to toluene and other inhalants affects ion channels in a very different manner than acute exposure. Indeed, prolonged exposure seems to evoke a counter-regulatory response in which the function and expression of $GABA_A$ receptors is reduced and the function and expression of NMDA receptors is enhanced (Beckley and Woodward, 2013). Such changes would tend to promote increased rather than decreased brain excitability, and they may also help account for the locomotor sensitization that occurs as a result of repeated toluene exposure.

Health risks have been associated with inhalant abuse

The health risks of inhalant use are significant. Even a single use can be fatal through several possible mechanisms such as cardiac arrhythmia (loss of normal heart rhythm). This outcome has been termed **sudden sniffing death syndrome** (Bowen, 2011; Phatak and Walterscheid, 2012). Although most users don't experience this syndrome, repeated inhalant use can damage not only the heart but also the liver, kidneys, respiratory tract, and bone marrow (Ford et al., 2014) (**FIGURE 16.3**). Bone marrow toxicity is responsible for the severe anemia observed in some chronic users of many inhalants. A different mechanism can cause anemia in people exposed repeatedly to high doses of nitrous oxide. In this case, anemia is a secondary consequence of vitamin B_{12} deficiency resulting from a direct inactivation of the vitamin by the gaseous inhalant (van Amsterdam et al., 2015b). Of additional concern is that over time, chronic inhalant use by juveniles or adolescents can impair growth. This effect was seen in a recent study of young Indigenous Australian men sniffing petrol (gasoline). The petrol users, who had begun their habit at an average age of about 13 years and who were tested at about 20 years of age, had significantly lower height and body weight than age-matched controls selected from the same communities in northern Australia (Crossin et al., 2017).

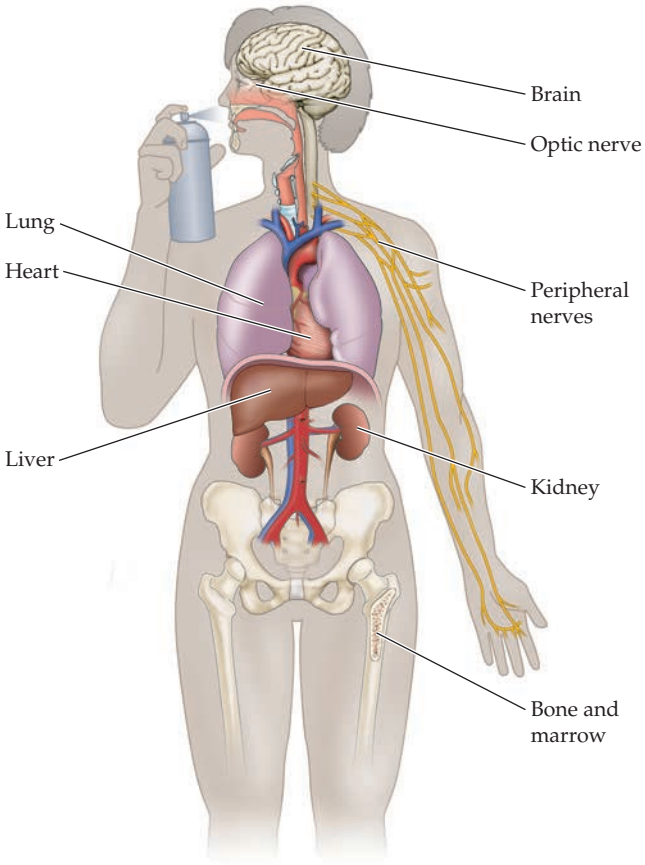


FIGURE 16.3 Organs and tissues damaged by repeated inhalant use (After Howard et al., 2011.)

Of special concern to neuropharmacologists is the brain's vulnerability to inhalant toxicity. The brain tends to concentrate inhalants because of its high lipid content (especially myelin-rich white matter) and the tendency of these substances to readily dissolve in lipids. Consequently, chronic inhalant abuse has been linked to myelin degeneration, cerebellar dysfunction, and damage to both the cranial and peripheral nerves (Takagi et al., 2011). Loss of myelin subsequently leads to pathological changes in axons and even loss of axons that had previously been covered by myelin sheaths (Licata and Renshaw, 2010). The brain damage caused by chronic abuse of inhalants, particularly toluene, has been studied extensively using magnetic resonance imaging (MRI), and the observed damage has been associated with cognitive and motivational impairment (**TABLE 16.2**) (Filley, 2013). Over a period of years, cognitive function may recover in chronic inhalant users if they maintain abstinence during this time (Dingwall and Cairney, 2011; Cairney et al., 2013); however, one reported exception is related to the sniffing of leaded gasoline, which can cause a "lead encephalopathy" responsible for persistent brain damage and neurological deficits (Cairney et al., 2013).

Finally, it is important to note that significant fetal exposure to inhalants has been documented in cases where the mother either was an inhalant abuser or was exposed to inhalants occupationally. Offspring of these women are often born prematurely and with reduced

TABLE 16.2 Neurological Symptoms and Associated MRI Findings in Chronic Toluene Abusers

Neurological symptoms	MRI findings
Apathy and inattention	Cerebral, cerebellar, and brainstem atrophy
Memory impairment	Lateral, third, and fourth ventricle enlargement
Visuospatial dysfunction	Loss of gray–white matter differentiation

Source: After Filley, 2013.

birth weight. In the most extreme cases, the infants exhibit physical malformations of the head and face that are similar to the abnormalities seen in newborn offspring of some alcoholic women (see Chapter 10). In parallel with the concept of a fetal alcohol syndrome, investigators in the inhalant field have coined the term **fetal solvent syndrome** to describe the craniofacial anomalies seen in the affected infants (Hannigan and Bowen, 2010; Bowen, 2011). It is well established that infants suffering from fetal alcohol syndrome show significant cognitive deficits, including severe mental retardation in the most extreme cases. Some research indicating developmental delays and cognitive impairment in inhalant-exposed offspring has been published, but long-term outcome studies are still lacking at the time of this writing.

Section Summary

- Inhalants are abused substances that are often obtained from everyday household items. These substances are volatile liquids or gases at room temperature; are used by sniffing, inhaling, or spraying the substance; and do not belong to another defined class of abused drugs.
- Inhalants comprise five categories of substances: volatile solvents, fuels, halogenated hydrocarbons, anesthetics, and nitrites. They are rapidly absorbed from the lungs and readily enter the brain because of their high lipid solubility. In contrast to most other abused substances, inhalant doses are calculated in parts per million (ppm).
- Inhalants are most commonly used by children and adolescents, and they are often the first substance of abuse tried by a child.
- Low doses of volatile and gaseous inhalants produce effects resembling those seen with alcohol intoxication. Users exposed to greater amounts of these substances show stronger depressant effects, including slurred speech, poor coordination, ataxia, sleepiness, and at very high doses, loss of consciousness and even coma. Sensory distortions, including hallucinations, may also occur.
- Repeated inhalant use can lead to tolerance, dependence, and an abstinence syndrome when drug use is stopped. Withdrawal symptoms include nausea, fatigue, irritability, anxiety, sleep disturbances, and craving.
- Inhalants are reinforcing to humans because of their euphoric effects, and laboratory animal studies have also established the rewarding and reinforcing properties of these substances by means of self-administration, place conditioning, and electrical stimulation of the brain. Studies using toluene as a representative inhalant have found that this compound activates ascending dopaminergic systems, causing increased firing of VTA dopaminergic neurons and enhanced DA release in forebrain projection areas. Activation of the mesolimbic dopaminergic system may play an important role in toluene's reinforcing and rewarding properties.
- Toluene exerts biphasic effects on locomotor activity in rodents, that is, locomotor activation at low concentrations and locomotor inhibition/sedation at higher concentrations. In drug discrimination studies, toluene and related volatile solvents fully substitute for ethanol and pentobarbital, indicating that these substances produce interoceptive cues like those of other sedative–hypnotic drugs.
- The depressant effects of acute exposure to high concentrations of inhalants such as toluene can generally be attributed to a combination of enhanced activity of inhibitory $GABA_A$ and glycine receptors and inhibited activity of excitatory NMDA and nicotinic receptors. In contrast, prolonged inhalant exposure induces a counter-regulatory response characterized by increased $GABA_A$ receptor expression and function and decreased function and expression of NMDA receptors. These changes favor greater brain excitability and may also contribute to locomotor sensitization produced by repeated toluene exposure.
- Inhalants present serious health risks including damage to the liver, kidneys, respiratory tract, and bone marrow. Severe anemia in chronic inhalant users can result from bone marrow toxicity or, in the case of nitrous oxide, as a secondary consequence of vitamin B_{12} deficiency. Myelin-rich white matter in the brain is particularly vulnerable to toxicity from repeated exposure to inhalants. Loss of myelin and subsequent damage to the previously myelin-sheathed axons is reflected by

abnormal MRI scans and can lead to significant cognitive impairment; however, some evidence exists for neurological recovery in most users following long-term abstinence.

- Inhalant use can additionally lead to a rare disorder called sudden sniffing death syndrome, which in some cases results from an inhalant-induced cardiac arrhythmia. Moreover, some offspring exposed to inhalants in utero reportedly suffer from fetal solvent syndrome, which involves craniofacial abnormalities and possibly also cognitive deficits. These effects resemble those seen in individuals diagnosed with fetal alcohol syndrome.

Gamma-Hydroxybutyrate

Background

The second part of this chapter concerns **γ-hydroxybutyrate** (**GHB**), a simple chemical with a complicated history. As can be seen in **FIGURE 16.4A**, GHB is closely related structurally to the important inhibitory neurotransmitter GABA. In fact, GHB is actually produced in the brain as a by-product of GABA metabolism (**FIGURE 16.4B**) and is thought to function as a novel neurotransmitter/neuromodulator (Kamal et al., 2016; Maitre et al., 2016). In the human brain, the highest levels of endogenous GBH are found in the striatum and the lowest levels are found in the cerebellum. A rare genetic disorder leads to overproduction of GHB and a characteristic cluster of symptoms that may be related to excess levels of this substance (see Web Box 16.1). GHB can be taken up into GABAergic vesicles by the vesicular inhibitory amino acid transporter (VIAAT; see Chapter 8), which has led to the idea that some GHB may be coreleased with GABA when the neuron fires and vesicular exocytosis occurs at the nerve terminal. GHB uptake into neurons has also been demonstrated, although the system responsible for this uptake is not yet fully characterized (Maitre et al., 2016).

The pharmacologic properties of exogenous GHB were first reported in 1960 by a French team headed by Henri Laborit, the same scientist who discovered the antipsychotic drug chlorpromazine. Laborit was looking for a GABA analog that would cross the blood–brain barrier more efficiently than GABA and thus might have therapeutic potential as a CNS depressant. Although early studies did confirm the ability of GHB to produce sedation and even anesthesia in laboratory animals and humans, it later fell into disfavor as an anesthetic and is currently used for other therapeutic purposes (see later in this chapter).

In the United States, little attention was paid to GHB until the 1980s, when it began to be marketed by health food stores as a nutritional supplement for bodybuilders. Despite a lack of supporting evidence, GHB was claimed by manufacturers to reduce body fat and enhance muscle mass through its ability to stimulate growth hormone secretion by the pituitary gland. As time went on, the Food and Drug Administration (FDA) began to receive a number of reports of GHB-related illness, and in 1990 the FDA declared the drug to be unsafe and banned over-the-counter sales.

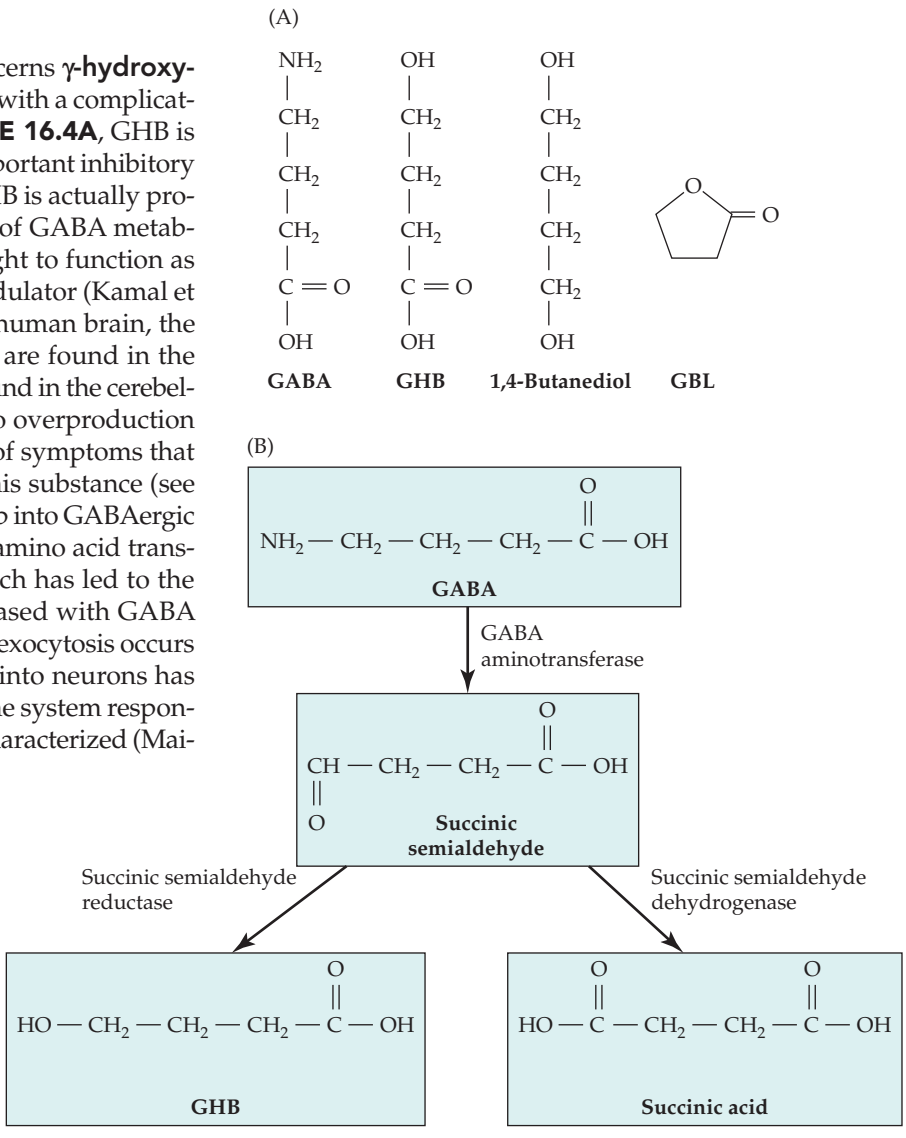


FIGURE 16.4 Relationship of GHB and its precursors to the neurotransmitter GABA (A) Chemical structures of GABA, GHB, 1,4-butanediol, and γ-butyrolactone (GBL). (B) Endogenous synthesis of GHB from GABA.

Despite the FDA ban, GHB use continued to grow, largely as the result of an upsurge in recreational use under various street names such as "liquid X," "liquid E," "Georgia home boy," "grievous bodily harm," "Heaven," "cherry meth," "organic quaalude," and "nature's quaalude." Some stores as well as internet sites began to sell GHB-containing formulations, and even now it's easy to find internet sellers who offer this product to customers. The GHB precursors γ-butyrolactone (GBL) and 1,4-butanediol (1,4-BD; see Figure 16.4A) are also available for purchase either for the purpose of synthesizing GHB at home or for direct consumption. Indeed, many users have begun to consume GBL directly instead of GHB because this precursor is rapidly converted to GHB within the body.

Recreational users typically report that they take GHB or its precursors for relaxation, euphoria, and/or sexual arousal (Bosch and Seifritz, 2016). Additionally, GHB is still thought by some users to help build muscle mass. In accordance with these real or perceived benefits, current evidence suggests that GHB is most likely to be consumed by three different subgroups of users: (1) attendees at nightclubs and dances or "raves," (2) gay men, and (3) bodybuilders (Brennan and Van Hout, 2014). Because of its availability and consumption at nightclubs and dances, GHB has sometimes been called a **club drug**.[2] Unfortunately, GHB has also been used as a "date rape" drug because of its intoxicating and heavily sedating effects at high doses (**Web Box 16.2**). Increasing concerns over the problems associated with illicit GHB use led to its designation as a Schedule I controlled substance in 2000, thus making possession of the drug illegal except for medicinal use with a prescription.

Behavioral and Neural Effects

The sections below will discuss the subjective and behavioral effects of GHB and its proposed mechanisms of action.

GHB produces behavioral sedation, intoxication, and learning deficits

Pure GHB is a solid powdery material; however, it is usually sold as a solution in water. GHB-containing solutions are clear, odorless, and almost tasteless. Such solutions are often packaged in small bottles similar to those used to package shampoo in hotel rooms. Typical recreational doses range from 1 to 3 g (approximately 14 to 42 mg/kg for a 70 kg adult), although some regular users may take as much as 4 or 5 g (about 57 to 71 mg/kg) at a time.

When a GHB-containing solution is drunk, the drug is rapidly absorbed from the gastrointestinal tract, enters the bloodstream, and crosses the blood–brain barrier without difficulty. The ensuing psychological and physiological effects are strongly dose related. Low doses of GHB produce an alcohol-like experience. Users report mild euphoria, relaxation, and social disinhibition beginning approximately 15 minutes following drug ingestion (Miotto et al., 2001; Abanades et al., 2006). Indeed, these are among the experiences sought after by typical recreational users of GHB, GBL, or 1,4-BD, although some users have also mentioned enhanced sexual arousal (Bosch and Seifritz, 2016). Higher doses of GHB can cause lethargy, ataxia, slurring of speech, dizziness, nausea, and vomiting. Memory impairment has also been reported following a high dose of GHB (Carter et al., 2006). Finally, overdosing on this compound is very dangerous, since respiration is depressed and the user may become unconscious or even comatose (Dyer, 1991; Brennan and Van Hout, 2014). Alternatively, the individual may exhibit signs of seizure activity. Overdosing can readily occur if the user takes multiple doses over a short period of time, if there is a greater than expected concentration of GHB in the bottle, or if the drug is taken in combination with alcohol or another sedative–hypnotic drug.

In laboratory animals, acute GHB administration causes sedation, reduced locomotor activity, and decreased anxiety in certain tests such as the elevated plus-maze. At higher doses, animals show signs of catalepsy. Some of these behavioral effects, particularly hypolocomotion and catalepsy, are thought to be due to GHB-induced inhibition of DA release. Other studies found impaired spatial learning in young rats repeatedly given moderate doses of GHB (Pedraza et al., 2009; Sircar et al., 2010) (**FIGURE 16.5**). Such impairment may be related to neurotoxic effects of the drug treatment on the hippocampus, including reduced NMDA receptor expression and neuronal cell loss (Pedraza et al., 2009; Sircar et al., 2011).

Electroencephalographic (EEG) recordings have revealed the presence of a paradoxical CNS excitation at high doses of GHB that some investigators have likened to absence (petit mal) seizures in humans and that may be related to the occasional reports of seizure activity in human overdose cases (Binienda et al., 2011). Absence seizures involve a temporary loss of consciousness and cessation of activity without the whole-body convulsions seen in a grand mal seizure. Researchers have investigated the possibility of developing an animal model of absence seizures based on GHB or GBL administration. Of the various species tested thus far, it appears that laboratory rats are the best choice for such a model (Venzi et al., 2015).

GHB and its precursors have reinforcing properties

We mentioned above that recreational users report a mild euphoria upon consumption of a low dose of

[2]Two other substances considered to be club drugs are 3,4-methylenedioxymethamphetamine (MDMA; see Chapter 6) and ketamine (see Chapter 15).

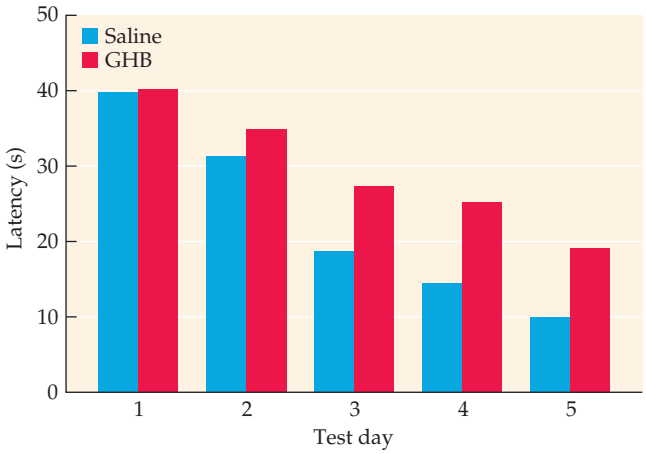


FIGURE 16.5 GHB impairs spatial navigation learning in adolescent female rats Female rats were trained in the Morris water maze for 5 days beginning at 30 days of age. The graph shows the latency to find the hidden platform by animals given either GHB (100 mg/kg IP) or saline vehicle 30 minutes before training each day. Beginning on day 3, the drug-treated group took significantly longer than the controls to find the platform. This impairment was not observed in adult rats treated in the same way, indicating greater vulnerability to GHB-induced learning impairment during adolescence. (After Sircar et al., 2010.)

GHB. This phenomenon was studied experimentally by Johnson and Griffiths (2013), who assessed the subjective effects of a range of GHB doses, compared with the effects elicited by ethanol. The participants consisted of a small group of regular users of sedative–hypnotic drugs, including ethanol but not necessarily GHB. Using several different questionnaires, the investigators showed dose-dependent positive effects of GHB that were similar to the effects of ethanol.

Consistent with these findings in humans, GHB also exerts rewarding and reinforcing effects in laboratory animals when tested at appropriate doses and under the right experimental conditions. Such effects were first shown in studies with rats and mice using standard place conditioning and self-administration methods (Nicholson and Balster, 2001; Itzhak and Ali, 2002). A subsequent study additionally showed that GHB can induce a conditioned place preference when microinjected directly into the VTA (Watson et al., 2010). More recent research has further demonstrated IV self-administration of GHB, GBL, and 1,4-BD by baboons (Goodwin et al., 2011; 2013). However, the experiment required that the subjects initially be trained to lever press for infusions of cocaine, after which GHB or one of its precursors was substituted for cocaine as the infusion drug. Not all of the baboons responded reliably for GHB, and even in those baboons for which GHB served as an effective reinforcer, daily numbers of lever presses for GHB were often lower than the numbers of presses elicited by cocaine (**FIGURE 16.6**). Moreover, for some of the baboons switched to one of the substitute drugs, lever pressing dropped to the low response rates elicited by vehicle infusions (data not shown). From these findings we can conclude that while GHB, GBL, and 1,4-BD show some ability to serve as reinforcers in animal models, the effects seen with this class of drugs are not nearly as robust as those seen with many other major drugs of abuse such as cocaine, opioids, and ethanol.

Effects of GHB are mediated by multiple mechanisms

Historically there has been much confusion regarding the mechanism of action of GHB. Fortunately, recent findings have begun to clarify the situation, although some uncertainties remain. It's important that we first differentiate between potential sites of action of endogenous versus exogenous GHB. If GHB does function as a signaling agent in the brain, the receptors that mediate such signaling must have a high affinity for GHB because of the relatively low endogenous levels of this compound. Radioligand binding studies have, indeed, revealed the existence of sites in the rat brain that bind both GHB and the selective GHB antagonist NCS-382 with high affinity (reviewed in Kamal et al., 2016; Maitre et al., 2016); however, the nature of these sites continues to be controversial. A proposed GHB-specific receptor was initially cloned from rat brain; this was followed several years later by the isolation of two clones from human brain tissue that were proposed to code for GHB receptor isoforms. These putative GHB receptors have the functional properties of metabotropic receptors and are expressed at highest levels in the hippocampus, cerebral cortex, and nucleus accumbens. Subsequent studies showed that GHB can act as a high-affinity partial agonist at $GABA_A$ receptor subtypes containing α4, β (particularly β1), and δ subunits. It is interesting to note that $GABA_A$ receptors containing α4, β, and δ subunits are located at extrasynaptic sites and exert a tonic inhibition of the neurons in which they're expressed (see Chapter 8). Thus, it is possible that the inhibitory effect mediated by these extrasynaptic $GABA_A$ receptors is produced not only by GABA but also by coreleased GHB.

Exogenous administration of GHB, as occurs in human recreational or therapeutic use and in experimental animal studies, considerably elevates brain GHB concentrations. This increase permits an additional mechanism to come into play, namely, the activation of $GABA_B$ receptors (Kamal et al., 2016; Maitre et al., 2016). GHB is a weak agonist at $GABA_B$ receptors, and therefore at high tissue concentrations it could activate these receptors directly. The importance of $GABA_B$ receptor activation in the effects of exogenously

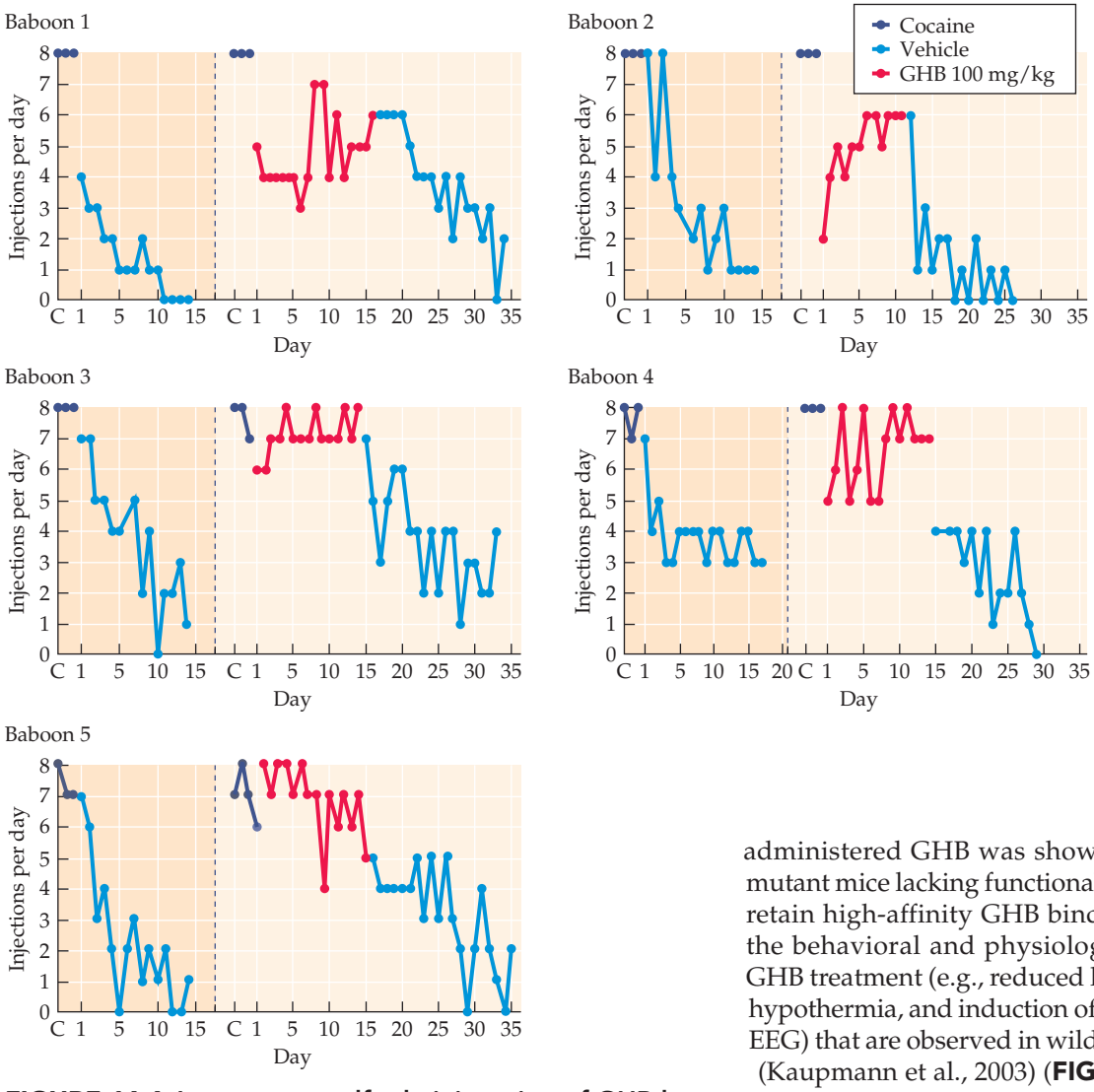


FIGURE 16.6 Intravenous self-administration of GHB by baboons Nine adult male baboons were implanted with intravenous catheters and subsequently trained to respond on a pull-and-release lever for IV injections of cocaine (0.32 mg/kg). Researchers gradually increased the task demands until the subjects were reliably responding on a fixed-ratio-160 (FR-160) schedule of reinforcement (i.e., 160 responses were required to obtain each cocaine infusion). Five of the nine subjects trained to self-administer cocaine exhibited strong evidence for GHB reinforcement when given the opportunity to inject the latter drug, and the panels of the figure depict the individual response patterns measured in injections per day for these animals. The first set of data points in each panel (purple circles) shows that animal's injection rate for the last 3 days of cocaine (C) self-administration, which served as a baseline response measure. The subsequent blue circles show the expected falloff of responding when the drug vehicle was substituted for cocaine for 15 days (extinction). Cocaine availability was then restored for 3 to 4 days (data points immediately after the vertical dashed line) to induce a restoration of lever responding. Finally, GHB (100 mg/kg; red circles) was substituted for cocaine for up to about 15 days, followed by vehicle again. The data show that GHB supported a response rate greater than that for vehicle (demonstrating a reinforcing effect) but generally lower than that for cocaine. (After Goodwin et al., 2011.)

administered GHB was shown by the fact that mutant mice lacking functional $GABA_B$ receptors retain high-affinity GHB binding sites but lack the behavioral and physiological responses to GHB treatment (e.g., reduced locomotor activity, hypothermia, and induction of delta waves in the EEG) that are observed in wild-type control mice (Kaupmann et al., 2003) (**FIGURE 16.7**). Moreover, most of the effects of GHB administration are blocked by $GABA_B$ antagonists but not by the GHB receptor antagonist NCS-382 (Carter et al., 2009a; Castelli et al., 2004). However, experimental animals can be trained to discriminate GHB from the $GABA_B$ agonist baclofen, which is consistent with the notion that non-$GABA_B$ receptors like those discussed above may contribute to some of the effects of GHB.

To summarize, GHB effects are mediated by three different kinds of receptors: GHB-specific receptors, a type of extrasynaptic $GABA_A$ receptor, and $GABA_B$ receptors. The first two kinds of receptors are activated at relatively low GHB concentrations, whereas only high concentrations of GHB are able to activate $GABA_B$ receptors. Keep in mind, however, that GHB can also be metabolized to GABA. The GABA produced from this metabolic pathway is capable of activating any GABA receptors that are accessible to the transmitter.

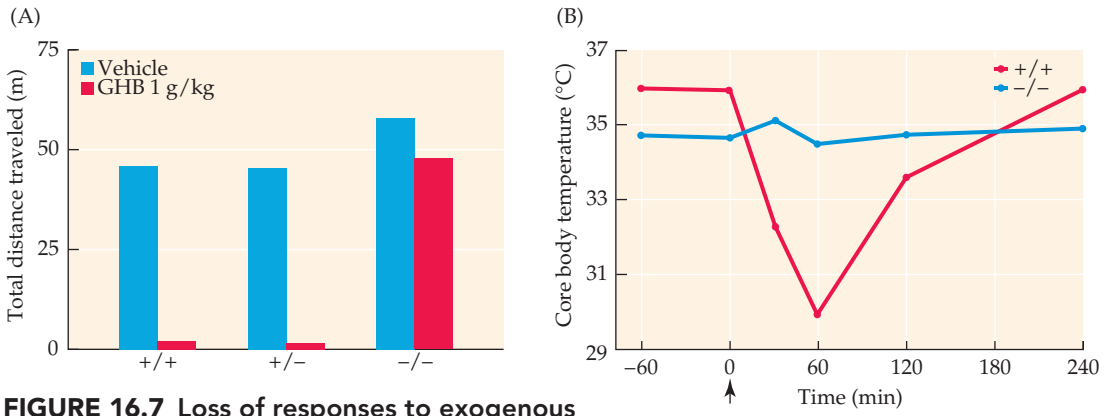


FIGURE 16.7 Loss of responses to exogenous GHB in mutant mice lacking functional $GABA_B$ receptors GHB (1 g/kg) was administered orally to wild-type mice (+/+), to mice that were homozygous for a null mutation of the $GABA_{B1}$ receptor subunit that is necessary for the formation of functional $GABA_B$ receptors (–/–) (see Chapter 8), and in one experiment, to heterozygous $GABA_{B1}$ knockouts (+/–). The wild-type mice but not the homozygous mutants showed (A) a strong sedative effect of GHB as indicated by distance traveled in a locomotor activity cage, and (B) a transient reduction in core body temperature after dosing (shown by the arrow at time 0). (After Kaupmann et al., 2003.)

Medical and Recreational Uses of GHB

Although GHB is discussed in the popular press primarily with regard to its recreational use and abuse, this compound also has recognized medical uses.

GHB is used therapeutically for the treatment of narcolepsy and alcoholism

Early clinical studies of GHB in the 1960s and 1970s showed that bedtime administration of this compound promoted normal sleep. These findings led to the first trials of GHB assessing its potential usefulness for treating narcolepsy, a sleep disorder previously discussed in Box 3.1. GHB was administered in the form of a sodium salt under the alternate name **sodium oxybate** (trade name **Xyrem**). The clinical trials showed that over time, sodium oxybate not only improved nighttime sleep, but also reduced daytime sleepiness and attacks of cataplexy. Unfortunately, the rise in recreational GHB use and abuse, evidence implicating GHB in some cases of sexual assault (see **Web Box 16.2**), and the subsequent scheduling of GHB as a controlled substance in 1990 all contributed to a delay in the clinical development and FDA approval of sodium oxybate for the treatment of narcolepsy. Despite these hurdles, FDA approval was granted in 2002, and the introduction of this pharmacotherapy has led to significant symptom reduction in patients with narcolepsy (Robinson and Keating, 2007; Boscolo-Berto et al., 2012).

Given the abuse potential of GHB, it was important to address the possible diversion of sodium oxybate for recreational instead of therapeutic use. Consequently, when the medicinal formulation was introduced to the U.S. market, it was accompanied by a unique national risk management program called the Xyrem Success Program, designed to minimize diversion and misuse of Xyrem. This program involves such factors as single-source manufacture (Jazz Pharmaceuticals), distribution through a central pharmacy, education of physicians and patients on appropriate use of the medication, and patient and physician registries. The program, which appears to have been successful in achieving its aims (Carter et al., 2009b), continues at the present time under the new name Xyrem REMS Program. It is interesting to compare sodium oxybate with modafinil, a stimulant that is the only other drug currently approved for treating narcolepsy (see Chapter 12). Considering the differing mechanisms of action of these two compounds, one wouldn't have predicted that both would have efficacy in reducing symptomatology of the same clinical disorder.

Sodium oxybate is approved for other medical applications in several European countries. In France and Germany, sodium oxybate is used as an IV anesthetic, which is understandable in light of the drug's sedating properties at high doses. This compound has also been administered for many years in Italy and Austria as a pharmacotherapeutic agent for treating alcohol use disorder (Keating, 2014; Caputo et al., 2016). Clinical studies have indicated that the drug is particularly useful in dealing with alcohol withdrawal symptoms and in helping to prevent relapse by reducing craving. Some of these studies suggest that sodium oxybate compares favorably in therapeutic efficacy with the pharmacotherapies currently licensed in the United States, namely, disulfiram, naltrexone, and acamprosate (see Chapter 10 for more information on these medications). On the other hand, some investigators have argued that there is greater risk of misuse of sodium oxybate by patients with alcohol use disorder than by individuals with narcolepsy (Sewell and Petrakis, 2011). Thus, if this compound is eventually accepted for the treatment

of alcoholism in this country, it will probably be used under carefully controlled conditions (e.g., in inpatient settings).

GHB has significant abuse potential when used recreationally

Miotto and coworkers (2001) surveyed 42 regular GHB users in the Los Angeles area about their experiences while under the influence of GHB and the effects that occurred after drug use. As shown in **TABLE 16.3**, GHB users reported the expected feelings of euphoria and well-being, heightened sexuality and sensory perception, and feelings of relaxation and disinhibition during the period of drug intoxication. Loss of consciousness, which is a sign of GHB overdose, sometimes also occurred. In contrast to the (mostly) subjectively positive aspects of GHB intoxication, the period of drug "comedown" seemed to be rather unpleasant. Users commonly reported sluggishness, mental confusion and amnesia, weakness, and increased arousal, as indicated by feelings of anxiety and agitation and difficulty sleeping during the period following GHB consumption.

TABLE 16.3 Commonly Reported Experiences during and after Recreational GHB Use

During GHB use	After GHB use
Euphoria	Sluggishness and exhaustion
Enhanced sexual experience	Amnesia
Increased feelings of well-being	Confusion
Feelings of relaxation and tranquility	Anxiety
Heightened sensory perception	Insomnia
Disinhibition	Weakness
Loss of consciousness	Agitation

Source: From Miotto et al., 2001.

Numerous risks have been associated with recreational GHB use. The intoxicated state produced by this compound is similar to alcohol intoxication (i.e., drunkenness), and indeed GHB has been implicated in some instances of "driving under the influence" (Andresen et al., 2011). Excessive consumption (overdose) of GHB can cause an acute toxic reaction that includes unconsciousness or even coma, respiratory depression, bradycardia (slowed heart rate), and hypotension (Drasbek et al., 2006; Wood et al., 2011). Concurrent use of alcohol results in an additive response that is extremely dangerous. Most people who experience a GHB overdose recover without obvious adverse consequences (Munir et al., 2008); however, fatalities have been reported, mostly as the result of cardiorespiratory arrest (Zvosec et al., 2011).

Repeated GHB exposure can lead to the development of tolerance. For example, daily treatment of mice with 200 mg/kg GHB produced tolerance to the activity-suppressing effects of the drug (Itzhak and Ali, 2002) (**FIGURE 16.8**). Chronic GHB use by humans can elicit not only tolerance but also a syndrome of dependence and withdrawal when abstinence is attempted. Indeed, some dependent users engage in binges characterized by GHB consumption every 2 to 4 hours around the clock. Symptoms of GHB withdrawal include anxiety, agitation, tremor, tachycardia (increased heart rate), insomnia, confusion, and, in extreme cases, hallucinations and a state of delirium (Brunt et al., 2014; Busardò and Jones, 2015). Withdrawal starts to occur within a few hours of the last dose and, in severe cases, can last up to a few weeks. Clinicians typically treat the symptoms of GHB withdrawal with high doses of a benzodiazepine such as diazepam, but such treatment is not always effective. Consequently, the Netherlands instituted a different protocol for detoxifying GHB-dependent patients by administering Xyrem at a high starting dose and then tapering the medication until the individual is symptom-free. This treatment program has proved effective in the short-term management of GHB dependence, but unfortunately the majority of treated patients relapsed to either occasional or frequent GHB use within 3 months (Brunt et al., 2014).

At the present time, little is known about the long-term effects of heavy GHB use. Nevertheless, concerns have been raised about potential neurotoxic effects in patients who have overdosed and gone into a drug-induced coma (van Amsterdam et al., 2012). Additional research is needed to determine whether such concerns are warranted.

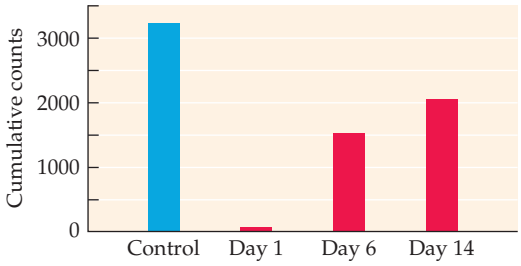


FIGURE 16.8 Tolerance to the locomotor suppressant effects of GHB in mice Mice were given 200 mg/kg GHB IP for 14 days. Locomotor activity in a photobeam apparatus was measured for 2 hours following drug administration on days 1, 6, and 14 of treatment. The figure illustrates the number of beam breaks recorded from 30 to 60 minutes post-treatment on each of these days. Compared with control mice administered saline, the GHB-treated animals showed a nearly complete cessation of locomotor activity on day 1, whereas a partial return of activity was observed on subsequent test days. (After Itzhak and Ali, 2002.)

Section Summary

- GHB is an analog of the inhibitory neurotransmitter GABA. It is synthesized in the brain in small amounts and is thought to function as a neurotransmitter/neuromodulator that may be coreleased with GABA at some synapses.
- GHB was developed pharmacologically as a CNS depressant. It was later marketed in the United States as a bodybuilding supplement and for recreational use.
- Over-the-counter sales of GHB were banned in 1990, and the drug was designated as a Schedule I controlled substance in 2000. Nevertheless, GHB and its precursors GBL and 1,4-BD continue to be available for sale over the internet. GHB is most likely to be consumed by three different subgroups of users: attendees at dances and raves, gay men, and bodybuilders. The compound has also occasionally been used as a date rape drug at clubs.
- GHB is usually taken orally in the form of an aqueous solution. Low doses produce alcohol-like effects including mild euphoria, relaxation, and social disinhibition. These effects, along with enhancement of sexual arousal, are the experiences sought after by typical recreational users of GHB, GBL, or 1,4-BD. Higher doses of these compounds are associated with stronger sedating effects, as well as dizziness, nausea, vomiting, and memory impairment. Severe overdosing with GHB causes severe respiratory depression, unconsciousness, and even coma.
- Animals treated with GHB exhibit sedation, reduced locomotor activity, decreased anxiety behavior, and catalepsy at high doses. Impaired spatial learning has also been reported, which may be related to neurotoxic effects on the hippocampus.
- High doses of GHB can lead to EEG excitation resembling absence (petit mal) seizures in humans. A rat model of absence seizures has been developed based on administration of the appropriate doses of GHB or GBL.
- In regular users of sedative–hypnotic drugs, GHB ingestion produced positive subjective effects similar to those produced by ethanol. In laboratory animals, GHB and its precursors exert rewarding and reinforcing effects when tested under the appropriate dose and experimental conditions. However, these effects are not as robust as those seen with many other major drugs of abuse.
- The functional effects of GHB are mediated by multiple mechanisms. The low levels of endogenous GHB are believed to act on putative high-affinity GBH-specific receptors and on extrasynaptic $GABA_A$ receptors containing $\alpha 4$, β, and δ subunits. High levels of exogenously administered GHB are additionally capable of activating $GABA_B$ receptors, which mediate many of the behavioral and physiological effects observed in GHB-treated animals.
- The sodium salt of GHB (also called sodium oxybate; trade name Xyrem) is approved in the United States for the treatment of the sleep disorder narcolepsy. This treatment improves nighttime sleep and reduces the incidence of daytime sleepiness and attacks of catalepsy. In several European countries, sodium oxybate is approved for use as an IV anesthetic or for the treatment of alcoholism. In the latter application, the drug has been shown to ameliorate alcohol withdrawal symptoms and to reduce craving during abstinence from alcohol.
- Recreational GHB users reportedly experience euphoria, heightened sexuality, and feelings of relaxation during drug intoxication. In several respects, GHB intoxication resembles the state of alcohol intoxication, including impairment of psychomotor function and even loss of consciousness with overdose. The "comedown" following GHB use is characterized by sluggishness, mental confusion and amnesia, weakness, and increased arousal.
- Repeated GHB use can lead to tolerance, dependence, and withdrawal. Consumption patterns may escalate to dosing every 2 to 4 hours around the clock. In heavy GHB users, withdrawal symptoms can start within a few hours after the last dose and can persist for up to a few weeks. Typical withdrawal symptoms include insomnia, anxiety, and tremors, although use of extremely high doses can apparently cause a psychotic reaction involving hallucinations, delirium, and extreme agitation.
- Patients diagnosed with GHB dependence are typically treated with high doses of a benzodiazepine such as diazepam to help them get through the withdrawal period. However, an alternative approach developed in the Netherlands is to administer Xyrem starting with a high dose and then gradually tapering the medication until the patient is symptom free. Unfortunately, the majority of dependent users were found to relapse within 3 months after Xyrem treatment.

Anabolic–Androgenic Steroids

In recent years, we have been inundated with numerous reports of formerly revered sports heroes being found guilty of taking various performance-enhancing substances (sometimes also called **doping agents**). Use of specific foods or drugs to enhance performance dates back at least to the ancient Greek and Roman athletes, who consumed a variety of natural substances

thought to provide increased energy, strength, and endurance (Conti, 2010). Although many doping agents are currently available, especially to a world-class athlete, one particularly important category of performance enhancers is a group of compounds called **anabolic–androgenic steroids** (**AAS**). These are defined as steroid hormones that increase muscle mass (the "anabolic" part) and also have masculinizing, or testosterone-like, properties (the "androgenic" part).[3] On the street, these substances are usually just called anabolic steroids, but there are no members of the group that aren't also androgenic.

Why are AAS being discussed in a chapter on substances of abuse? There is significant evidence that these hormones are abused by some users, and some researchers have theorized that AAS can produce an addiction-like dependence. Before we discuss these ideas, however, we will present basic information on these substances and how they entered the realm of bodybuilding and athletic competition.

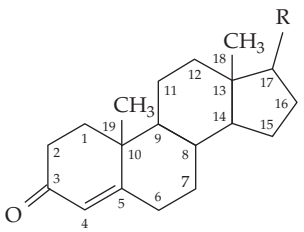
[3]One of the first recorded uses of anabolic steroids was that of the sixth-century BCE Olympian wrestler Milo of Croton, who ate a diet that included bull testicles in order to maintain his award-winning performance (Conti, 2010).

Background and History

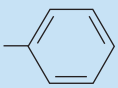
Compared with other substances of abuse, AAS have a unique structure and mechanism of action. However, they share the ability to cause dependence when used regularly in large amounts.

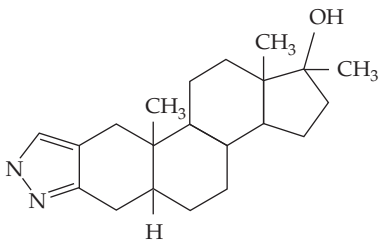
Anabolic–androgenic steroids are structurally related to testosterone

The chemical and trade names of some common AAS are presented in **TABLE 16.4**. These compounds are taken by several different routes. Steroids being used for muscle building and performance enhancement are most often taken orally or by intramuscular injection. AAS prescribed for medicinal purposes are sometimes formulated for buccal or transdermal absorption from a topical gel or skin patch. Intramuscularly administered steroids are formulated for depot injection and maintain their potency for periods ranging from several days to 3 weeks, depending on the steroid. As shown in **FIGURE 16.9**, these compounds are all structurally related to testosterone, the principal androgen synthesized by the testes. However, because it is the anabolic rather than androgenic effects that are desired by most users, the

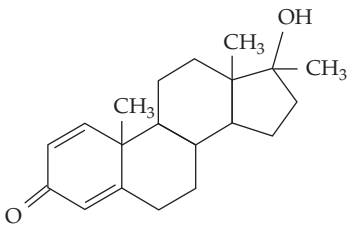


Core structure of testosterone-related steroids

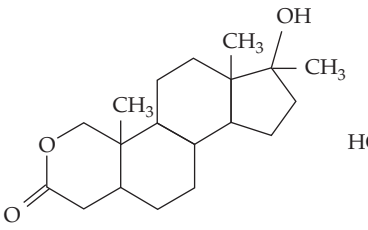
Compound	R
Testosterone	— OH
Testosterone enanthate	— O — $CO(CH_2)_5CH_3$
Testosterone undecanoate	— O — $CO(CH_2)_9CH_3$
Testosterone cypionate	— O — $COCH_2CH_2$
Nandrolone decanoate	— O — $CO(CH_2)_8CH_3$ (no methyl group at position 19)
Nandrolone phenproprionate	— O — $CO(CH_2)_2$  (no methyl group at position 19)



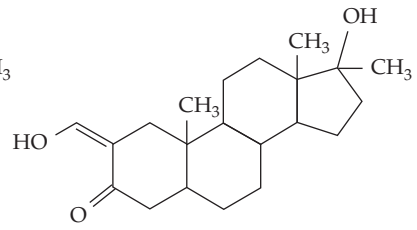
Stanozolol



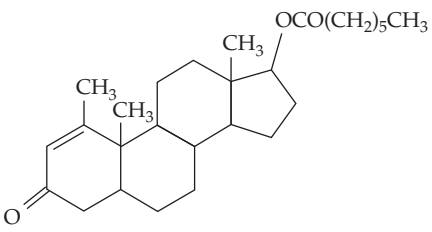
Methandrostenolone



Oxandrolone



Oxymetholone



Methenolone enanthate

FIGURE 16.9 Chemical structures of some commonly abused anabolic–androgenic steroids

chemical modifications that differentiate various synthetic steroids from testosterone are aimed at selectively enhancing their anabolic potency. Because the oral steroids are potentially vulnerable to first-pass metabolism in the liver, these compounds are chemically designed to minimize this problem and thus retain adequate bioavailability.

TABLE **16.4** Some Common Anabolic–Androgenic Steroids

Generic name	Trade name	Route of administration
Methandrostenolone	Dianabol	Oral
Testosterone undecanoate	Andriol	Oral
Oxandrolone	Oxandrin	Oral
Oxymetholone	Anadrol	Oral
Stanozolol	Winstrol	Oral or injection
Tetrahydrogestrinone	"The Clear" (street name)	Oral or injection
Testosterone cypionate	Depot-Testosterone	Injection
Testosterone enanthate	Primoteston	Injection
Testerone propionate	Testoprop	Injection
Nandrolone phenylpropionate	Durabolin	Injection
Nandrolone decanoate	Deca-Durabolin	Injection
Methenolone acetate	Primobolan	Injection
Methenolone enanthate	Primobolan Depot	Injection
Trenbolone enanthate	None	Injection
Testosterone	Striant	Buccal
Testosterone	AndroGel	Topical gel
Testosterone	"The Cream" (street name)	Topical gel
Testosterone	Androderm	Transdermal (skin patch)

Anabolic–androgenic steroids were developed to help build muscle mass and enhance athletic performance

American athletes knew little about these compounds before the 1954 World Weightlifting Championships held in Vienna, Austria. Until 1953, American weight lifters had routinely beaten teams from what was then the Soviet Union (USSR), but the Soviets outscored the Americans in that year and again in 1954. During the Vienna competition, the U.S. and Soviet team physicians reportedly went out in the evening for entertainment, and after a few drinks, the physician for the Soviet Union squad confided that some of his men were using testosterone. Dr. John Ziegler, who was the American physician, went back home and began to experiment with testosterone, but he didn't like the strong androgenic side effects. Ziegler expressed to the giant pharmaceutical company Ciba the need for a more anabolic, less androgenic compound. Within a few years, Ciba introduced Dianabol, an orally active compound with enhanced anabolic properties. When Dianabol was administered to elite weight lifters at the famous York Barbell Club in Pennsylvania, the drug produced spectacular results. Once the news got out, many similar compounds quickly followed, and strength athletes began to view steroids as the only way to reach the highest level of achievement. According to a 1969 article in the magazine *Track and Field News* entitled "Steroids: Breakfast of Champions," these substances were readily available to athletes either from physicians who were willing to write the necessary prescription or even from some pharmacists who dispensed steroids without requiring a prescription (Hendershott, 1969). The "win at all costs" mentality of elite athletes can be seen in the results of a 1995 *Sports Illustrated* poll of almost 200 individuals who either had competed in the Olympics or were aspiring Olympians. When offered a scenario in which the use of a banned performance-enhancing substance would (without risk of being caught) guarantee victory in every competition for the next 5 years but would subsequently result in death due to side effects, more than 50% of the respondents said that they would take the substance (Bamberger and Yaeger, 1997). Ziegler later recognized the monster that he had helped create, and by the time of his death in 1984, he profoundly regretted that part of his life.

Besides the Soviet Union, the German Democratic Republic (GDR, or East Germany) began secretly giving AAS to its elite athletes in the 1960s, and this doping program continued until the fall of communist rule in the GDR in 1989. Overall, approximately 10,000 athletes (!) were administered steroids in order to enhance their performance at national and international competitions (Franke and Beredonk, 1997; Nieschlag and Vorona, 2015a). The most commonly used compound in the GDR was chlordehydromethyltestosterone, known as Oral Turinabol. The East Germans had especially great success with their female athletes, who won many Olympic and world championships with the aid of AAS. **FIGURE 16.10** illustrates the improved performance of a female shot-putter over an 11-week period of Oral Turinabol treatment. Unfortunately, these competitors paid a high price for their achievements, in part because of the powerful side effects of AAS in women. As we will discuss later, one of these side effects is the development of male secondary sex characteristics such as facial hair and an Adam's apple. Indeed, the masculine appearance of many female athletes from the GDR and other Soviet bloc countries during this time led to speculation that

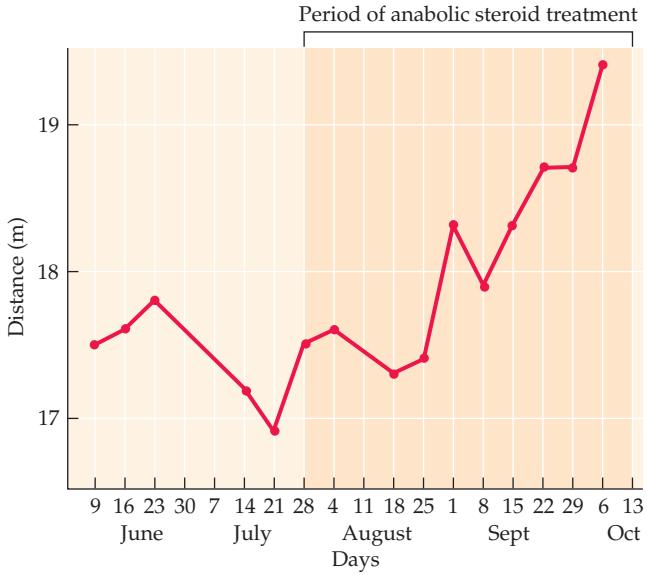


FIGURE 16.10 Performance enhancement of a female shot-putter in former East Germany Shot-put distance increased markedly over the 11-week period during which the athlete was chronically treated with an anabolic–androgenic steroid. (After Bauersfeld et al, 1973 and Franke and Berendonk, 1997.)

these competitors either were hermaphrodites (individuals possessing both sets of sex organs) or were men disguising themselves as women. As a result, officials for the 1967 European Championship soccer tournament required testing to verify the chromosomal sex of the participants (Bird et al., 2016).

Widespread public awareness of AAS use by elite athletes can be traced to the revelation that Ben Johnson, a Canadian sprinter and world record setter in the 100-meter dash at the 1988 Seoul Olympic Games, had been taking the banned steroid stanozolol during his training. Johnson was stripped of his Olympic gold medal and was suspended from athletic competition. Later, a San Francisco–area enterprise called the Bay Area Laboratory Cooperative (BALCO) was reported to have provided AAS to a number of famous athletes, most notably Barry Bonds, who is the Major League Baseball career leader in home runs per season and total home runs. Among the steroids provided by BALCO were preparations that came to be called "the Cream" and "the Clear" by athletes seeking to enhance their performance with these substances (see Table 16.4). Members of the public and sports journalists have voiced their concerns over allegations of steroid use, which has been reflected in recent balloting for the Baseball Hall of Fame. Thus far, no former player who is strongly suspected of steroid use, including Bonds and the all-time great pitcher Roger Clemens, has been elected to the Hall.

By the mid-1980s, there were increasing reports of rampant AAS use not only in professional sports but also reaching down into colleges and even high schools. This progression was aided by the publication of practical guides to steroid use, such as Duchaine's *Underground Steroid Handbook* and Phillips's *Anabolic Reference Guide* (Kanayama et al., 2010). In response, the U.S. Congress held a series of hearings between 1988 and 1990 that culminated in the Anabolic Steroids Control Act of 1990. This legislation classified 27 specific AAS preparations as Schedule III controlled substances, thus making their use illegal without a medical prescription. In addition, these substances are banned by many amateur and professional sports organizations, including the National Collegiate Athletic Association, the International Olympic Committee, the National Football League, the National Basketball Association, and Major League Baseball.

Anabolic–androgenic steroids are currently taken by many adolescent and adult men

Despite legal restrictions governing the availability of AAS for nonmedical purposes, these compounds continue to be used and abused because of their availability from various websites without a prescription. For a number of years, the popular media have promoted an image of muscularity as one of the defining characteristics of masculinity, and this image has contributed to the expansion of AAS use beyond the traditional groups of strength athletes and body builders. Indeed, a web-based survey of male AAS users found that improving one's physical appearance was rated just as important as increasing one's strength as a motivation for such use (Ip et al., 2011). Studies on the prevalence of AAS use estimate that roughly 3 to 4 million Americans between the ages of 13 and 50 have taken one or more of these substances at some time in their lives (Pope et al., 2014a). First exposure to AAS generally occurs later than for most other abused substances. Nevertheless, for some individuals steroid use begins while they are in high school or even earlier (Nicholls et al., 2017). For this reason, programs such as ATLAS (Adolescents Training and Learning to Avoid Steroids) have been developed to educate young people about the dangers of steroids and to deter their use (Mulcahey et al., 2010).

Anabolic–androgenic steroids are taken in specific patterns and combinations

AAS are taken in a variety of doses, patterns, and combinations (Mottram and George, 2000). Endurance athletes (e.g., marathon runners) and sprinters tend to take relatively low doses of steroids, whereas body builders and strength athletes such as weight lifters may take up to 100 times the therapeutic doses of these hormones. AAS are often used in patterns called **cycling**. Cycles are typically 6 to 12 weeks in duration, with periods of abstinence between successive cycles. Athletes use cycling for the following reasons:

- To minimize the development of tolerance to the drug

- To reduce the occurrence of adverse side effects
- To maximize performance at an athletic competition
- To avoid detection of a banned substance

Cycling is sometimes combined with **pyramiding**, in which the steroid dose is gradually increased until the midpoint of the cycle and then is gradually decreased as the cycle is completed. Pyramiding is thought to reduce possible withdrawal effects resulting from sudden termination of steroid use. Another common feature of steroid use is **stacking**, which refers to the simultaneous use of two or more AAS. Stacking is often done by combining a short-acting oral steroid with a long-acting injectable preparation. **FIGURE 16.11** illustrates some typical patterns of concomitant steroid use demonstrating the phenomena of cycling, pyramiding, and stacking. Users who prefer to cycle without abstaining from use may engage in **bridging** (also called "blast and cruise"), a process in which a low dose (the "cruise") is used to bridge between each high dose of the steroid (the "blast"). It is important to note that many of the reasons offered for using AAS in these various patterns are based on anecdotal information rather than on controlled scientific studies.

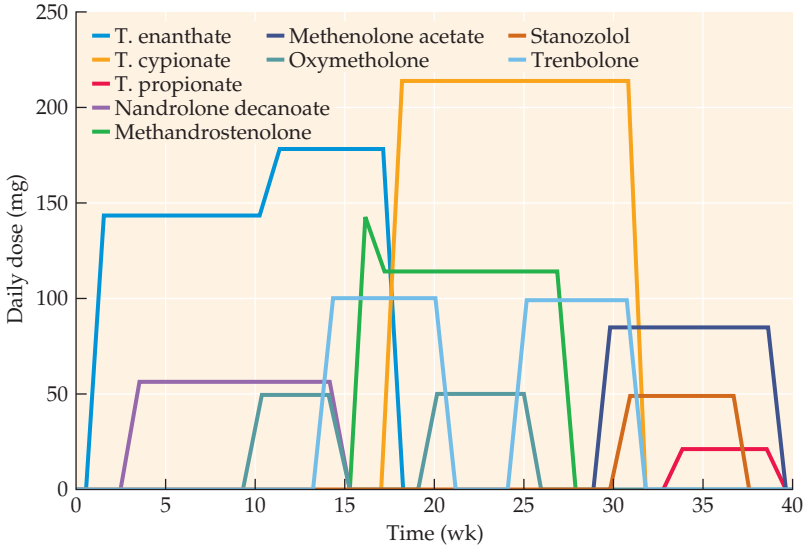


FIGURE 16.11 Typical dosing patterns of different anabolic–androgenic steroids Each line depicts time-related increases and decreases in use of a particular steroid, sometimes in combination with another steroid. Together, these dosing regimens are illustrative of cycling, pyramiding, and stacking. T, testosterone. (After Oberlander and Henderson, 2012.)

Finally, AAS users frequently engage in **polypharmacy**, in which additional substances are taken to augment the performance-enhancing properties of the steroids, to attempt to mask their presence in the individual's system, and/or to minimize some of the undesirable side effects that will be discussed below. Several different masking strategies are discussed in Alquraini and Auchus (2017). One relatively simple approach is to take high doses of a diuretic, which is a drug that increases urine production. This may permit the user to pass a urine test because the concentration of the banned steroid has been diluted to a level below the limit of detection of the assay being performed by the testing lab. Other masking strategies involve biochemical approaches that are beyond the scope of this book. The World Anti-Doping Agency (WADA) maintains a record of positive doping results from accredited testing laboratories around the world. The 2013 WADA report reveals that 63% of positive doping tests that year were for anabolic agents (mainly steroids); 10% for stimulants such as amphetamine, methylphenidate, or cocaine; 8% for diuretics and other masking agents; 6% for glucocorticosteroids (taken for their anti-inflammatory and analgesic effects); 4% for peptide hormones, growth factors, and related substances; 4% for cannabinoids;

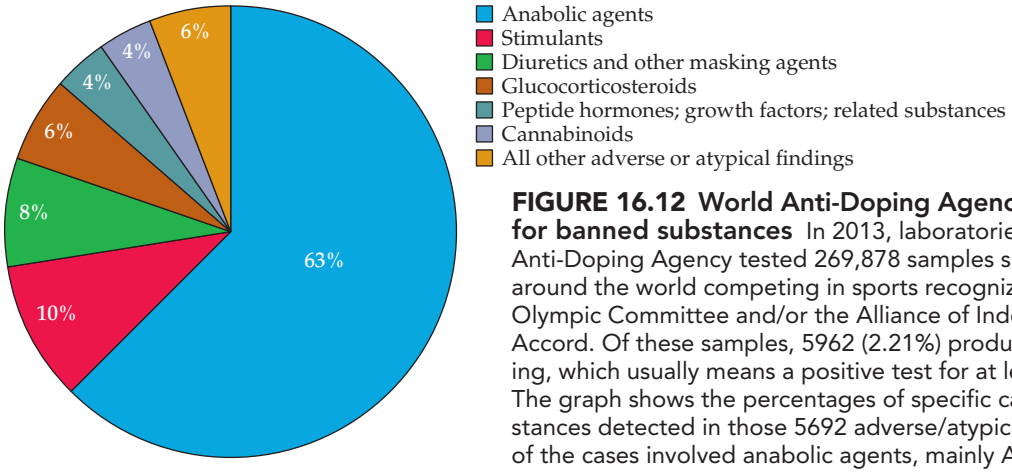


FIGURE 16.12 World Anti-Doping Agency 2013 analytical findings for banned substances In 2013, laboratories accredited by the World Anti-Doping Agency tested 269,878 samples submitted by athletes from around the world competing in sports recognized by the International Olympic Committee and/or the Alliance of Independent Members of SportAccord. Of these samples, 5962 (2.21%) produced an adverse/atypical finding, which usually means a positive test for at least one banned substance. The graph shows the percentages of specific categories of banned substances detected in those 5692 adverse/atypical findings. More than 60% of the cases involved anabolic agents, mainly AAS. (After Bird et al., 2016.)

and 6% for other substances (Bird et al., 2016) (**FIGURE 16.12**). These findings confirm that doping by elite athletes involves the use of a variety of other banned drugs in addition to AAS.

Pharmacology of Anabolic–Androgenic Steroids

AAS are unlike other abused substances discussed in this book because of their powerful action on muscle. However, they are similar to other abused substances in their ability to produce adverse health effects and dependence in some users.

Research is beginning to unravel the mechanism of action of anabolic–androgenic steroids on muscle

Although users had long touted the beneficial effects of AAS on muscle mass and strength, many researchers remained unconvinced by these anecdotal reports because properly controlled scientific studies were lacking and arguments could be made that placebo effects, increased training motivation, or other confounding factors might be responsible for the enhanced performance of AAS users. However, a series of studies by Bhasin and colleagues showed that giving high doses of testosterone to healthy young men leads to muscle fiber hypertrophy (increased size), increased muscle mass, and enhanced strength (Bhasin et al., 1996, 2001; Sinha-Hikim et al., 2002). Some of the results from one of these studies are presented in Figure 16.13 (Bhasin et al., 2001). The men were given weekly injections of testosterone enanthate at different doses for a period of 20 weeks. They also received another drug at the same time to suppress endogenous testosterone secretion so that their testosterone levels would depend solely on the exogenous treatment. The lowest doses (25 and 50 mg per week) produced subnormal circulating testosterone concentrations, the 125 mg dose produced concentrations in the normal range, and the 600 mg dose produced testosterone levels that were at least four times the average pretreatment concentration. As shown in **FIGURE 16.13A–C**, AAS administration caused dose-dependent increases in muscle volume and strength. In contrast, sexual function was unchanged (**FIGURE 16.13D**), indicating that this aspect of androgen action is not influenced by testosterone level within the dose range used and over the time period of testing. The findings of Bhasin's group are very important because they were obtained under carefully controlled conditions. However, it is worth

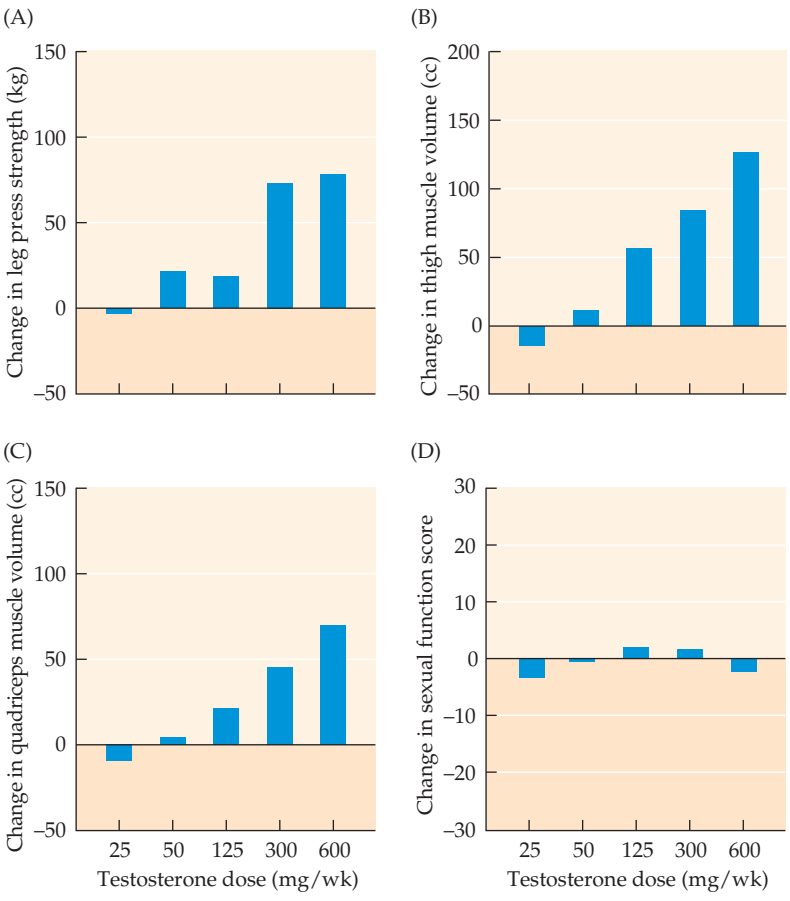


FIGURE 16.13 Increased muscle strength and volume in men following chronic testosterone administration
The study participants were healthy men, 18 to 35 years of age, who had prior weight lifting experience but who had not previously taken AAS. All participants were given monthly treatments with a long-acting drug to suppress their endogenous testosterone synthesis. Matched groups were also administered weekly intramuscular injections of testosterone enanthate for 20 weeks at doses ranging from 25 to 600 mg per injection. A comparison of circulating testosterone levels at the beginning of the study (baseline) with levels present at the 16-week time point showed that the 25 and 50 mg doses produced testosterone concentrations significantly below baseline, the 125 mg dose produced concentrations similar to baseline, and the 300 and 600 mg doses produced testosterone levels that were approximately two to four times baseline, respectively (data not shown). Leg press strength (A), thigh muscle volume (B), quadriceps muscle volume (C), and sexual function as determined by sexual activity and desire (D) were assessed at baseline and at the end of the 20-week dosing period. Muscle strength and volume were enhanced by increasing doses of testosterone, whereas sexual function remained relatively constant regardless of dose. (After Bhasin et al., 2001.)

noting that even greater effects may be obtained by users taking still higher doses of steroids and combining the treatment with intensive strength training.

Determining the mechanisms underlying the strength-enhancing effects of AAS has also proven challenging; however, researchers have begun to reach consensus on the relevant processes. AAS all have androgenic action, which means that they are agonists at the **androgen receptor**. These receptors are present in many different tissues, including skeletal muscle. In the inactive state, androgen receptors are located in the cytoplasm of the cell (**FIGURE 16.14**). Androgen molecules diffuse across the cell membrane and bind to the receptor, thereby activating it. The activated hormone–receptor complex then translocates into the cell nucleus, where it regulates the transcription of specific genes, depending on the cell type (Matsumoto et al., 2013). The result in this case is to increase overall muscle protein synthesis with a particular influence on proteins needed for muscle growth.

AAS exert several additional effects that contribute to muscle growth, two of which will be mentioned here. One important effect is an increase in the proliferation of so-called satellite cells that are necessary for the formation of new myotubes (developing muscle fibers). A second effect is promotion of the differentiation of local stem cells into muscle cells while inhibiting an alternate differentiation pathway into adipose (fat) cells (Dubois et al., 2012). The discovery of these mechanisms has given researchers considerable insight into how the use of AAS can enhance muscle growth, increase strength, and improve athletic performance.

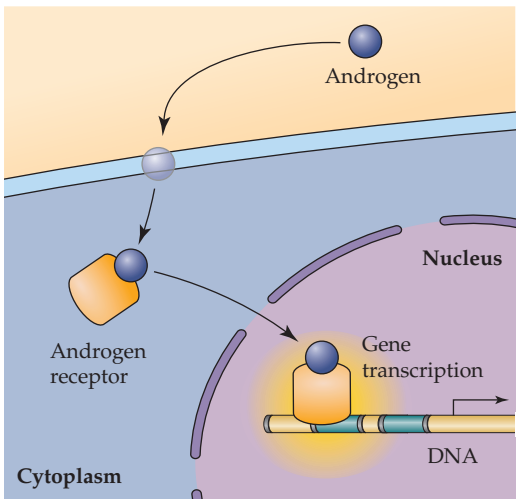


FIGURE 16.14 Mechanism of action of androgens in gene transcription Androgens enter target cells by diffusing across the cell membrane. After the hormone binds to androgen receptors in the cell cytoplasm, the hormone–receptor complex translocates to the cell nucleus, where it alters the transcription of specific genes.

Many adverse side effects are associated with anabolic–androgenic steroid use

Nieschlag and Vorona (2015a) discuss some of the problems encountered by researchers trying to identify the adverse effects of AAS use. We mentioned earlier that steroid users often take other substances, legal or illegal, which complicates the attribution of any observed health problem to a specific effect of the steroid. Equally important is that the doses taken by athletes and body builders are usually many times greater than the doses of testosterone prescribed medicinally (see the section below on the use of anabolic steroids to treat hypogonadism). Controlled studies involving supraphysiological steroid doses are not ethically permissible, and therefore the data must be obtained by documenting the occurrence of adverse health outcomes in steroid users without the benefit of matched controls. With these caveats in mind, researchers have accumulated sufficient evidence to show a number of side effects of AAS, which are listed in **TABLE 16.5**. Indeed, this evidence led the Endocrine Society, the leading international society of physicians and researchers concerned with hormonal function and disease, to issue a policy statement identifying the use of performance-enhancing drugs (mainly AAS) as a serious public health problem (Pope et al., 2014b). We will first discuss side effects that involve the user's physical condition, after which we will consider behavioral and psychological effects.

PHYSICAL HEALTH Abnormalities in heart structure and function, high blood pressure, and reduced high-density lipoprotein (HDL) cholesterol have been reported in some AAS users (Nieschlag and Vorona, 2015a). Nascimento and Medei (2011) suggest that these cardiotoxic effects increase the risk of sudden cardiac death, and other authors recently reviewed a number of fatalities in steroid users that may have been caused by cardiac damage (Frati et al., 2015). Liver toxicity can occur with chronic use of a particular chemical class of AAS, members of which include the oral steroids methandrostenolone, oxandrolone, oxymetholone, and stanozolol. Liver toxicity ranges from mild effects such as elevated liver enzymes in the bloodstream to more serious but rarer complications including peliosis hepatis and liver tumors. The kidneys play an important role in the clearance of AAS metabolites. As with liver toxicity, steroid-associated renal toxicity typically manifests in a mild, unsymptomatic form characterized by elevations in creatinine and blood urea nitrogen (BUN) levels in the bloodstream. On the other hand, excessive steroid use has been linked to severe impairment in renal function. Skin and hair problems are common in AAS users, with a particularly high risk of outbreaks of acne (Melnik et al., 2007) (**FIGURE 16.15A**). The effects of steroid use on reproductive function has been studied extensively

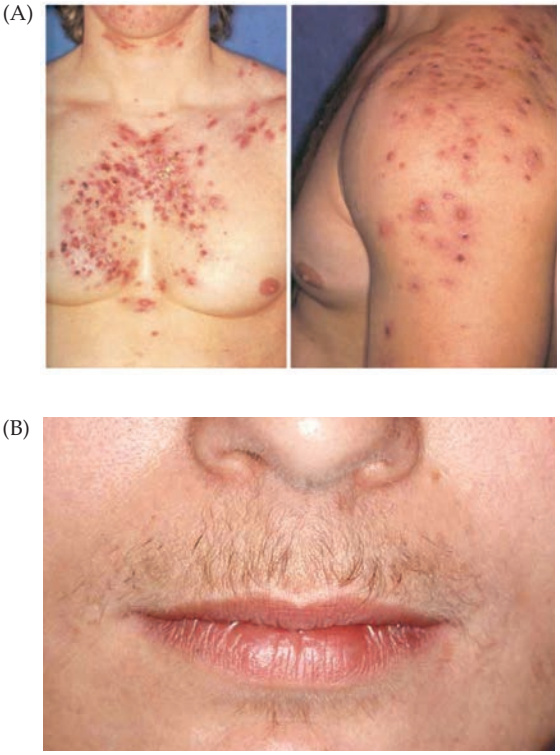


FIGURE 16.15 Facial acne and facial hair growth in anabolic–androgenic steroid users
Anabolic steroids can cause severe acne in users (A) and can also stimulate the growth of facial hair in women (B). (A from Melnik et al., 2007; B from Kopera, 2012.)

TABLE 16.5 Potential Health Consequences of Anabolic–Androgenic Steroid Use

Category	Effects
Cardiovascular effects	Left ventricular hypertrophy
	Abnormal cardiac structure and electrical activity
	Hypertension (high blood pressure)
	Decreased HDL cholesterol (the "good" kind of cholesterol)
Effects on the liver (not all anabolic steroids)	Jaundice
	Peliosis hepatis (blood-filled cysts in the liver)
	Tumors
Effects on the kidneys	Elevated serum creatinine and blood urea nitrogen (BUN) levels
	Impaired renal function
Effects on the skin and hair	Oily skin and scalp
	Severe acne
	Male-pattern baldness
Growth effects	Growth stunting in adolescents due to premature epiphyseal closure
Behavioral effects	Increased libido (sex drive)
	Mood changes (depression and mania)
	Increased anxiety, irritability, and aggressiveness
	Muscle dysmorphia
	Dependence
Specific effects on men	Testicular shrinkage
	Reduced sperm counts and possible infertility
	Prostate enlargement
	Gynecomastia (breast development)
Specific effects on women	Menstrual abnormalities
	Growth of an Adam's Apple and deepening of the voice
	Excessive hair growth, especially on the face
	Enlargement of the clitoris
	Decreased breast size

(Nieschlag and Vorona, 2015b; Christou et al., 2017). In men, chronic use of AAS leads to a persistently suppressed release of the gonadotrophins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland (see Chapter 3). This occurs because the androgenic actions of these steroids engage the negative feedback system that controls normal androgen and estrogen secretion in men and women. In men, the consequences of suppressed LH and FSH release can include testicular shrinkage, low testosterone levels, and reduced sperm counts (which, in turn, can cause infertility). Steroid use also influences secondary sex characteristics in both men and women. For example, **FIGURE 16.15B** illustrates the masculinizing effects of these compounds in women, which are irreversible. Young people who are still growing also need to be concerned about the possible stunting effects of AAS produced by premature closure of the epiphyses.[4] Which side effects occur depends on a number of factors, including the age and sex of the user, the type of steroid used (especially oral versus injectable), the dose, and the pattern and duration of use. Regardless, the long list of potential adverse health effects, some of which can be quite serious, certainly provides adequate warning to anyone who is contemplating the use of these substances.

[4]These are the end regions of long bones, which retain the capacity to further lengthen the bone. Once the epiphyses are "closed," the individual stops growing.

BEHAVIORAL AND PSYCHOLOGICAL PROBLEMS

Chronic use of AAS has also been associated with a variety of adverse psychological and behavioral effects. We will focus here on abnormalities in mood (ranging from mania to depression), body image, and anxiety/irritability and aggressiveness.

The relationship between AAS and psychological and behavioral variables has been investigated using prospective studies (controlled administration of steroids or placebo to volunteers), naturalistic studies (investigation of steroid users compared with controls), and individual case reports. Naturalistic studies and case reports suffer from the limitation that differences observed between steroid users and nonusers may have preceded the onset of use. Keeping this limitation in mind, current findings from the AAS literature suggest that a relatively small subset of vulnerable individuals does experience psychological and behavioral abnormalities following repeated exposure to high doses of these compounds. In such vulnerable individuals, heavy steroid use can lead to extreme mood changes typically consisting of mania (abnormally elevated or irritable mood, racing thoughts, grandiosity, and sometimes psychotic delusions) or hypomania (less intense manic symptoms with no psychosis) during periods of active use that contrast with depressive episodes during steroid withdrawal (Kanayama et al., 2008; Piacentino et al., 2015). In severe cases, depressed mood has included suicidal ideation and behavior. Almost all of the human studies regarding the mood-altering effects of AAS have been conducted with males. Future studies need to take into account potential effects on women, given that experimental animal studies have revealed significant sex differences in the affective responses to steroid administration (Onakomaiya and Henderson, 2016).

Mood changes in heavy users of AAS are not confined to feelings of anxiety or depression. Another reported effect is the development of increased anxiety/irritability and aggressiveness. This may lead, in the most extreme cases, to violent outbursts known on the street as "roid rage." The relationship between steroids and aggressiveness is discussed in **BOX 16.1**.

Finally, some AAS users suffer from an unusual body image disorder called **muscle dysmorphia**, also known as "reverse anorexia" (Rohman, 2009; Tod et al. 2016). In the fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-5*), muscle dysmorphia is listed as belonging to a more general diagnostic category called body dysmorphic disorder. The *DSM-5* describes this disorder as follows: "Muscle dysmorphia … consists of preoccupation with the idea that one's body is too small or insufficiently lean or muscular. Individuals with this form of [body dysmorphic disorder] actually have a normal-looking body or are even very muscular. They may also be preoccupied with other body areas, such as skin or hair. A majority (but not all) diet, exercise, and/or lift weights excessively, sometimes causing bodily damage"[5] (American Psychiatric Association, 2013, p. 243). Although muscle dysmorphia is almost always diagnosed in men, occasional cases in women have been reported. In addition, the symptoms of muscle dysmorphia are more prevalent in bodybuilders than in non-bodybuilder resistance trainers (Mitchell et al., 2017). The *DSM-5* classification of muscle dysmorphia places it within the context of obsessive-compulsive and related disorders; however, some researchers have proposed that muscle dysmorphia might be better classified as an addiction to body image, thus placing it within the broader category of behavioral addictions (Foster et al., 2015) (see Chapter 9).

Regular anabolic–androgenic steroid use causes dependence in some individuals

By the late 1980s and early 1990s, reports began to appear suggesting that some AAS users met *DSM* criteria for dependence on these substances. Case studies and self-reports by bodybuilders and athletes provide some insight into the nature of steroid dependence. One such case is that of Lyle Alzado, a three-time All-Pro NFL defensive end who played for 15 seasons with the Denver Broncos, Cleveland Browns, and Los Angeles Raiders before coming down with brain cancer and dying in 1992 at the age of 43. In a 1991 guest article for *Sports Illustrated*, Alzado admitted taking AAS beginning in his college days and continuing throughout his NFL career and beyond. At the height of his steroid use, he would cycle 10 to 12 weeks on steroids and then go off the drugs for only 2 to 3 weeks before starting again. In his own words, "It was addicting, mentally addicting. I just didn't feel strong unless I was taking something." Alzado also described the aggressiveness produced by his steroid use. "I became very violent on the field. Off it, too. I did things only crazy people do. Once in 1979 in Denver, a guy side-swiped my car, and I chased him up and down hills through the neighborhoods. I did that a lot. I'd chase a guy, pull him out of his car, beat the hell out of him" (Alzado, 1991).

Kanayama and coworkers (2009) summarized the results of five field studies that assessed the prevalence of *DSM* dependence criteria in 426 AAS users. When these studies were taken together, the percentages of users exhibiting specific criteria were as follows: tolerance, 20.7%; withdrawal, 44.6%; taking the substance in larger amounts than intended, 27.9%; inability to cut down or control use of the substance, 12.0%; significant amount of time spent on substance-related activity, 28.6%; other activities reduced in favor of substance use, 17.8%; and continued substance use despite

[5]Reprinted with permission from the *Diagnostic and Statistical Manual of Mental Disorders*, Fifth Edition, © 2013. American Psychiatric Association. All rights reserved.

BOX 16.1 Of Special Interest

Anabolic–Androgenic Steroids and "Roid Rage"

You may have heard the term *roid rage* used to describe a sudden eruption of intense anger or violent behavior by someone taking AAS. Case reports have documented instances in which violent outbursts appear to be linked to heavy steroid use. But before we discuss such extreme examples of violent behavior, it's important to consider information about the general relationship between AAS and irritability and aggression. One source of information is a study conducted in Australia that included personal interviews with 60 male users of performance- and image-enhancing drugs (PIEDs), including but not restricted to AAS (Larance et al., 2005). When questioned about events that had occurred during the 6-month period prior to their interview, 37% of these PIED users reported episodes of "roid rage," typically associated with domestic or automobile-related conflicts. Study participants described these episodes as "requiring a specific trigger, a sudden rush of anger or arousal, and possible escalation to verbal or physical aggression" (Larance et al., 2005, p. 94). Indeed, 23% of the participants had been in a situation that evoked aggressive behavior or violence. Other studies by Pagonis and coworkers support a specific association between AAS use and aggressiveness. One of these studies found large increases in hostility, phobic anxiety, paranoid ideation, and psychoticism (measured by means of the Symptom Checklist-90 [SCL-90]) following a cycle of steroid use, compared with precycle levels, in a group of amateur and recreational athletes and body builders (Pagonis et al., 2006a). These changes were most marked in a subgroup characterized as heavy (ab)users, thereby demonstrating a dose dependency of the mood-altering effects of AAS. A second study reported on two pairs of male monozygotic twins, of which one member of each pair was a steroid user. The SCL-90 again revealed noteworthy differences between users and nonusers, with consistently higher hostility and paranoid ideation scores in the users (Pagonis et al., 2006b). Among other studies that compared steroid users with nonusers, several (though not all) found evidence for increased agitation, irritability, feelings of aggression, aggressive behavior (including both verbal and nonverbal), and violence in the user group (Trenton and Currier, 2005).

Despite the ability of AAS to increase hostility and aggressive tendencies, outbursts of violent behavior are less common. Nonetheless, data on over 6000 survey respondents from the National Longitudinal Study of Adolescent Health found a statistically significant association between steroid use and violent behaviors such as physical fighting (Beaver et al., 2008). Of course, a mere association doesn't demonstrate causation, as it is possible that individuals with a propensity to engage in violence also tend to be attracted to AAS to enhance their strength and physical appearance. Individual case studies may help in this regard, particularly if information is available to compare the person's behavior prior to the onset of steroid use with his behavior afterward. One extreme case of this kind is described in Katz and Pope (1990). Mr. X, as he is referred to, was a 23-year-old male who had been bodybuilding for 5 years. While in high school and prior to beginning his use of steroids, Mr. X drank alcohol socially and occasionally snorted cocaine with friends. He had no history of psychiatric illness, nor was he known to have ever committed a violent act. Indeed, his father was a minister, and Mr. X himself had been an active member of the church's youth ministry. He was described by friends as a considerate, religious person. At the age of 21, Mr. X began the first of two cycles of AAS use in order to improve his competitive standing as a bodybuilder. During this time, he started to experience severe mood swings, including noticeable increases in irritability and argumentativeness:

> *On more than one occasion he tore chunks of aluminum out of cans with his teeth to intimidate bystanders. He also ripped telephones out of the wall on impulse. At this time, he met* DSM-III-R *criteria for a manic episode with decreased desire and need for sleep, explosive temper, extremely reckless behaviors with a high potential for dangerous and undesirable consequences, continued irritability, and grandiosity that reached delusional proportions. While out one weekend evening with some friends during the second course of anabolic steroids, the group stopped at a small market. While in the parking lot, Mr. X, without known or observed provocation, suddenly wrapped his arms around the telephone booth, tore it from its base, and threw it across the lot. The group left immediately and soon thereafter saw a hitchhiker on the road. Mr. X told the driver to stop. After the hitchhiker, a stranger to all present, entered the vehicle, Mr. X instructed the driver to drive to a remote spot in the woods. Once there, without instigation, Mr. X beat the victim repeatedly, tied him between two poles,*

(*Continued*)

BOX 16.1 Of Special Interest (*continued*)

smashed a wooden board over his back, and kicked him. The hitchhiker was found dead the next morning.

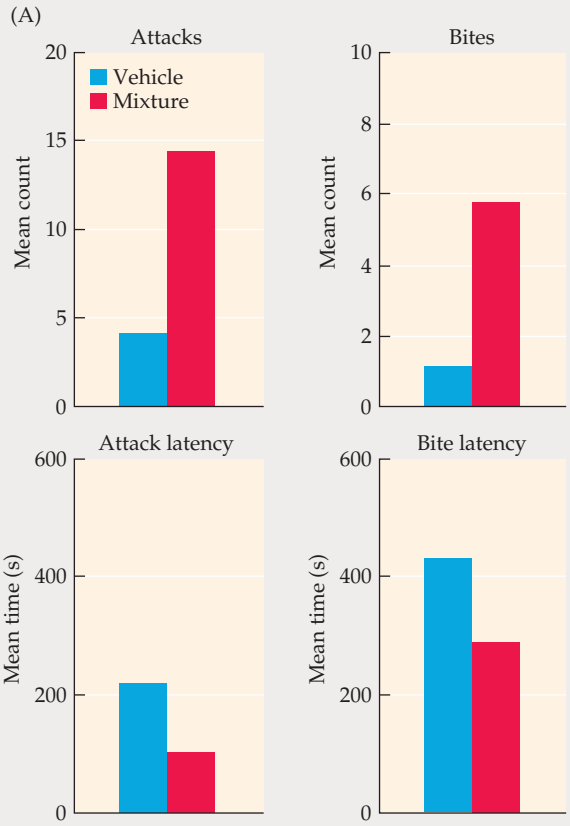
Katz and Pope, 1990, p. 220

Mr. X was arrested, convicted, and incarcerated for his crime. Once off steroids, however, he reverted to his previous personality traits. In prison he was described as quiet, modest, and accommodating. Indeed, he was astonished at the acts he had committed that fateful evening.

While case reports such as this do not prove a causal effect of AAS on violent behavior, they raise the possibility that such an effect may occasionally occur. On the other hand, this kind of extreme "roid rage" is a rare event even among heavy users. We may speculate that some individuals are particularly susceptible to steroid-induced aggressiveness and that steroid users who engage in violent acts (such as Mr. X) are among the susceptible group.

The mechanisms by which AAS may increase irritability and aggression in humans are not yet understood. Therefore, researchers have turned to experimental animal models to investigate such mechanisms. Studies using electrical stimulation and lesion methods have identified neural circuits that underlie several different kinds of aggressive behaviors (e.g., predatory and defensive aggression) in rats, mice, and cats (Haller, 2013). Laboratory rats and mice have also been used to investigate aggression- and anxiety-related behaviors induced by AAS administration (Cunningham et al., 2013; Bertozzi et al., 2017). However, the most well-characterized model is one developed by Melloni and colleagues that involves chronic administration of AAS to adolescent Syrian hamsters, followed by assessment of aggressive behaviors using the resident–intruder paradigm, which was described in Chapter 6 (Melloni and Ricci, 2010; Ricci et al., 2012). In a typical study, individually housed male hamsters are injected daily for 30 days with an AAS mixture containing several steroids that are commonly taken by human AAS users. Control subjects are injected daily with vehicle only. On the day after the last injection, each of the experimental and control hamsters (the "residents") is challenged by the introduction of an unfamiliar, similar-sized male hamster (the "intruder") into the animal's cage. As is typical of the resident–intruder paradigm, the resident animal behaves aggressively toward the intruder and almost always dominates the encounter. Among the agonistic behaviors recorded during the encounter are upright postures that signify aggressive intent, lateral attacks (attacks directed to the intruder's flank or rump), and flank/rump bites by the resident. **Figure A** shows the results of a typical experiment demonstrating the aggression-inducing effects of repeated AAS exposure in the hamster model (Morrison et al. 2016). Not only are the numbers of attacks and bites substantially increased by AAS treatment, but the latencies to first attack and first bite are reduced. These shorter latencies are suggestive of greater impulsivity in the treated resident hamsters compared with the control residents.

The hamster model has additionally provided the most detailed information on the neurobiological underpinnings of AAS-induced aggression and anxiety. Researchers have identified the anterior hypothalamus as the key component of a complex

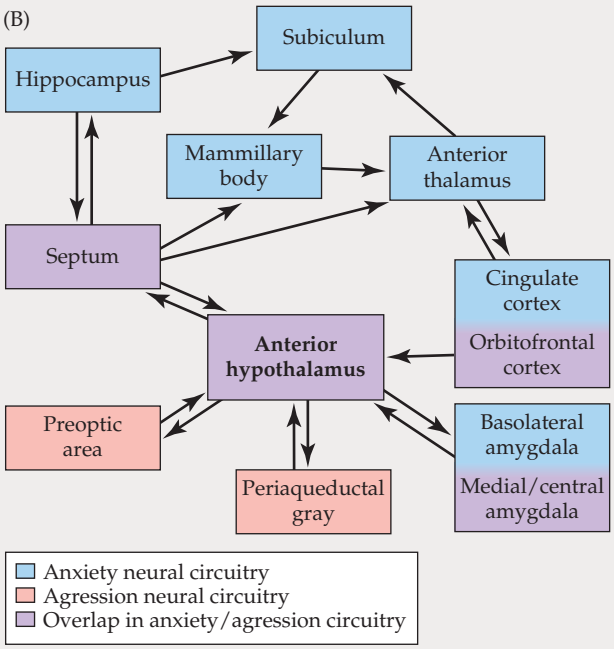


(A) Effects of repeated AAS administration on attack and biting behaviors in Syrian hamsters Individually housed male hamsters received daily SC injections of either an AAS mixture (2 mg/kg testosterone cypionate, 2 mg/kg nandrolone decanoate, and 1 mg/kg boldenone undecylenate) or sesame oil vehicle. Injections were given from postnatal day 27 to postnatal day 56. On day 57, all animals were subjected to a resident–intruder test of aggressive behavior during which number of attack behaviors (aggressive upright postures plus lateral attacks), number of bites, and latencies to attack and to bite were recorded over a 10-minute period. AAS administration increased the mean numbers of attacks and bites and also reduced the mean latencies to the first attack and the first bite. (After Morrison et al., 2016.)

BOX 16.1 Of Special Interest (*continued*)

neural circuit that helps regulate these two behavioral functions (**Figure B**; Ricci et al., 2012). Overlap between the aggression-mediating and anxiety-mediating brain areas may help explain why AAS users can experience both symptoms. Current findings additionally indicate that chronic AAS administration to hamsters alters the activity of several neurotransmitter systems known to modulate this circuit, including the dopaminergic, serotonergic, and vasopressin systems (reviewed in Morrison et al., 2016). Whether the neuroanatomical and neurochemical mechanisms of AAS-induced aggression in humans are similar to those of hamsters remains to be determined.

To summarize, repeated exposure to AAS at the high doses taken by human users appears to elevate levels of irritability and/or aggressiveness in some individuals. Why some users are affected this way and others are not is not yet known; however, it is likely that this difference is related to parameters of steroid dosing, along with individual susceptibility and/or preexisting tendencies toward aggressive behaviors. The occurrence of violence among AAS users may also be confounded by the use of other substances (i.e., polysubstance use) (Lundholm et al., 2015). Examples of extreme "roid rage" have been reported but fortunately are quite rare. Until more research is conducted on violent human AAS users, we will have to continue relying on animal models to help us understand the neurobiology of AAS-induced aggression.



(B) Model of the neural circuit subserving offensive aggression and anxiety in hamsters The anterior hypothalamus has been identified as a key area within a larger circuit involved in regulating both aggression and anxiety. As shown in the figure, some of the structures within this circuit participate only in aggressive behavior, others participate only in anxiety, and still others participate in both. (After Ricci et al., 2012.)

recognition of use-related problems, 24.9%. Most important, 33.8% of the users studied met three or more of the above criteria, indicating that they were classified as being dependent on AAS.

There are both similarities and differences between the characteristics of AAS dependence and the characteristics of dependence on typical drugs of abuse. One major similarity is the occurrence of withdrawal symptoms when substance use is discontinued. In the case of AAS, withdrawal symptoms can include fatigue, depressed mood, insomnia, restlessness, anorexia, decreased libido, dissatisfaction with body image, and a desire for more steroids (Brower et al., 1990; 1991). Anxiety is another symptom that is frequently observed during AAS withdrawal (reviewed in Ricci et al., 2012). Increased feelings of anxiety may occur at the end of a cycle of AAS use or following a longer period of abstinence. Differences between AAS dependence and dependence produced by most other drugs of abuse include the lack of an immediate steroid-induced euphoria or other intoxicating effect, relatively little impairment in the performance of daily activities, and less frequent occurrence of tolerance (Kanayama et al., 2009). Moreover, even heavy steroid users rarely seek treatment for their problem, and specific treatment protocols for steroid dependence have yet to be developed and tested empirically (Brower, 2009; Kanayama et al., 2010).

The potential abuse liability of AAS has been extensively studied using experimental animal models. These animal studies have shown that acute or chronic AAS administration can produce various neurotransmitter changes in the brain's reward circuitry (Mhillaj et al., 2015). More importantly, the reinforcing and rewarding properties of these compounds have been demonstrated by self-administration and place-conditioning studies in rats, mice, and hamsters (Wood, 2008; Grönbladh et al., 2016). For example, hamsters will self-administer testosterone or various synthetic AAS directly into the cerebral ventricles (Wood et al., 2004; Ballard and Wood, 2005). The demonstration of AAS reinforcement by the intracerebroventricular route is important in showing that this effect is mediated by mechanisms within the brain, not by peripheral anabolic activity. On the other hand, the data also indicate that

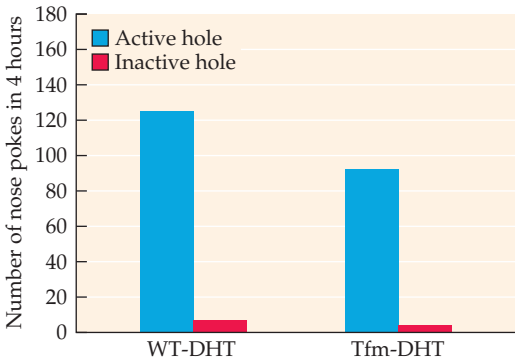


FIGURE 16.16 Intracerebroventricular self-administration of dihydrotestosterone in rats Wild-type rats (WT) and rats carrying a testicular feminization mutation that codes for a nonfunctional androgen receptor (Tfm) were trained to perform a nose-poke response. They received an intracerebroventricular infusion of 1 μg of dihydrotestosterone (DHT) per every five responses (FR-5 schedule of reinforcement). One hole for nose poking was active (associated with steroid delivery), and the other was inactive (responses in that hole had no consequence for the animal). Although the Tfm rats responded somewhat less than the WT animals, both groups clearly acquired DHT self-administration (i.e., they responded much more to the active hole than to the inactive hole), indicating a positive reinforcing effect of the hormone in the absence of classical androgen receptors. (After Sato et al., 2008.)

AAS are not as strongly reinforcing as highly addictive drugs like cocaine, methamphetamine, or heroin, which is consistent with the lack of a rapid euphoric effect experienced by human AAS users.

Relatively little is known about the mechanisms underlying AAS reinforcement. One obvious possibility is the classical intracellular androgen receptor described earlier; however, other possibilities must also be considered because of several contrary experimental findings. For example, when different steroids were tested in self-administration or place conditioning studies, their effects in these behavioral models were not closely related to their affinity for the androgen receptor. More important, steroid self-administration still occurs in rats and mice carrying genetic mutations that greatly decrease androgen receptor functioning (Kohtz and Frye, 2012; Sato et al., 2008) (**FIGURE 16.16**). One alternative mechanism involves some kind of androgen receptor located on the cell membrane instead of within the cell. The existence of a membrane-bound androgen receptor has been hypothesized but not yet confirmed by cloning (Sato et al., 2010). A second possible mechanism is related to AAS metabolism within the brain. Testosterone can be metabolized by two different enzymes (**FIGURE 16.17**). One enzyme, called **5α-reductase**, converts testosterone to 5α-dihydrotesterone (DHT), which is even more potent than testosterone itself as an androgen receptor agonist. A second enzyme, **aromatase**, converts testosterone to the estrogenic hormone estradiol. Many synthetic steroids are also substrates for aromatase. Consequently, we must consider that some of the subjective and behavioral effects of AAS are mediated not only by androgen receptors but also by estrogen receptors expressed in the brain. A third possible mechanism is mediation by one or more kinds of neurotransmitter receptor, such as the $GABA_A$ receptor (Oberlander et al., 2012). Keep in mind that the development of severe mood disturbances and/or dependence in AAS users is typically associated with long-term exposure to very high steroid doses. Such exposure is likely to cause interaction of these substances with other molecular targets besides androgen or estrogen receptors. From this discussion, it should be clear that much more research is needed to clarify the mechanisms by which AAS produce their behavioral effects.

Testosterone has an important role in treating hypogonadism

As we have discussed in previous chapters, substances that are abused recreationally may, nevertheless, have legitimate therapeutic applications. The same is true for AAS, especially testosterone. The principal medical use of testosterone is for hormone replacement in cases of **male hypogonadism** (Basaria, 2014). **FIGURE 16.18A** shows that in a healthy man, the hypothalamus stimulates the pituitary gland to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which together act on the testes to promote testosterone

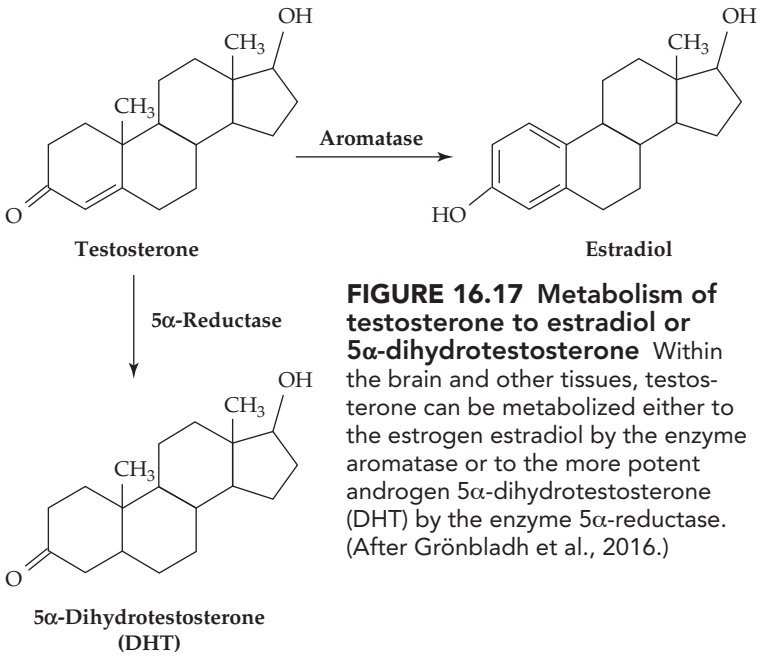


FIGURE 16.17 Metabolism of testosterone to estradiol or 5α-dihydrotestosterone Within the brain and other tissues, testosterone can be metabolized either to the estrogen estradiol by the enzyme aromatase or to the more potent androgen 5α-dihydrotestosterone (DHT) by the enzyme 5α-reductase. (After Grönbladh et al., 2016.)

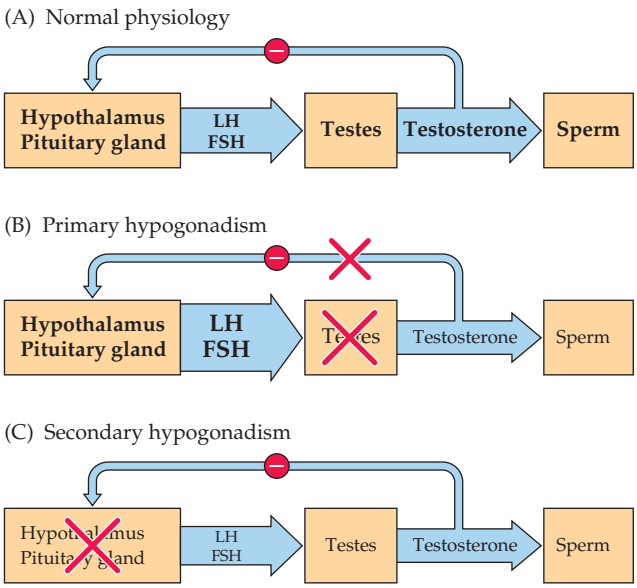


FIGURE 16.18 Functioning of the male hypothalamic–pituitary–gonadal axis in health and in primary and secondary hypogonadism (A) In men with normal physiology, the hypothalamus stimulates the pituitary gland to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which together stimulate the testes to secrete testosterone and to produce sperm. Through a negative feedback loop (–), testosterone acts back on the hypothalamus and pituitary gland to prevent excessive LH and FSH release. (B) In primary hypogonadism, the testes are unresponsive to LH and FSH, which leads to reduced testosterone levels and sperm counts and to increased LH and FSH secretion due to loss of the negative feedback signal. (C) In secondary hypogonadism, an abnormality in either the hypothalamus or the pituitary gland causes reduced LH and FSH secretion, which leads to effects on testosterone and sperm production like those in primary hypogonadism. (After Christie and Meier, 2015.)

synthesis and secretion along with spermatogenesis. Damage to the testes, certain rare genetic mutations, or several diseases can render the testes unresponsive to LH and FSH. This condition is called **primary hypogonadism** (**FIGURE 16.18B**). Notice that a lack of the normal negative feedback of testosterone on the hypothalamus and pituitary gland leads to hypersecretion of LH and FSH, despite the inability of these hormones to stimulate testicular activity. Alternatively, a condition called **secondary hypogonadism** can occur in which pituitary damage, trauma to the hypothalamus, or other mutations or diseases can cause a severe reduction in LH and FSH secretion from the pituitary (**FIGURE 16.18C**). Although the testes remain functional in such cases, the loss of stimulation again leads to reduced testosterone levels and deficient spermatogenesis.

The consequences of either kind of hypogonadism can be deduced from the biological actions of testosterone on multiple organs throughout the body (**FIGURE 16.19**). Thus, hypogonadal men may exhibit poor libido (sex drive), erectile dysfunction, and sterility. They additionally may show loss of muscle mass and bone density, and they may suffer from adverse effects on the kidneys and skin. For these reasons, testosterone replacement therapy is routinely administered to hypogonadal men. The hormone may be given in an injectable form, in a tablet or pill, as a pellet that is surgically implanted under the skin, as a transdermal patch or gel, or as a solution that is applied to the underarms (Basaria, 2014).

Besides the severe forms of hypogonadism associated with disease, tissue damage, or genetic abnormalities, a more moderate loss of testosterone typically occurs in elderly men (Christie and Meier, 2015). If the reduction in testosterone is great enough to cause symptoms, then the individual may seek medical attention. Not only can the effects of aging-related hypogonadism be psychologically distressful to men who

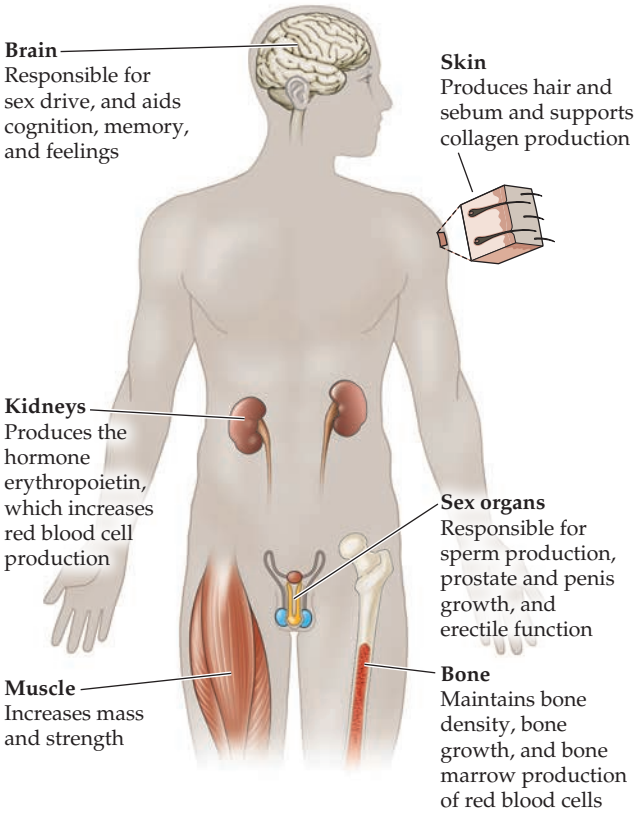


FIGURE 16.19 Multiple effects of testosterone on the body Testosterone acts on many organs of the body. These actions occur in both men and women except for those that are specific to the male sex organs. (After Kloner et al., 2016.)

wish to continue having active sexual relations, reduced strength (due to loss of muscle mass) and bone density cause an increased risk of dangerous falls. Consequently, testosterone replacement may be prescribed for aged men who show these symptoms and who have been diagnosed with hypogonadism based on clinical testing.

Because testosterone is thought of as the "male" sex hormone, its therapeutic use has been restricted almost entirely to men. Yet women also possess significant amounts of testosterone in their bloodstream, and some studies have associated testosterone with sexual function in women as well as men (Davis et al., 2016). Furthermore, women undergo a sharp decline in levels of gonadal hormones after menopause. Because many of the actions of testosterone are similar in men and women (see Figure 16.19 legend), researchers have begun to consider the potential benefits of testosterone administration for improving both sexual function and musculoskeletal health in postmenopausal women (Davis and Wahlin-Jacobsen, 2015).

Section Summary

- Anabolic–androgenic steroids (AAS) are hormones that increase muscle mass and strength and also produce masculinizing effects in the user. These substances either contain the naturally occurring male sex hormone testosterone or are similar to testosterone in their chemical structure. Some AAS are taken orally, others by intramuscular injection.
- AAS were initially developed for their muscle-building and performance-enhancing effects, but some current users take these substances mainly to attain a more muscular physical appearance. The Soviet Union was the first country in which steroids were administered to athletic competitors; however, the practice quickly spread to other countries. When the use and abuse of these substances became more widespread and numerous adverse side effects began to emerge, steroids were classified as Schedule III controlled substances in the United States. They were also banned by a variety of national and international athletic organizations.
- AAS are usually taken in specific patterns and combinations. In the case of cycling, the steroid is taken in alternating on and off periods. Cycling can be combined with pyramiding, in which the dose is increased during the early part of the cycle and then is gradually decreased after the peak dose is reached at the midpoint of the cycle. Bridging is a pattern that avoids periods of abstinence, since the user continues to take a low steroid dose in between the high doses of one cycle and the next. Some users also engage in stacking, which refers to combining two or more steroids (often one that is injected and another that is taken orally). Steroid users frequently practice polypharmacy, in which additional substances (e.g., stimulants or masking agents like diuretics) are taken along with the steroid.
- Controlled studies have confirmed that AAS such as testosterone dose-dependently enhance muscle fiber size, muscle mass, and strength. These effects are mediated by intracellular androgen receptors and involve increased protein synthesis, proliferation of satellite cells that can form new myotubes, and differentiation of local stem cells into muscle cells.
- There are a number of adverse side effects of AAS. Chronic steroid users may exhibit cardiovascular problems such as abnormalities in heart structure and function, high blood pressure, and reduced circulating HDL. Other physiological side effects may include renal toxicity, skin and hair problems (e.g., severe acne), liver toxicity (particularly with the use of certain oral steroids), and risk of stunted growth in young users. Women are vulnerable to significant masculinizing effects of AAS use.
- Reproductive function can be disrupted by AAS use. Men show suppressed release of LH and FSH from the pituitary gland because the androgenic properties of AAS activate the negative feedback system that controls gonadotropin release. Chronically reduced LH and FSH causes testicular shrinkage, low circulating testosterone, and low sperm counts, which can lead to infertility.
- AAS have also been associated with a variety of psychological side effects. Mood shifts may range from depression to mania. Some individuals suffer from an unusual male body image disorder known as muscle dysmorphia. Case reports as well as controlled and naturalistic studies have also linked AAS with heightened irritability and aggressiveness in a significant percentage of users. In rare cases, a heavy AAS user may exhibit severe aggressive outbursts colloquially known as "roid rage."
- Research with experimental animals has yielded important information regarding the neurobiological mechanisms of AAS-related aggression. A particularly well-studied model involves daily administration of an AAS mixture to adolescent male Syrian hamsters, after which the animals are tested in the resident–intruder paradigm. The treated

animals show substantially elevated amounts of aggressive behaviors compared with controls, and they additionally show increased anxiety-like behaviors. Researchers have identified a complex neural circuit mediating these behaviors, with the anterior hypothalamus serving as a point of convergence of both aggression- and anxiety-related neural pathways.

- A certain percentage of steroid users develop a characteristic pattern of dependence and withdrawal related to these substances. Feelings of anxiety are frequently experienced either at the end of a cycle of AAS use or after a period of prolonged abstinence and withdrawal. Intravenous and intracerebroventricular steroid self-administration have been demonstrated in laboratory animals, although steroids are not as strongly reinforcing as classical addictive drugs like cocaine or heroin. The mechanisms underlying steroid reinforcement are not yet well understood; however, several possible mechanisms can be considered. These mechanisms include activation of the following: the classical intracellular androgen receptor, a membrane form of the receptor, estrogen receptors after conversion of androgens (e.g., testosterone) to estrogens (e.g., estradiol) by aromatase, and receptors for neurotransmitters such as GABA.
- Testosterone has an important medical role in the treatment of male hypogonadism. Primary hypogonadism occurs when the testes are unresponsive to LH and FSH. Secondary hypogonadism occurs when an abnormal condition causes reduced secretion of LH and FSH. Both types of hypogonadism result in low testosterone levels, which in turn can cause poor libido, erectile dysfunction, sterility, and loss of muscle mass and bone density. Fortunately, these symptoms are usually responsive to testosterone replacement therapy. Besides the severe hypogonadism that may develop at a relatively young age, a more moderate reduction in circulating testosterone typically occurs in elderly men, who may seek medical attention if the symptoms are sufficiently distressing. Researchers are additionally beginning to consider the potential benefits of testosterone administration for improving sexual function and musculoskeletal health in postmenopausal women.

STUDY QUESTIONS

1. Name the five classes of substances that comprise the category of inhalants.
2. What are the characteristics common to all types of inhalants?
3. How are inhalant doses calculated, and how do these substances enter the brain?
4. Why are inhalants often the first substances of abuse used by children and adolescents?
5. Describe the acute effects of inhalants, including how these effects are influenced by dose and by frequency of use.
6. Discuss the phenomena of inhalant tolerance and dependence, including the symptoms of inhalant withdrawal.
7. Describe (a) the evidence for inhalant reward and reinforcement based on experimental animal studies and (b) current information on the neural mechanisms underlying inhalant reward and reinforcement.
8. (a) Describe the acute effects of inhalants such as toluene on locomotor activity in animals. (b) How can these effects be explained by the action of these substances on ionotropic receptors and voltage-gated ion channels? (c) How do the effects of prolonged inhalant exposure on ion channels differ from the effects of acute exposure?
9. Discuss the health risks associated with inhalant exposure, including complications involving developmental exposure to these substances.
10. Discuss the characteristics of GHB in the brain, including the origin of GHB, its localization, and its relationship to the neurotransmitter GABA.
11. For what purposes and by what user groups is GHB consumed recreationally?

(Continued)

STUDY QUESTIONS (*continued*)

12. Describe the acute behavioral and electrophysiological effects of GHB in humans and in laboratory animals.
13. What is the evidence for rewarding and reinforcing effects of GHB? How does this compound compare with cocaine, opioids, and ethanol with respect to its efficacy as a reinforcing agent?
14. Discuss the various mechanisms thought to underlie GHB's behavioral actions, making sure to differentiate between mechanisms requiring high GHB doses and those that may mediate lower doses of GHB.
15. Describe the therapeutic use of GHB in the treatment of narcolepsy and alcoholism.
16. Discuss the features of recreational use of GHB, including the symptoms of GHB intoxication and overdose, and evidence for GHB tolerance and dependence.
17. What are anabolic–androgenic steroids (AAS), and why are these substances given that general name?
18. List the major routes of administration of AAS.
19. (a) For what purposes were AAS developed historically? (b) Who are the primary users of AAS at the current time?
20. Describe the various patterns and combinations in which AAS are taken. Make sure to include information on the rationale for these different patterns.
21. What are the mechanisms by which AAS increase muscle mass and physical strength?
22. Describe the adverse effects of chronic AAS use on physical health.
23. What kinds of behavioral and psychological problems (apart from irritability and aggressiveness) have been reported in studies of long-term and/or high-dose AAS use? Be sure to include a discussion of muscle dysmorphia in your answer.
24. (a) What is the current evidence regarding the association of AAS use with heightened irritability and aggression (including "roid rage")? (b) Discuss the Syrian hamster model of AAS-induced aggression and anxiety, including information on the neural circuitry mediating these behaviors.
25. Discuss AAS dependence and withdrawal, including (a) information based on human AAS users, (b) evidence for AAS reward and reinforcement from laboratory animal studies, and (c) hypothesized mechanisms underlying AAS reinforcement.
26. (a) What is hypogonadism in males? Include in your answer a discussion of the physiology of primary and secondary hypogonadism. (b) Describe the symptoms of hypogonadism and the use of testosterone replacement therapy to treat this disorder.

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CHAPTER 17

Virtual reality can be effective in treating PTSD and phobias. (© Erika Schultz/TNS/ZUMAPRESS.com. PC inset, © Bart Sadowski/Shutterstock.com.)

Disorders of Anxiety and Impulsivity and the Drugs Used to Treat These Disorders

A NUMBER OF YEARS AGO, a psychologist was asked to make a house call to a woman having panic disorder with agoraphobia. Mrs. M. was 67 years old and lived in a lower-middle-class section of the city. Her adult daughter was one of her few remaining contacts with the world and had requested the evaluation. Mrs. M. was friendly and was glad to see the clinician. He was the first person she had seen in 3 weeks. Mrs. M. had not set foot out of that apartment in 20 years, and she had suffered from panic disorder with agoraphobia for over 30 years.

As her story was told, she provided vivid images of the tragedy of a wasted life. Early in her very stressful marriage to a man who abused alcohol and her, Mrs. M. had her first panic attack and had gradually withdrawn from the world. Even areas of her apartment signaled the potential for terrifying panic attacks. She did not answer the door herself, because she had not looked out in her hallway for the past 15 years. She reported that she could enter her kitchen and go into the areas containing her stove and refrigerator but that she had not been to the back of her kitchen overlooking the backyard for the past 10 years. Thus, her life for the past decade had revolved around her bedroom, her living room, and the front half of her kitchen. She relied on her daughter to bring in groceries and visit once a week. Her only remaining contact with the outside world was through the television and the radio. As long as she stayed within her apartment, she was relatively free of panic. In her mind, there were few reasons left in her life to venture out, and she declined treatment (adapted from Barlow and Durand, 1995). ■

Neurobiology of Anxiety

We turn now to a class of maladaptive behaviors that produce an enormous amount of suffering, contribute to low productivity, and generate a poor quality of life for a large number of individuals. The *Diagnostic and Statistical Manual of Mental Disorders*, 5th ed. (*DSM-5*) has reclassified the anxiety disorders into three major categories: anxiety disorders, obsessive-compulsive disorder (OCD) and related disorders, and trauma-related disorders including post-traumatic stress disorder (PTSD). Although specific symptoms vary, all three of the major categories have anxiety or more severe stress response as a major component. Furthermore, as you will see, there are a number of common neurobiological features, so we will continue to include OCD and PTSD in this chapter. Although the incidence of each syndrome varies, it has been estimated that 10% to 30% of Americans will suffer from debilitating anxiety at some point in their lives. The ways in which that anxiety is expressed vary greatly and include episodes of panic, phobic avoidance of anxiety-eliciting stimuli, intrusive thoughts or compulsive behaviors, and damaging negative thinking patterns. In addition, anxiety is a factor commonly associated with other psychopathology, particularly clinical depression. The link between anxiety and depression is well documented: according to the National Comorbidity Survey, 58% of patients with major depression also show signs of anxiety disorder (Ninan, 1999). Furthermore, both neurobiological and pharmacological evidence support the idea of a common link between the two. Before considering some specific psychiatric disorders in the Characteristics of Anxiety Disorders section, let's look a little closer at the neurobiology of anxiety itself. The final section of this chapter will discuss the drugs that can be used to treat anxiety disorders—barbiturates, benzodiazepines, and second-generation anxiolytics[1]—as well as some of the potential new approaches to treatment.

What is anxiety?

Most anxiety manifests as a subjectively unsettling feeling of concern or worry that is displayed by behaviors such as having a worried facial expression, as well as by bodily responses such as increased muscle tension, restlessness, impaired concentration, sleep disturbances, and irritability. In addition, activation of the sympathetic branch of the autonomic nervous system (ANS) produces increased heart rate, sweating, shortness of breath, and other signs of the "fight-or-flight response" (see Chapter 2). Anxiety can vary in intensity from feelings of vague discomfort to intense sensations of terror.

Evolutionarily, anxiety is important to survival since it warns us of danger and activates the fight-or-flight response, enabling us to cope with impending emergency. Unfortunately, many of the dangers we face in the modern world do not involve fighting off or running from predators like the saber-toothed tiger, when increased heart rate and blood pressure, elevated blood glucose, and surges of adrenaline would be beneficial. Most of the anxiety-provoking situations that we face demand instead that we restrain our aggressive impulses (wanting to attack our hostile boss), think clearly (during a difficult exam), and remain in the anxiety-producing situation (giving a speech to a large group) until a resolution occurs. In these circumstances, the fight-or-flight response is not helpful and may impair our ability to perform at our best.

Nevertheless, despite its unpleasantness, anxiety in small doses is clearly a necessary stimulus for optimum performance in many everyday situations. If it is contextually appropriate, anxiety is a highly adaptive response to threat. Anxiety before an exam encourages more study; anxiety before public speaking forces us to practice our presentation one more time; anxiety before a first date prompts us to recheck our plans for the evening. Regardless of whether we are students, factory workers, or business people, anxiety boosts our energy level and pushes us to work harder and longer. But sometimes we experience too much of a good thing. When anxiety increases beyond a certain level, performance deteriorates noticeably, particularly on complex tasks. What begins as increasing alertness and focus becomes preoccupation with our own agitation that distracts us from our task. The ANS prepares our bodies for emergency; our muscles are tense, and we may suffer from digestive problems, sleep disturbances leading to fatigue, and psychosomatic illness. The overanxious student often cannot focus on an important exam, because he is too preoccupied with thoughts of how awful it would be to fail. Worst of all, because high anxiety has damaged our performance, our failures provide more reason to be anxious, creating an escalating circular pattern (**FIGURE 17.1**). Once we begin to have negative feelings about ourselves and our lack of productivity, depression may develop or the initial stages of substance abuse may begin.

Brief episodes of anxiety, even when rather intense, are not likely to be harmful and may be quite rational in many situations. Acute anxiety occurs in response to real-life stressors, and symptoms occur only in response to these events. Pharmacological treatment is very effective in providing relief from the anxiety associated with major life changes such as death of a loved one, divorce, or permanent disability or with sudden stressors like major surgery that trigger intense anxiety. The anxiolytics in the benzodiazepine class are extremely effective for relieving this type of anxiety.

Although anxiety is related to the negative emotion fear, it is also somewhat different. Anxiety is

[1] An anxiolytic is a drug that reduces anxiety.

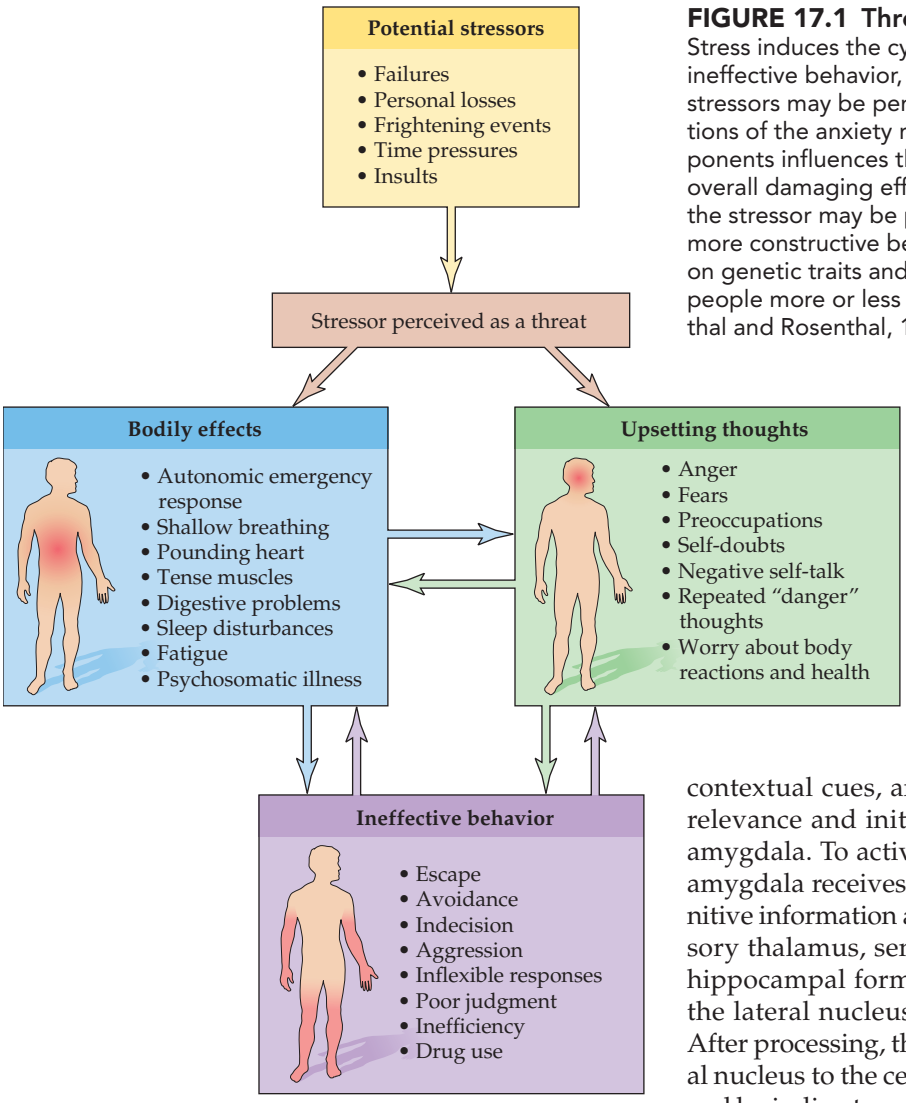


FIGURE 17.1 Three-component model of anxiety Stress induces the cyclical interaction of bodily response, ineffective behavior, and upsetting thoughts. Potential stressors may be perceived as a threat that initiates portions of the anxiety responses. Each of the three components influences the others, potentially escalating the overall damaging effect of anxiety. For other individuals, the stressor may be perceived as a challenge that engages more constructive behavior. Individual differences based on genetic traits and early experience may make some people more or less vulnerable to stressors. (After Rosenthal and Rosenthal, 1980.)

apprehension about possible future events or misfortune and concern about the ability to predict or deal with these events. In contrast, fear is an emotional response to clear and current danger, as occurs when you are confronted by a threatening bear. It is characterized by a strong urge to escape, and it elicits a strong activation of the ANS to mobilize the energy for fight or flight. These two emotions differ not only in their temporal nature, but also in terms of psychological and neurobiological consequences.

The amygdala is important to emotion-processing circuits

The amygdaloid complex, a structure deep within the temporal lobes, is a major component of several emotion-processing circuits. These circuits have many components in addition to the amygdala and include structures of the limbic cortex (insula and anterior cingulate cortex [ACC]), hypothalamus, and hippocampus. The circuits evaluate environmental stimuli, contextual cues, and cognitions that have emotional relevance and initiate appropriate responses via the amygdala. To activate these emotional responses, the amygdala receives highly processed sensory and cognitive information about the environment from the sensory thalamus, sensory and association cortices, and hippocampal formation. These brain areas project to the lateral nucleus of the amygdala (**FIGURE 17.2**). After processing, the information is sent from the lateral nucleus to the central nucleus by direct connections, and by indirect connections via the basolateral nucleus. Negative emotional stimuli activate the amygdala in healthy humans, as visualized with positron emission tomography (PET) scanning and functional magnetic resonance imaging (fMRI). This activation is significantly greater in individuals with anxiety disorders. In laboratory animals, electrical stimulation of the amygdala produces signs of anxiety and fear, while bilateral lesions produce deficits in fearful responses.

The central nucleus of the amygdala orchestrates the components of fear: ANS activation, enhanced reflexes, increased vigilance, activation of the hypothalamic–pituitary–adrenal (HPA) axis, and other responses (LeDoux, 1996). Stimulation of the central nucleus has such widespread effects because of its multiple connections with brain areas that are responsible for how emotion is expressed. For example, projections to the lateral hypothalamus activate the sympathetic nervous system, those to the periaqueductal gray cause freezing, others that activate the locus coeruleus initiate arousal and vigilance, and so forth (**FIGURE 17.3**). Damaging selected brain areas that receive projection

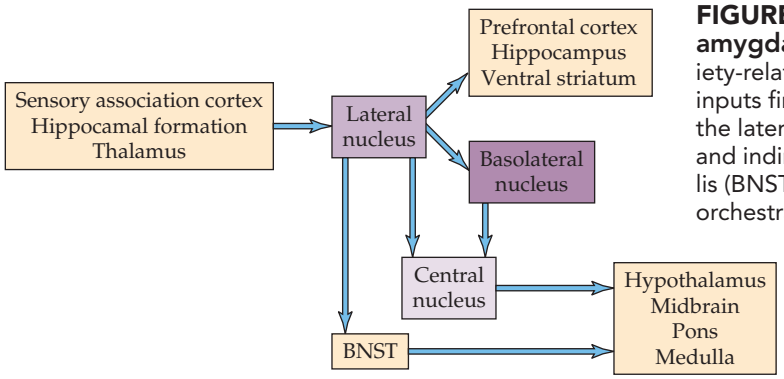
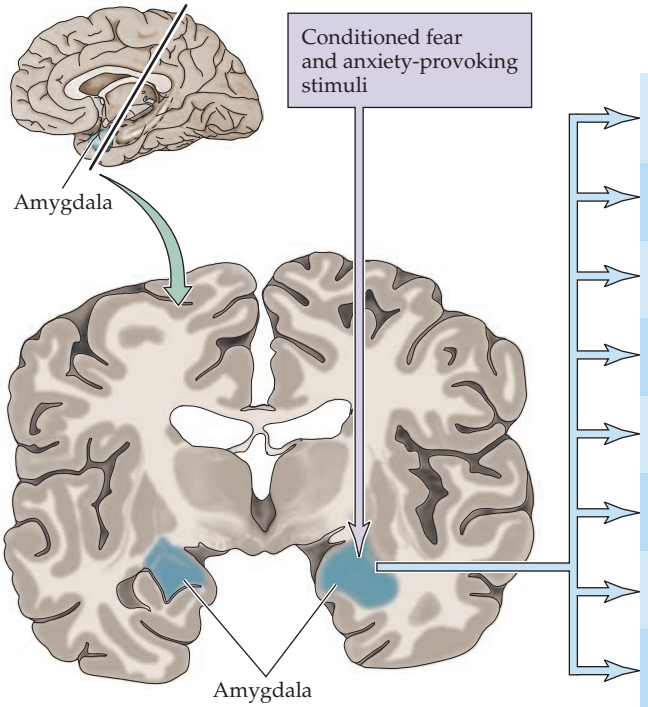


FIGURE 17.2 Flow diagram of connections of the amygdala This much simplified diagram shows how anxiety-related information processed by several brain areas inputs first to the lateral nucleus of the amygdala. From the lateral nucleus, it goes to the central nucleus (directly and indirectly) and the bed nucleus of the stria terminalis (BNST), which innervate widespread brain regions to orchestrate the components of emotion.

neurons from the central nucleus can eliminate specific components of the anxiety response, and damaging the central nucleus itself reduces or eliminates most emotional behaviors and physiological responses. Furthermore, intracerebral injection of various drugs into the amygdala reduces anxiety in a number of animal tests.

Although the amygdala plays a central role, it may be the plasticity of the connections among regions in the emotion-processing circuits that is responsible for the development of various psychopathologies. Using resting state fMRI (rs-fMRI; see Chapter 4), van Marle and colleagues (2010) evaluated regional brain connectivity in healthy human volunteers immediately *after* (not during) experiencing a stressful, violent video. They found that the stressed volunteers, compared with controls who saw nonarousing movie clips, showed enhanced connectivity between the amygdala and locus coeruleus (LC), as well as other structures in emotion-processing circuits, including the insula and anterior cingulate cortex. Although their results showed normal responses to stress, the authors suggest that such studies may provide insight into the early stages of stress-related psychopathologies because after intense stress, prolonged activation of the stress circuit may lead to an extended pathological response to trauma, including overconsolidated fear memories and hypervigilance. It also may



Brain area	Physiological effect	Component of emotional response
Lateral hypothalamus	Sympathetic activation	Increased heart rate and blood pressure, paleness, pupil dilation
Dorsal motor nucleus of vagus	Parasympathetic activation	Ulcers, urination, defecation
Parabrachial nucleus	Increased respiration	Panting, gasping for breath
VTA, LC, PPN	Activation of DA, NE, and ACh	Behavioral arousal and increased vigilance
Nucleus reticularis	Increased reflexes	Increased startle response
Periaqueductal gray	Cessation of behavior	Freezing
Trigeminal and facial nuclei	Mouth open and jaw movements	Facial fear expression
Paraventricular nucleus of hypothalamus	ACTH release	Glucocorticoid release (stress response)

FIGURE 17.3 The amygdala coordinates components of emotion The amygdala has neuronal connections with all of these brain areas that produce individual pieces of emotional expression. ACh, acetylcholine; ACTH, adrenocorticotropic hormone; DA, dopamine; LC, locus coeruleus; NE, norepinephrine; PPN, pedunculopontine nucleus; VTA, ventral tegmental area.

help to explain why individuals exposed to stress are more vulnerable to various anxiety disorders.

Although the central nucleus is vital for fear responses, evidence suggests that the physiology of anxiety may be somewhat different from that of fear (Walker et al., 2009). Although amygdaloid processing still plays a central role in the emotional circuit, the behavioral responses in anxiety may be initiated by a brain area referred to as the "extended amygdala," the bed nucleus of the stria terminalis (BNST). It is possible that each of these areas may be involved in different types of anxiety disorders. The BNST is similar to the central nucleus in anatomy, cytoarchitecture, and neurochemistry. It projects to the same brain regions as the central nucleus (see Figure 17.2) but functions somewhat differently. The central nucleus plays a role in the fear response when threatening stimuli are distinct cues that appear suddenly and predict an aversive event, which also ends abruptly. This is modeled by conditioned fear-potentiated startle paradigm in rats (see Chapter 4). In contrast to the rapid response system mediated by the central nucleus, the BNST initiates components of the emotional response when the stimuli are less precise predictors of a potentially dangerous situation. It produces a state of sustained preparedness for an unclear danger and a prolonged period of anticipation that something unpleasant might occur. In addition, this response persists long after the initial stimulus is ended and resembles the state of anxiety.

Anxiety in several animal tests (see Chapter 4) is modulated by manipulations of the BNST. One such manipulation is optogenetic control of that brain region. Activation of the BNST in mice expressing the light-sensitive protein channelrhodopsin-2 reduced exploration in the open field test and the open arms in the elevated plus-maze. In contrast, optogenetic-induced inhibition reduced the anxiety-like behaviors in those tests. The researchers further investigated the microcircuitry, using a combination of optogenetics and electrical recording. They found that subregions of BNST with distinct projections have opposite effects in modulating anxiety. Those multiple projections may explain how the various features of the anxious behavioral state are selected and coordinated for a given situation (Kim et al., 2013). It is especially interesting to note that chronic restraint stress or chronic unpredictable stress increases dendritic length and branching, as well as volume of the BNST. This stress-induced plasticity was accompanied by increased anxiety in the elevated plus-maze. It is tempting to speculate that similar stress-induced plasticity may increase vulnerability to anxiety disorders. Additionally, the increased vulnerability is significant because BNST-dependent anxiety is modulated by sex hormones and may explain gender differences in anxiety disorders. Gender differences in stress response are discussed more fully later in this section.

In addition to identifying the emotional significance of events, the amygdala aids in the formation of emotional memories, sometimes called conditioned fear or conditioned emotional response (CER). An emotional memory, or CER, involves making an association between an environmental stimulus and an aversive stimulus, for example, the sound of a tone preceding the onset of a brief electrical shock. After only a few pairings, hearing the tone will lead to elevated heart rate, rapid breathing, secretion of stress hormones, and other signs of anxiety. Emotional memories are established quickly and are long lasting. Formation of the classically conditioned CER is significant for survival because it allows an animal to anticipate danger and to be prepared physiologically and behaviorally to cope with the situation. The association is formed in the lateral amygdala, which activates the central nucleus that in turn activates those brain areas responsible for individual components of the response. Although this emotional memory is important for survival, it is clear that if the emotional response generalizes to many similar stimuli, a chronic state of anxiety could result. Of special significance is the fact that emotional memories can be learned vicariously; that is, we do not have to have personal experience with every aversive event but can learn to fear by watching another person experience fear or harmful consequences. Although vicarious conditioning is generally highly beneficial, can you think of times when it is harmful?

The amygdala also contributes to the enhancement of memory consolidation through its connections with the hippocampus (shown in Figure 17.2). Memories of events and their contexts are established in the hippocampus, and memory consolidation is significantly improved for events with strong emotional relevance. In addition to memory consolidation, the hippocampus may have a role in some anxiety disorders because reciprocal connections with the amygdala modulate emotional responses on the basis of context. The anxious behaviors of people suffering from the disorders described in this chapter are not abnormal in character, but they are expressed in contextually inappropriate situations (Davidson et al., 2000).

Although activation of the amygdala elicits emotional responses, the prefrontal cortex (PFC), particularly the orbitofrontal and medial prefrontal, and the subgenual anterior cingulate cortex exert inhibitory control over the more primitive responses of the subcortical regions. Without control by the PFC, which is responsible for planning, decision making, and evaluating consequences of behavior, the anxiety response produces more limited patterns of behavior that may not be suitable for coping with modern stressors that are not resolved by fighting or running away. Furthermore, the medial PFC is important for fear extinction, that is, for learning that a cue that once predicted danger

no longer does so. The disorders in this chapter are frequently considered to arise from an imbalance between emotion-generating centers and higher cortical control (see the section that describes the characteristics of anxiety below). Neuroimaging has become increasingly important in generating neurocircuitry models of the disorders (see Farb and Ratner, 2014; Martin et al., 2010 for reviews of this important topic).

Multiple neurotransmitters mediate anxiety

Neurobiological hypotheses of anxiety disorders have been tested in animal studies and in clinical evaluation. The anatomical complexity of emotion and its importance to survival makes it highly likely that many neurotransmitters modulate the anxiety response.

ROLE OF CORTICOTROPIN-RELEASING FACTOR IN ANXIETY Corticotropin-releasing factor (CRF; sometimes called corticotropin-releasing hormone, or CRH) is a small neuropeptide that controls the neuroendocrine (HPA axis), autonomic, and behavioral responses to stress. As you know from Chapter 2, the HPA axis is activated by the release of CRF from the paraventricular nucleus of the hypothalamus in response to stress. CRF is responsible for inducing the anterior pituitary to release the stress hormone adrenocorticotropic hormone (ACTH) into the blood, which in turn increases the release of glucocorticoids such as cortisol from the adrenal cortex. These hormones induce a variety of physiological changes that provide the means to adapt to environmental challenges. In addition, elevated cortisol initiates a negative feedback loop by binding to glucocorticoid receptors on the hippocampus, hypothalamus, and pituitary, which inhibits HPA axis function and brings cortisol levels back to normal. In addition to its role in stress hormone regulation and body physiology, CRF acts as a neurotransmitter in neural circuits involved in the stress response including the amygdala (**FIGURE 17.4**). Therefore, the release of this tiny peptide may help coordinate the distinct behavioral strategies needed to adapt to stressful events and hence may contribute to disorders with prominent signs of anxiety (see Joels, 2011).

Intraventricular administration of CRF stimulates the sympathetic nervous system and causes increases in plasma adrenaline and increased heart rate and blood pressure. These effects are not due to HPA axis activation, because removing the adrenal gland does not change this response to cerebral CRF. Therefore, the effects must be mediated by extrahypothalamic neural control such as excitation of the LC (see the following section on the role of norepinephrine). The dual role for CRF means that the endocrine response to stress via the HPA axis is well coordinated with the autonomic and adaptive cognitive portion of the stress response. CRF also has wide-ranging effects on

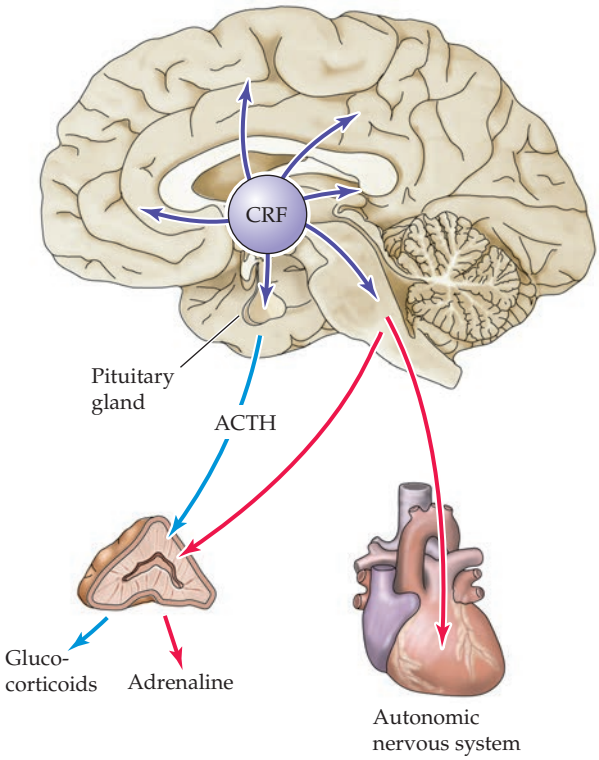


FIGURE 17.4 Dual role for CRF Corticotropin-releasing factor (CRF) is released by the hypothalamus to act on the pituitary gland to control the release of glucocorticoids from the adrenal cortex (HPA axis) in response to stress (blue pathway). CRF also acts as a neurotransmitter in multiple brain areas associated with anxiety (purple) and is released following threatening stimuli. Intracerebral CRF causes behavioral signs of anxiety as well as altered autonomic nervous system function (red pathways). ACTH, adrenocorticotropic hormone.

feeding, gastrointestinal activity, and energy balance that are consistent with preparation for dealing with environmental stressors. CRF causes increased anxiety, as seen in conflict tests, social interaction, exploration in novel open fields, and the elevated plus-maze (these and other tests of anxiety are described in Chapter 4). Direct neuronal application of CRF produces strong excitatory effects in many brain areas that contain significant numbers of CRF receptors, including the hippocampus, amygdala, LC, cortex, and hypothalamus, all of which are parts of the neural circuit for anxiety. Of particular significance are the large numbers of CRF nerve endings and CRF receptors found in the amygdala. Further, individual differences in anxiety level may be due to differences in the amount of CRF in the amygdala (Davis, 1997). For example, rats that show greater "freezing" responses to stress and show enhanced physiological signs also have higher levels of CRF mRNA in the central nucleus of the amygdala compared with less anxious rat strains. Clinical studies show that CRF levels are higher in the cerebrospinal

fluid of combat veterans with PTSD than in veterans without the anxiety disorder.

Stressful stimuli such as physical restraint cause a release of CRF in the amygdala, as measured by microdialysis. The increased extracellular CRF is accompanied by a variety of signs of anxiety. Many of these effects can be prevented by pretreatment with the CRF antagonist α-helical CRF9–41. However, not all measures of anxiety are attenuated by intra-amygdaloid infusion of the CRF antagonist, which suggests that other areas within the central nervous system (CNS) such as the LC must also mediate aspects of anxiety.

CRF neurons originating in the central nucleus of the amygdala project to the LC and activate the adrenergic component of the stress response (reviewed in Valentino and Van Bockstaele, 2008). Intracranial injection of CRF increases the firing rate of cells in the LC and increases the turnover of norepinephrine (NE), as determined by the increase in the NE metabolite MHPG in the amygdala (via reciprocal connections), hypothalamus, hippocampus, and PFC. It should be no surprise that infusion of CRF into the LC produces anxiety, as measured by time spent in the novel open field. For all these reasons, the neuroendocrine and CNS effects of CRF are generating a great deal of research in neuroscience, and the neuropeptide is a target for new drug development. **Web Box 17.1** describes CRF antagonists and several other novel approaches to treating anxiety, including neuropeptide Y agonists and glutamate antagonists.

ROLE OF NOREPINEPHRINE IN ANXIETY Reciprocal connections between the amygdala and the LC provide a mechanism for generating arousal, orienting, and responding to fear-evoking stimuli. The LC is a major cluster of noradrenergic cell bodies in the dorsal pons that has a widely distributed network of axons to many brain areas involved in emotional processing, vigilance, and attention to physiologically relevant stimuli. The LC consists of several histologically differentiated neurons having distinct electrophysiological properties. One type fires at a low tonic (i.e., continuous) rate (1–2 Hz) that corresponds to general wakefulness, while other neurons show phasic bursts of activity (i.e., responding strongly, but briefly, followed by a longer period of inhibition) in response to non-noxious environmental stimuli, such as a brief tone, a flash of light, or light touch. A third type of cell responds to stressors and stress-induced CRF release with high rates (3–8 Hz) of tonic activity. McCall and colleagues (2015) evaluated the importance of neurons showing high tonic activity during stress and identified the neural mechanism responsible. They performed a series of experiments using several of the genetic (optogenetic and chemogenetic) and behavioral (open field test and elevated zero maze) techniques described in Chapter 4. Their research showed that restraint stress increased tonic activity of a discrete population of adrenergic cells in the LC. The cell firing was responsible for causing stress-induced anxiety behaviors that were prevented by chemogenetically inhibiting the LC neurons. Further, the increased LC activity was generated by photostimulation of CRF terminals of neurons originating in the central nucleus of the amygdala, demonstrating a role for CRF in stress-induced anxiety (see **BOX 17.1** for details).

Of particular interest is that the magnitude of phasic firing depends on the level of tonic activity. Hence if tonic firing changes from a moderate level to either low or high firing, phasic bursting in response to an environmental stimulus is reduced. This differential responding may reflect a role for the LC in establishing the appropriate level of arousal and sensory processing needed to produce optimal behavioral adjustments under changing conditions. For instance, during a stressful episode, visual scanning of the environment may be more beneficial than responding to a discrete sensory stimulus that would normally cause a phasic burst.

The CRF-induced high tonic firing of LC neurons is of further interest because male rats with a history of stress show sensitization to low doses of CRF. That means that lower levels of CRF, not normally significant, are able to activate the LC that in turn enhances NE release in LC target regions of the brain. That sensitization may help to explain why previous exposure to stress increases the vulnerability to anxiety disorders. Such a mechanism may also contribute to the hypervigilance seen in PTSD. However, although female rats show greater LC activation to CRF at all stages of their estrous cycle compared with male rats, they do not develop the same stress-related sensitization to CRF. It would appear that gender differences need to be considered both in diagnosis and treatment of anxiety disorders. (Refer to Valentino and Van Bockstaele, 2008, for further discussion of the regulation of stress-induced LC activity by CRF, endogenous opioids, and glutamate).

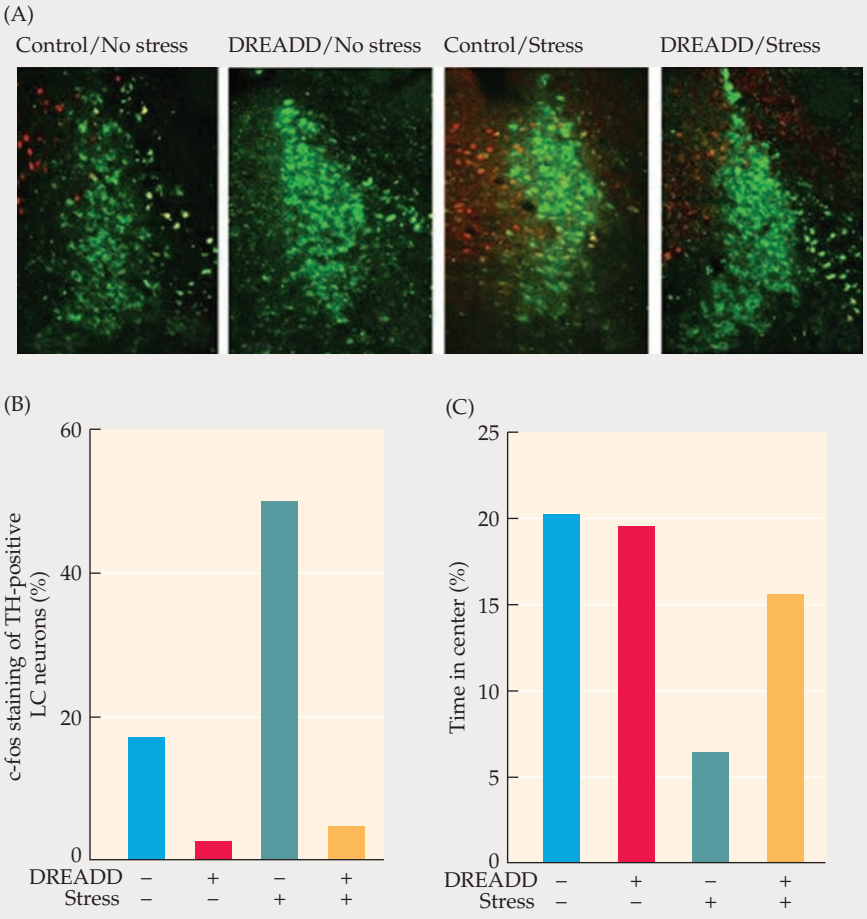
Several other lines of evidence demonstrate the importance of NE in anxiety. Numerous studies over many years have indicated the significant role of the LC as a mediator of stress-induced anxiety. Exposing animals to novel stimuli that signal threat or subjecting them to various forms of acute stressors causes increased electrical activity and enhanced expression of the immediate early gene c-fos, another indicator of neuronal activation in LC. Electrical stimulation of the LC or administration of the α_2-autoreceptor antagonist yohimbine (which increases NE release) induces a wide range of alerting and fear responses accompanied by pupil dilation, piloerection, and increased heart rate in primates. Yohimbine likewise produces panic attacks in patients with panic disorder or PTSD, but not in

BOX 17.1 The Cutting Edge

Neural Mechanism Responsible for High Tonic Cell Firing Mediating Anxiety

In their initial experiment, McCall and colleagues (2015) measured anxiety in an open field test after exposing mice to 30 minutes of restraint stress. The mice showed the expected stress-induced anxiety response; that is, they spent significantly less time in the center of the open field than nonstressed control mice. The stressed mice also showed increased c-fos expression in LC, which indicates elevated neural activity. Although in past studies the anxious behavior could be prevented by lesioning the LC, these researchers wanted to more precisely identify the neural mechanism. In order to block the stress-induced c-fos expression and anxiety, they used chemogenetics (DREADDs; see Chapter 4). To precisely target the NE cells, they genetically engineered mice to express an inhibitory DREADD *just* in those cells in the LC that were positive for tyrosine hydroxylase (TH; note that these must be the noradrenergic neurons since there are no dopaminergic neurons, which also express TH, in the LC). When this artificial receptor is present in a neuron, activation of the receptor by the drug clozapine N-oxide (CNO) leads to a powerful suppression of firing. **Figures A–C** show that when CNO was administered to the genetically engineered and control mice prior to restraint stress, not only was c-fos expression reduced (see Figures A and B), but stress-induced anxiety in the open field test was reduced to control levels (see Figure C). These results show that LC neuron activity is necessary for the anxious behavior following stress. It is interesting that the neuronal inhibition was selective for stress-induced behavior without altering other stress-related functions such as fecal output.

Although it is known that LC neurons respond to stress with increased tonic firing rate, the behavioral consequences are less clear. This question led to another set of experiments using the related technique of optogenetics. In these experiments, mice were genetically engineered to express the excitatory light-sensitive protein channelrhodopsin-2 (ChR2) selectively in the noradrenergic neurons of the LC. The mice were subsequently implanted with a fiber-optic

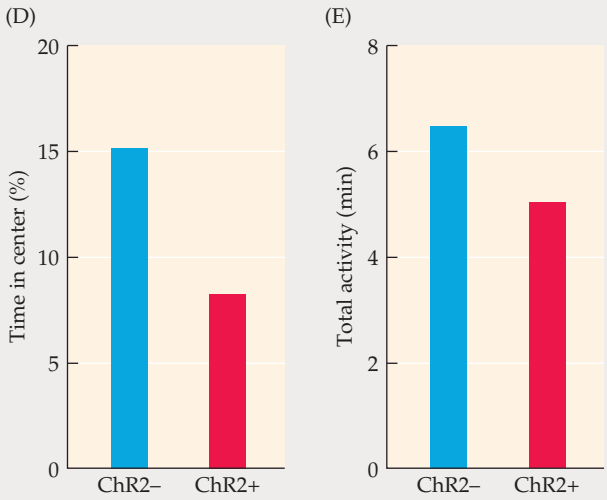


Chemogenetic inhibition of LC adrenergic neurons (A) Mice were genetically engineered to express an inhibitory DREADD, responsive to CNO, selectively in the noradrenergic neurons of the LC. Representative immunohistochemistry (IHC) shows that stress stimulated c-fos expression in the LC neurons of control mice above nonstressed levels (control/no stress versus control/stress), but the stress effect was blocked by administration of CNO to the mice genetically engineered to express the DREADD (DREADD/no stress versus DREADD/stress). Red indicates c-fos immunoreactivity; green, tyrosine hydroxylase (TH) immunoreactivity. (B) Quantification of the IHC showing significant differences between the groups. The *y*-axis corresponds to the percentage of total TH-positive neurons that also stained for c-fos. (C) DREADD-mediated inhibition of LC adrenergic neurons during stress reduces stress-induced anxious behavior in the open field test. + indicates the presence of DREADD and/or stress; – indicates the absence of DREADD and/or stress. (After McCall et al., 2015.)

BOX 17.1 The Cutting Edge (*continued*)

probe in the LC, which enabled the researchers to stimulate the ChR2-expressing cells with a laser emitting blue light with a wavelength of 473 nm. The results showed that optogenetic stimulation of the LC produced sustained (30 minutes) high rates (5 Hz) of tonic firing, similar to that seen after exposure to stress. After the 30 minutes of stimulation, the animals were placed in the open field test. The increased neural activity induced anxious behavior in the open field test *in the absence of an exogenous stressor* (**Figure D**) and also reduced overall locomotion (**Figure E**), demonstrating that exogenous LC activation is anxiogenic and the anxiety state persists after stimulation is removed. The researchers further found that light-induced anxiety depends on norepinephrine activity at β-adrenergic receptors, because the anxious behavior could be prevented by systemic pretreatment with the β-adrenergic receptor blocker propranolol, but not by the α_1-receptor antagonist prazosin.

In order to determine the mechanism responsible for the increased tonic firing, McCall and colleagues first replicated earlier studies and showed that systemic administration of a CRF receptor 1 (CRF_1) antagonist prevented stress-induced anxiety. They then optogenetically stimulated the CRF (also called CRH) terminals that projected from the central nucleus of the amygdala to the LC. The specificity of the stimulation was achieved by using mice genetically engineered to express ChR2 only in CRF neurons located in the central amygdala. When the fiber-optic probe was implanted into the LC and the laser was turned on to stimulate the ChR2-expressing terminals, the researchers found both an increase in LC neuron firing and an increase in anxious behavior in the elevated zero maze. Since a CRF_1 antagonist injected locally into the LC completely prevented the effects of the optogenetic stimulation of CRF terminals, it was clear that CRF release in LC from the amygdaloid neurons acting on CRF_1 receptors was responsible for the light-stimulated anxious behaviors, reminiscent of direct LC norepinephrine cell stimulation or stress alone.



Optogenetic stimulation of LC neurons (D) Animals expressing the excitatory light-sensitive protein channelrhodopsin-2 (ChR2+) in LC neurons spent significantly less time in the center of the open field compared with controls (ChR2–) following a period of laser light stimulation of the neurons. (E) Locomotor activity was also less in ChR2+ mice. (After McCall et al., 2015.).

healthy individuals (Ninan, 1999). A wide variety of stressors also increase release of NE in LC target regions of the brain as shown by microdialysis.

Second, clinical studies of patients with a variety of anxiety disorders suggest that abnormal ANS response is a common key feature. NE is the neurotransmitter released at the target visceral organs, including the heart, during sympathetic activation. Additionally, the catecholamine adrenaline (epinephrine), released from the adrenal medulla, produces widespread effects that prepare the individual to respond to danger. Individuals with panic disorder and PTSD have especially dramatic body responses to anxiety-provoking stimuli. Furthermore, war veterans with PTSD have higher than normal circulating NE.

Third, both NE and epinephrine have a significant role in the formation of emotional memories that may contribute to disorders in which memories of past trauma or stress can influence future behavior (as in agoraphobia seen in the chapter opener, panic, and PTSD). The normal enhancement of memory by the catecholamines in basolateral amygdala may be a way to help us remember what is emotionally significant and therefore important for future use. Fortunately, modifying NE function with drugs may represent a useful treatment for PTSD. Animal studies have shown that β-adrenergic agonists injected into the amygdala improve the consolidation of memory into long-term storage, and β-adrenergic antagonists impair the formation of emotional memories and associated physiological changes. This means that in humans, it may be possible to interfere with the formation of traumatic memories by blocking β-adrenergic receptors right after a severe trauma. It may also be possible to modify existing traumatic memories, because in addition to helping consolidate new memories, β-adrenergic agonists may

have a role in reconsolidation processes. It is believed that recalling or retrieving a memory makes it temporarily unstable and susceptible to interference that would prevent the reconsolidation needed to maintain the memory. On this basis, it was suggested that the use of β-adrenergic antagonists may disrupt the already consolidated traumatic memories and associated physiological responses of those suffering from PTSD. Although clinical findings are not entirely consistent, a number of reports show that the β-adrenergic antagonist propranolol reduces the initial consolidation of emotional memories. In one study, survivors of auto accidents who received propranolol in the emergency room and for the next 6 days developed fewer cases of PTSD, and those who developed PTSD had fewer symptoms compared with those individuals who declined the propranolol treatment (Vaiva et al., 2003). Propranolol treatment also was effective in disrupting emotional memories that had been previously consolidated and retrieved in response to a learned fear cue or personalized trauma scripts (i.e., specific reminders of the individual's traumatic experience). The β-blocker given before retrieval of the emotional memory does not seem to impair the declarative memory of the association of the conditioned and unconditioned stimuli, but it does seem to significantly diminish the emotional effects. This means that the individual would still remember the traumatic event but would not respond emotionally to associated cues (Shad et al., 2011).

In addition to the enhancement of memory following rapid activation of the basolateral nucleus by NE from LC neurons, Joels and colleagues (2011) propose that cortisol following HPA axis activation acts on G protein–coupled receptors on that nucleus (albeit some minutes later) and reinforces the establishment of emotional memories. The reviewers provide neurophysiological, behavioral, and brain imaging evidence to support the synergistic interaction. For example, corticosterone (the rodent equivalent of cortisol) administered to rodents after an emotionally arousing training session enhanced the 24-hour memory of the event. However, corticosterone had no such effect in animals that did not show arousal-induced adrenergic activation because they had been previously habituated to the experimental event. Furthermore, the corticosterone-induced memory enhancement could be prevented by β-adrenergic antagonist administration into the basolateral amygdala, indicating that NE is necessary for the memory formation but the glucocorticoid enhances its action.

One intriguing aspect of their proposal is that the slower and more prolonged genomic effects of cortisol (see Chapter 3) several hours later gradually normalize the NE-induced activity of the basolateral nucleus. Furthermore, these slower effects make the basolateral cells less responsive to further stress, possibly by allowing greater mPFC control to develop gradually. Conceivably that means that pretreatment with a cortisol-like drug administered hours before a trauma could reduce the effects of the NE surge and prevent the formation of traumatic memories. It also could mean that low levels of cortisol, as seen in individuals with PTSD, are unable to reduce the sensitivity of the basolateral nucleus of the amygdala to further stress, allowing intense adrenergic response during the re-experiencing of their traumatic event (see the section on PTSD below). The memories become intrusive and these individuals re-experience the extreme anxiety associated with their trauma over and over, both in the day as flashbacks and at night as nightmares. The proposed model may also explain the somewhat curious finding that individuals with PTSD typically have low levels of cortisol, but high levels of circulating catecholamines.

Fourth, some of the therapeutic effects of anxiolytic drugs can be explained by modulation of LC firing (Sullivan et al., 1999). Noradrenergic cells in the LC are excited by CRF synaptic input and are inhibited by γ-aminobutyric acid (GABA) and serotonin (5-HT), as well as by stimulation of α_2-adrenergic somatodendritic autoreceptors (**FIGURE 17.5**). Since benzodiazepines enhance the inhibitory function of GABA, reduced LC firing may be responsible for at least some of the anxiolytic effects of these drugs. Serotonin reuptake blockade by selective serotonin reuptake inhibitors (SSRIs) and subsequent enhancement of serotonergic function would likewise reduce LC firing and explain some of their antianxiety effects. Tricyclic antidepressants such as desipramine and monoamine oxidase inhibitors (MAOIs) that are used to treat selective anxiety disorders enhance NE function, which inhibits firing of LC neurons by acting on the α_2-autoreceptors (see Figure 17.5). In support of the therapeutic evidence, clinical studies have found abnormal autoreceptor response in individuals with generalized anxiety disorder (GAD) and social phobia (Sullivan et al., 1999).

ROLE OF GABA IN ANXIETY The inhibitory amino acid neurotransmitter GABA has a major role in modulating anxiety. GABA-induced inhibition is important for controlling the excitability of local circuits, and it regulates the activation of the central nucleus of the amygdala. Further, glutamatergic neurons from the PFC stimulate these GABA neurons in the amygdala to provide top-down control of amygdaloid activity. Hence an impaired GABA function could both lead to overactive bottom-up signaling from the amygdala, which indicates a menacing or aversive event, and hinder the top-down control by the PFC, which would fail to control the emotional impact of the stimuli. As you recall from Chapter 8, GABA is the principal inhibitory neurotransmitter in the nervous system. The $GABA_A$ receptor complex comprises a chloride (Cl^-) channel that, when opened following GABA

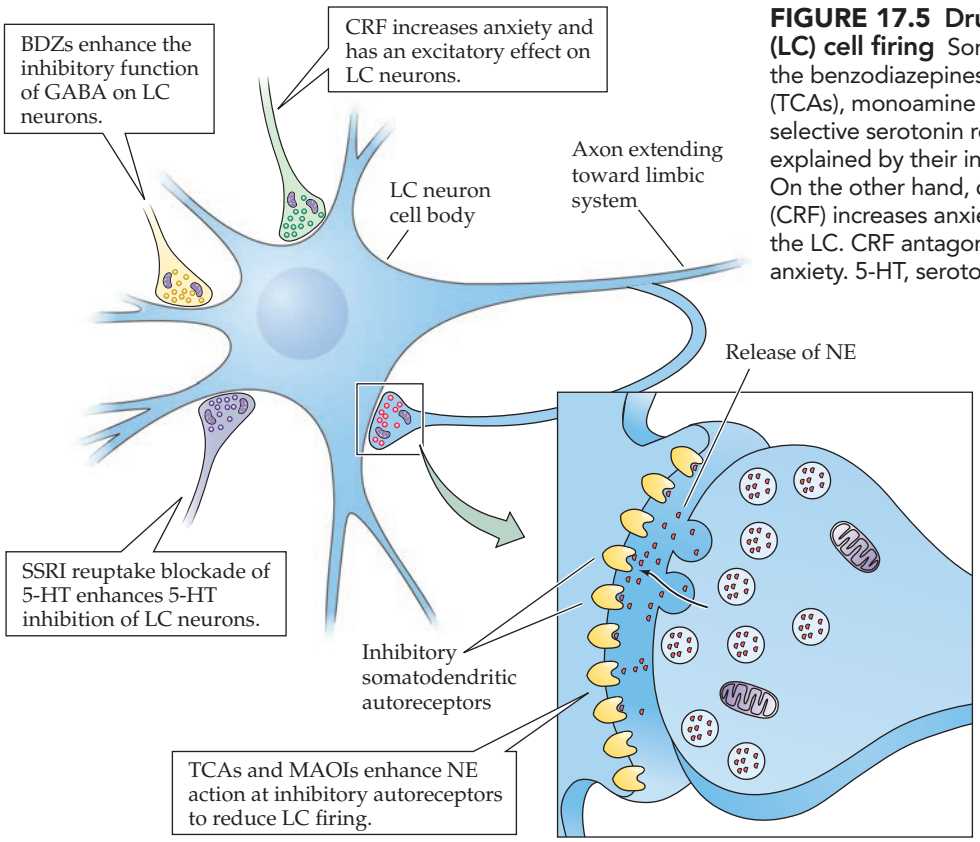


FIGURE 17.5 Drug effects on locus coeruleus (LC) cell firing Some of the anxiolytic effects of the benzodiazepines (BDZs), tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), and selective serotonin reuptake inhibitors (SSRIs) may be explained by their inhibitory effect on LC neurons. On the other hand, corticotropin-releasing factor (CRF) increases anxiety and has excitatory effects on the LC. CRF antagonists may be effective in reducing anxiety. 5-HT, serotonin.

binding, allows Cl^- to enter the cell, causing hyperpolarization. Several sedative–hypnotics enhance the function of GABA, causing sedation, reduced anxiety, and anticonvulsant effects. Benzodiazepines and barbiturates produce these effects by binding to distinct modulatory sites different from the GABA binding site on the receptor complex (further discussion of these drugs is found in the final section of this chapter). Ethyl alcohol also enhances GABA function, although its precise mechanism remains unclear (see Chapter 10). Naturally occurring neurosteroids such as allopregnanalone additionally act as positive modulators at distinct sites on the receptor complex and also produce sedative–hypnotic and anxiolytic effects. The reader is directed to Figure 8.20 for a schematic diagram of the GABA receptor complex and to an excellent, readable summary in Nuss (2015).

THE BENZODIAZEPINE BINDING SITE A great deal is known about the mechanism of action of the benzodiazepines (BDZs) because they are clinically useful GABA modulators, and their neuropharmacology has told us a great deal about the neurochemistry of anxiety. The BDZ binding sites were identified in 1977. When their location was mapped in the rat brain using autoradiography, it became clear that benzodiazepine modulatory sites are widely distributed and are found on many, but not all, GABA receptor complexes. A PET scan of BDZ modulatory sites in the human brain shows their wide distribution (**FIGURE 17.6**). Their high concentration in the amygdala and in other parts of the limbic system that regulate fear/anxiety responses, and in the cerebral cortex (particularly the frontal lobe), which exerts control over limbic structures, provides the first clue to their function.

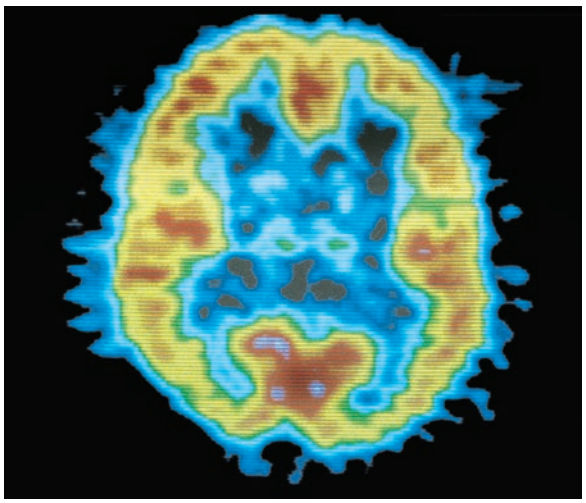


FIGURE 17.6 PET scan of benzodiazepine receptors in the human brain The highest concentrations are shown in orange and red. (Courtesy of Goran Sedvall.)

To clarify the role of the BDZ binding sites in neuropharmacological and behavioral experiments, a specific receptor antagonist, flumazenil, was developed. Flumazenil prevents the effects of BDZ binding but has no effect on the GABA receptor. A second group of substances have been found to bind to the BDZ sites and act as inverse agonists. They produce the opposite actions of the BDZ drugs, namely, increased anxiety, arousal, and seizures. One class of inverse agonists is the **β-carboline** family, which when administered to humans, produce extreme anxiety and an overwhelming sense of panic. β-Carbolines have been very useful tools in the study of the neurobiology of anxiety. These inverse agonists are presumed to uncouple the GABA receptors from the Cl^- channels so that GABA is less effective in causing entry of Cl^- into the cells (**FIGURE 17.7**), leading to increased membrane excitability.

The importance of GABA is directly shown by the reduction in anxiety produced by local administration of GABA or the GABA agonist muscimol into the amygdala. Intracranial administration of BDZs into the amygdala also has anxiolytic effects in several animal tests, including the light–dark crossing test, freezing response, elevated plus-maze, and operant conflict tests. The anticonflict effects indicative of reduced anxiety can be reversed by the benzodiazepine binding site antagonist flumazenil and also by coadministration of the GABA antagonist bicuculline into the amygdala. Furthermore, Sanders and Shekhar (1995) found that intra-amygdaloid injection of flumazenil or bicuculline also blocks *systemic* antianxiety effects of the benzodiazepine chlordiazepoxide in the social interaction test. The bicuculline effect demonstrates the necessity for GABA activity in the anxiolytic effects of BDZs. The amygdala, particularly the basolateral nucleus, is clearly an important site mediating the antianxiety effects of BDZs. However, since BDZs can still have anxiety-reducing effects following destruction of the amygdala, multiple redundant brain areas must be involved in the response to anxiety (see Davis, 2006, for an excellent summary).

Malizia and coworkers (1998) and others have shown with PET scans that patients with panic disorder show less benzodiazepine binding in the CNS, particularly in portions of the frontal lobes including the orbitofrontal cortex, medial prefrontal, and insula, as well as limbic structures involved in the anxiety neurocircuitry (**FIGURE 17.8**). These patients, compared with healthy individuals, are also less sensitive to BDZs on several psychophysiological measures such as eye movement to targets, and clinical evidence confirms that these patients require higher doses to reduce anxiety. Reductions in BDZ binding have also been found in selected brain areas of individuals with GAD and PTSD. Reduced [^{11}C]flumazenil binding in cortical areas, hippocampus, and thalamus was found in veterans with PTSD compared with veterans who did not have PTSD but had experienced similar traumatic events. From that one might conclude that trauma per se was not responsible for the reduced binding. Furthermore, care was taken to match the individuals in the two groups for age as well as the year and country of deployment, ensuring similar experiences. In addition, both groups were tested approximately 10 years after the trauma (Geuze et al., 2008). The reduced BDZ receptor binding in frontal cortex may reflect the deficits in working memory that are associated with abnormal GABA neurotransmission in that brain region. Reduced BDZ–$GABA_A$ function in hippocampus may impair hippocampal modulation of the stress response and prevent adequate evaluation of the context in which an emotional response is expressed. Nevertheless, it is not clear whether the differences reflect a predisposing characteristic in these individuals or are adaptive changes to trauma-induced stress. It seems reasonable that reduced BDZ binding sites may result in failure of GABA inhibition leading to uncontrolled

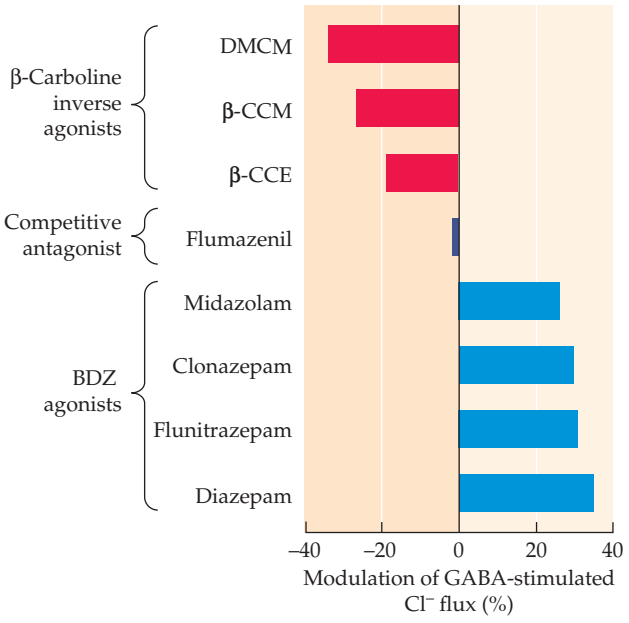


FIGURE 17.7 Modulation of GABA-induced chloride (Cl^-) flux The anxiolytic BDZs are agonists (blue bars) and enhance the effects of GABA. The competitive antagonist flumazenil binds to the BDZ receptor but has no action of its own. However, it prevents either the BDZs or the inverse agonists from acting. The anxiety-producing β-carbolines (red bars) are inverse agonists at the BDZ receptor. They not only prevent the effect of GABA on Cl^- flux but produce the opposite effect, which leads to cell excitation. β-CCE, β-carboline-3-carboxylic acid ethyl ester; β-CCM, methyl β-carboline-3-carboxylate; DMCM, methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate. (After Richards et al., 1991.)

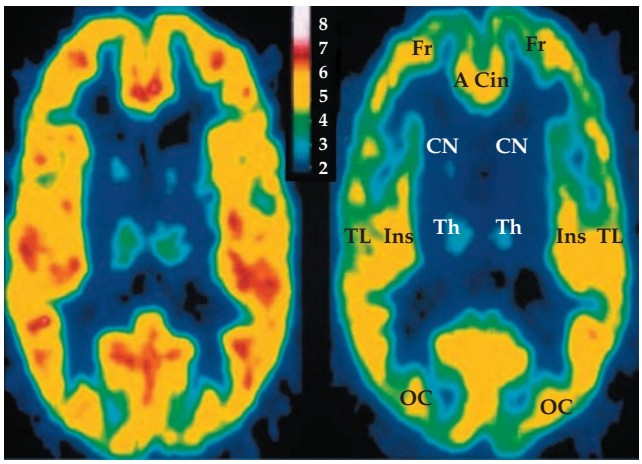


FIGURE 17.8 PET scans of a control individual (left) and a patient with panic disorder (right) The scans made in the horizontal plane with the frontal cortex at the top show decreased density of BDZ binding sites in the patient with panic disorder, particularly in the frontal cortex (Fr), anterior cingulate (A Cin), and insula (Ins), areas important in anxiety modulation. Other brain areas also show reduced numbers of BDZ binding sites. Warm colors indicate the most receptors; cool colors indicate fewer. Receptors were labeled with [^{11}C]flumazenil. CN, caudate nucleus; OC, occipital cortex; Th, thalamus; TL, temporal lobe. (From Malizia et al., 1998.)

panic attacks, phobias, generalized anxiety, and the hyperarousal of PTSD. Refer to a review by Liberzon and colleagues (2003) for more detail on the relationship between GABA and anxiety disorders based on animal studies, molecular pharmacology, and brain imaging.

ROLE OF NEUROSTEROID MODULATION OF GABA IN ANXIETY Substantial evidence indicates that neuroactive steroids provide an additional modulatory role in anxiety. A family of neurosteroids, including pregnenolone, allopregnanolone, tetrahydrodeoxycorticosterone, and dehydroepiandrosterone (DHEA), are synthesized from cholesterol in both the central and peripheral nervous systems. They are elevated by physiological stressors such as forced swim and foot shock stress and have anxiolytic effects in animal models, including the light–dark, open field, and water-lick suppression tests and the elevated plus-maze. Additionally, they have potent anticonvulsant effects. The anxiolytic effects occur following both systemic and intraventricular administration. The importance of amygdala mediation of these effects was shown by direct infusion into the central nucleus. Inhibiting the synthesis of allopregnanolone leads to increased emotional reactivity, impaired social interaction, and enhanced fear conditioning. Neuroactive steroids bind to a site on the $GABA_A$ receptor that is different from those sites described thus far and potentiate the effect of GABA by increasing the duration of GABA-induced chloride channel opening.

Surprisingly, baseline levels of neurosteroids are *elevated* in individuals with panic disorder compared with controls. However, some have suggested that the higher levels may represent a natural self-defense mechanism that protects against spontaneous panic attacks. When a panic attack was induced in these patients, the levels dropped; this signals reduced GABA control of the anxiety. Since there was no change in control individuals even when they experienced similar levels of panic anxiety, it suggests that the reduction in neurosteroid is not caused by anxiety itself but may represent the underlying pathophysiology of the disorder. In contrast, neurosteroid levels tend to be lower in individuals treated for GAD and generalized social phobia. Hence neurosteroid levels may represent a distinguishing biomarker among the individual anxiety disorders. Nuss (2015) and Schule and colleagues (2014) provide thorough discussions of the relationship of several neurosteroids to both anxiety disorders and depression. Evidence suggests that neurosteroids may be a potential target for new drug development. Unfortunately administering neurosteroids themselves does not seem feasible because they are rapidly metabolized and therefore have low bioavailability. Also, they have the potential to cause undesirable endocrine side effects. However, other potential strategies include creating synthetic neurosteroid-like agonists or manipulating neurosteroid synthesis or degradation. One such approach will be described briefly in a later section of this chapter.

ROLE OF SEROTONIN IN ANXIETY There is a rich literature on the serotonergic link with mood, suicide, violence, and impulsivity (Apter et al., 1990). Likewise, 5-HT plays a large role in anxiety modulation, although the precise role has been difficult to delineate, because of the large number of receptor subtypes and 5-HT's widespread innervation of the brain structures in the complex neurocircuitry regulating anxiety. Results from animal studies show that 5-HT modulates anxiety in a complex fashion and depends on manipulations of the particular receptor subtype, the behavioral measure of anxiety, the brain regions and neural circuits underlying the procedures, and genetic contributions (e.g., see Bauer, 2015). Among the many receptor subtypes, the 5-HT_{1A} has received the most attention. Knockout mice lacking the 5-HT_{1A} receptor represent a genetic model of anxiety, demonstrated by increased anxious behaviors in the light–dark, open field, elevated plus-maze, and novel object tests. In most cases 5-HT_{1A} agonists have anxiolytic effects in these measures of anxiety while antagonists are anxiogenic. The reduced anxious

behavior after administration of 5-HT_{1A} agonists and partial agonists is correlated with the inhibition of 5-HT cell firing and reduced 5-HT release onto postsynaptic heteroreceptors in target brain regions. Studies using intracerebral injections of 5-HT_{1A} agonists indicate that the site of injection determines the behavioral outcome. In general, direct injection of 5-HT_{1A} agonists into the hippocampus or amygdala where 5-HT_{1A} receptors are located postsynaptically increases anxious behaviors. In contrast, local injection of 5-HT_{1A} agonists into the raphe nuclei, where 5-HT_{1A} receptors are somatodendritic autoreceptors that inhibit firing rate of the serotonergic neurons and reduce 5-HT release in target regions, has an anxiolytic effect. Hence it would appear that the autoreceptors and the postsynaptic receptors have opposing roles in modulating anxious behavior (see Akimova et al., 2009; Garcia-Garcia et al., 2014).

It has long been known that inability to control a stressor, such as foot shock in animal studies, leads to amplification of a variety of anxious behaviors including greater fearfulness, less social interaction, avoidance of novel stimuli, and more submissive behaviors. In addition, the animals that have been exposed to uncontrollable stress fail to learn escape responses even when given the option. Those maladaptive behaviors do not occur in animals that are given the opportunity to control the shock (see *learned helplessness* in Chapter 4). The behavioral changes following uncontrollable shock are prevented by lesioning the dorsal raphe nucleus, indicating a role for serotonergic function. Other evidence shows that the inability to control the stressor increases the expression of the transcription factor c-fos in the dorsal raphe nucleus, indicating enhanced activation. That activation causes increased levels of extracellular 5-HT in the dorsal raphe nuclei as well as in projection regions. The intense activation makes the cells more sensitive, so the 5-HT neurons respond more vigorously and will under future challenge release more 5-HT in projection regions, causing exaggerated anxious behaviors (see Maier and Watkins, 2010). To further clarify the mechanism responsible for the increased sensitivity, researchers used extracellular single-unit recording of 5-HT neuronal activity in brain slices from the dorsal raphe nucleus that were prepared from brains of animals exposed to uncontrollable stress 24 hours before. They found desensitization of 5-HT_{1A} inhibitory somatodendritic autoreceptors in the raphe nuclei, demonstrated by the reduced effectiveness of the autoreceptor agonist ipsapirone in inhibiting cell firing (**FIGURE 17.9A**). The reduced inhibition was responsible for the increased neurotransmitter release. One intriguing possibility suggested by their research is that failure to control life stressors and the subsequent sensitizing of 5-HT neurons may make some individuals prone to developing anxiety disorders. Equally significant was the demonstration that giving the rats control over the shock (called behavioral immunization) by use of a running wheel not only prevented the exaggerated anxiety during subsequent uncontrollable stress events, but also prevented the desensitization of 5-HT_{1A} autoreceptors (**FIGURE 17.9B**; Rozeske et al., 2011). Clinical relevance of this research is supported by neuroimaging studies of individuals with panic disorder or social anxiety disorder who showed reduced 5-HT_{1A} receptor binding in the raphe nuclei, which would increase 5-HT function in limbic regions as well as in cortical circuits that regulate limbic-induced anxiety (see Akimova et al., 2009).

Another approach to understanding the role of 5-HT in anxiety is to examine the effectiveness of drug treatments. The first line of treatment for anxiety includes several SSRIs, drugs that acutely block the reuptake of 5-HT from the synapse, prolonging the neurotransmitter's

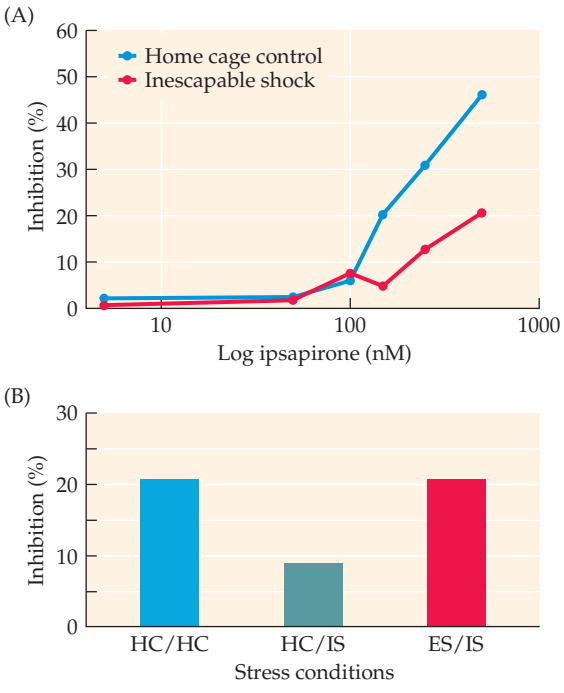


FIGURE 17.9 Autoreceptor-induced inhibition of dorsal raphe cell firing (A) Twenty-four hours after exposure to inescapable shock, rats showed less inhibition of dorsal raphe nucleus cell firing in response to the 5-HT_{1A} agonist ipsapirone compared with home cage control animals. Significant differences between the groups occurred at 150 and 250 nM doses of ipsapirone. (B) Behavioral immunization prevents desensitization of inhibitory 5-HT_{1A} autoreceptors caused by inescapable stress. Ipsapirone-induced inhibition (at 150 nM) of dorsal raphe cells was significantly reduced in rats that experienced home cage treatment followed by inescapable stress (HC/IS) compared with control rats that never experienced stress (HC/HC). Rats in the HC/IS condition also showed impaired ipsapirone-induced inhibition compared with rats provided with behavioral immunization (ES/IS), that is, those that were given control of the stressor in trials preceding an uncontrollable stress event. (After Rozeske et al., 2011.)

effects. This initial increase in 5-HT function paradoxically increases anxiety in the early phase of treatment for some individuals treated with SSRIs and also increases anxious behaviors in several animal tests including the noise-induced startle response and freezing. Of further interest was the finding that those animals that demonstrated high baseline anxiety were more likely to show robust anxiety responses to acute SSRI administration than the more laid-back rodents. Those results predict the clinical experience of patients with anxiety disorders such as panic disorder, who demonstrate increased anxiety in the early treatment phase while others show no such effect (Pettersson et al., 2015). Since the anxiolytic effects of the SSRIs require several weeks of chronic administration, it is apparent that long-term neuronal adaptations beyond the acute increase in 5-HT function are required for their clinical effectiveness (SSRI antidepressants will be discussed in Chapter 18).

In addition to the effects of 5-HT in the adult, 5-HT has a neurotrophic effect during fetal development and is critical for normal development of the anxiety circuitry. This means that appropriate regulation of both pre- and postnatal serotonergic function is needed to prevent anatomical, functional, and behavioral abnormalities. The amygdala and the PFC are two brain areas that show serotonin-dependent developmental differences in morphology and activity. It is of interest that infants and adults having a polymorphism of the promoter region of the serotonin reuptake transporter (SERT) gene show increased emotionality and anxiety. People with one or two short alleles had a significantly smaller volume of the amygdala and the subgenual anterior cingulate cortex. In addition, neuronal connectivity between the two regions was weaker. Similar reductions in white matter connections between the amygdala and several regions of the PFC were reported by others. It seems reasonable to assume that such differences may be responsible for variations in processing of emotional events. In fact, when anticipating public speaking, individuals with social phobia having one or two short alleles reported higher symptom severity and increased anxiety and showed greater neural activity in the amygdala than phobic individuals with two long alleles (Furmark et al., 2004).

Individuals having the short version of the 5-HT reuptake transporter gene express fewer reuptake transporters than those with long alleles, which means that more 5-HT remains in the synapse. Although elevated 5-HT following *chronic* treatment with SSRIs in the adult is associated with enhanced mood and reduced symptoms of depression, animal studies have shown that administration of SSRIs to animals prenatally increases anxiety and depression in the adult animal. It would seem that elevated 5-HT has distinctly different effects on anxiety circuitry depending on the maturity of the animal. It is likely that the polymorphism and the resulting elevation in synaptic 5-HT have their impact during early brain development and that this causes abnormalities in brain structure and emotional traits. Animal studies support this conclusion since similar increases in anxiety occur in knockout mice lacking SERT or 5-HT_{1A} receptors, suggesting that the 5-HT_{1A} receptor mediates the neurodevelopmental effect. In addition, reducing 5-HT reuptake during the first 2 weeks of life in rodents increases anxious behaviors in those animals as adults, indicating that the developmental effect of elevated 5-HT may extend to early postnatal periods as well. Since environmental events influence 5-HT function, it is possible that stress-induced alterations in 5-HT during prenatal or early postnatal development may in part explain why early life stress predisposes some individuals to anxiety disorders and depression later in life (more detail is provided in the section on gene–environment interactions below). For further information, the reader may refer to excellent reviews by Garcia-Garcia et al. (2014), Gross and Hen (2004), and Nordquist and Oreland (2010).

ROLE OF DOPAMINE IN ANXIETY A modulatory role for dopamine (DA) is suggested by the significant DA projections (mesocortical tract) from the ventral tegmental area (VTA) to the mPFC as well as the mesolimbic tract that connects the VTA and limbic regions including the amygdala. Stress such as forced swimming or administration of the anxiety-producing β-carboline FG7142 increases firing of mesocortical dopaminergic neurons and increases DA turnover in the prefrontal cortex. Further, the vicarious stress of observing another rat receiving foot shocks also increases DA turnover in the prefrontal neurons and elevates adrenal glucocorticoids. Enhancement of GABA function by the benzodiazepine diazepam blocks the anxiety responses to the stressors and to β-carboline treatment and also prevents the DA turnover (Kaneyuki et al., 1991).

Dopaminergic projections to the amygdala from the VTA apparently inhibit the normal descending inhibition from the mPFC and permit expression of adaptive anxiety responses. These mesolimbic DA cells are activated by stressful or threatening environmental stimuli. The released DA acting on D_1 and D_2 receptors in the amygdala reduces the inhibitory control of local GABA interneurons that are activated by the PFC. Decreasing inhibitory control increases amygdaloid activation. In support of this hypothesis, a variety of stressors increase release and turnover of DA in the central and basolateral nuclei of the amygdala, where D_2 and D_1 receptors, respectively, are most densely located. As would be expected, reducing dopaminergic transmission with neurotoxic lesions of the VTA–amygdala pathway reduces conditioned fear and fear-potentiated startle and impairs acquisition of avoidance behavior. Furthermore, intra-amygdaloid injection of D_1 receptor agonists increases anxiety

responses in a variety of rodent models, and antagonists at the D_1 receptor produce anxiolytic effects. The effects of D_2 agonists and antagonists are more difficult to interpret, since their effects vary depending on the type of fear/anxiety paradigm utilized, which may reflect the distinct role played by D_1 and D_2 receptors in fear and anxiety. De la Mora and colleagues (2010) provide an in-depth discussion of the structure and function of DA mechanisms in fear and anxiety.

Just as we saw for the LC, discrete populations of neurons in the VTA have distinctive firing patterns. Cells in the VTA have low-frequency tonic and high-frequency phasic firing properties. The phasic burst firing is increased by stress, including restraint, fear, and chronic social defeat stress, and is correlated with enhanced release of DA lasting several seconds, most notably in the nucleus accumbens (NAcc). Although the DA release may aid the animal by enhancing the salience of the environmental threat, chronic activation persisting when the threat is no longer present may predict vulnerability to dysfunctional responses in social interaction. Razzoli and colleagues (2011) exposed mice to 10 days of social defeat stress by aggressive resident mice and found the expected increase in phasic burst firing. Of special significance is that it lasted *at least 3 weeks* after the termination of the social defeat stress. Furthermore, the stressed animals at that time point still showed avoidance behaviors in the social interaction test as well as hyperphagia, that is, abnormally increased feeding. This chronic activation of VTA neurons is associated with neuroadaptive changes that may be responsible for enduring maladaptive responses to social cues. Of particular interest is that individual differences in stress responsiveness and coping behaviors exist among animals in various rodent strains. It is tempting to speculate that those animals that demonstrate the increased phasic firing and subsequent neuroadaptive modification of VTA neurons may be those most vulnerable to dysfunctional behavior, while those that do not show the prolonged changes may represent animals more resilient to stress.

Chaudhury and coworkers (2013) provided a more precise demonstration that increased phasic burst firing (but not tonic activity) is responsible for the dysfunctional behaviors occurring after social defeat stress. The researchers optogenetically activated the VTA neurons projecting to the NAcc and in real time evaluated social interaction and sucrose preference in animals previously exposed to subthreshold (i.e., a mild form that does not usually elicit abnormal behaviors) social defeat stress. Not only could they induce impaired social behaviors and reduced preference for sucrose in those animals, but mice that were initially resilient to the mild stress and showed normal VTA firing and behavior could be made to demonstrate the abnormal behaviors as much as 12 hours after optogenetic stimulation. As you might predict, optogenetic inhibition of the VTA cells projecting to NAcc could enhance the resilience of the mice.

Genes and environment interact to determine the tendency to express anxiety

Now that you have an appreciation for the complexities of the anatomy and neurochemistry of anxiety, we are left with the questions of why some individuals are more anxious than others, and why some develop anxiety disorders while others are resilient. Differences in neurosteroid levels, BDZ receptors, and 5-HT_{1A} receptors are several possible explanations described earlier. It is quite clear that there are large individual differences in the levels of trait anxiety (the enduring characteristic to experience anxiety in a variety of situations) among individuals and that this characteristic is reasonably consistent over one's lifetime. For example, evidence suggests that behavioral inhibition in young children is associated with a CRF gene polymorphism, and these individuals are more likely than average to develop social phobia and panic disorder as adolescents. Rodents also can vary in innate anxiety level, and inbred strains of rodents can be used to study the neurobiology and treatment of anxiety. Several rodent lines have been bred for anxiety as well as abnormal regulation of the HPA axis. These differences in trait anxiety levels and hormone responses indicate genetically determined brain differences, and these differences may predispose some individuals to anxiety disorders.

Another approach used to predict stress vulnerability and stress resilience among healthy individuals is the evaluation of HPA axis function by measuring both basal cortisol levels and stress-induced cortisol responses. Henckens and colleagues (2016) found in their research using healthy males that those individuals with the *highest* basal levels of cortisol were those most resilient to stress, as measured with a psychological trait questionnaire. In contrast, those individuals also demonstrated *less* stress-induced increase in amygdala activity, which the researchers contend reflects a protective function of basal cortisol that acts by reducing the hyperactivity of neural stress circuits. A neuroimaging study by the same research team had previously found that cortisol administration does indeed suppress hyperactivity in the amygdala. In contrast, individuals with the most robust cortisol response *following* stress had the strongest activation of the amygdala and showed high stress sensitivity in a psychological trait questionnaire. The proposed explanation for these findings is that stress-sensitive people (with lower basal cortisol levels) are more likely to demonstrate a hyperactive amygdala during stress and so need the greater cortisol response to help them recover. Further discussion of the potentially protective effect of cortisol and a meta-analysis of research showing an inverse relationship between cortisol levels and

the negative emotional arousal following acute stress is provided by Het et al. (2012).

As you will see, many of the anxiety disorders are more prevalent among individuals in the same family. Nevertheless, family and twin studies estimate heritability across the disorders at a modest range of 20% to 40%, which demonstrates that life experiences must interact with a genetic vulnerability in shaping the anxious individual (Smoller et al., 2009). A large volume of evidence has shown that aversive childhood experiences have multiple damaging effects on the adult body and brain and predispose the individual to health problems, such as cardiovascular disease, as well as to psychiatric disorders including depression, anxiety, schizophrenia, and others. Although the brain shows plasticity throughout the life span, animal studies suggest that adverse events occurring prenatally or during early development have the most profound effects because of maximal brain plasticity during those times.

Both animal and human studies have shown that *early* exposure to stress and neglect alters the developing brain and can produce a lifelong tendency to respond to stimuli with enhanced anxiety. Both pre- and postnatal stressors have been shown to produce behaviors resembling anxiety, such as enhanced freezing, increased fear-potentiated startle, hyperarousal, reduced feeding and increased defecation under stress, and avoidance of novel stimuli. In addition, early stress alters the programming of the HPA axis, causing a hyperactive hormonal response to challenge that persists throughout adulthood. The stability of these changes suggests epigenetic factors at work. Evidence exists for changes in DNA methylation of promoter elements that control the expression of genes within the stress circuits (see Chapter 2 for a discussion on epigenetic effects). For example, Mueller and Bale (2008) studied male offspring of mice who had been exposed to chronic, variable stressors during early fetal development (days 1 through 7 of gestation). As adults these mice not only showed heightened HPA axis responsivity to stress compared with controls (**FIGURE 17.10A**), but also showed long-term increases in the expression of CRF in the central nucleus of the amygdala (**FIGURE 17.10B,C**), indicating an enhancement of stress neurocircuitry. These adult male mice also showed decreased expression of glucocorticoid receptors in several regions of the hippocampus that are normally responsible for restoring HPA activation to normal via the negative feedback mechanism (**FIGURE 17.10D,E**). Epigenetic effects were associated with these changes, including reduced methylation of the CRF promoter region in the hypothalamus and central nucleus of the amygdala and increased methylation of the glucocorticoid receptor promoter region. These may be expected to contribute to stress circuit programming during fetal development. As you will see

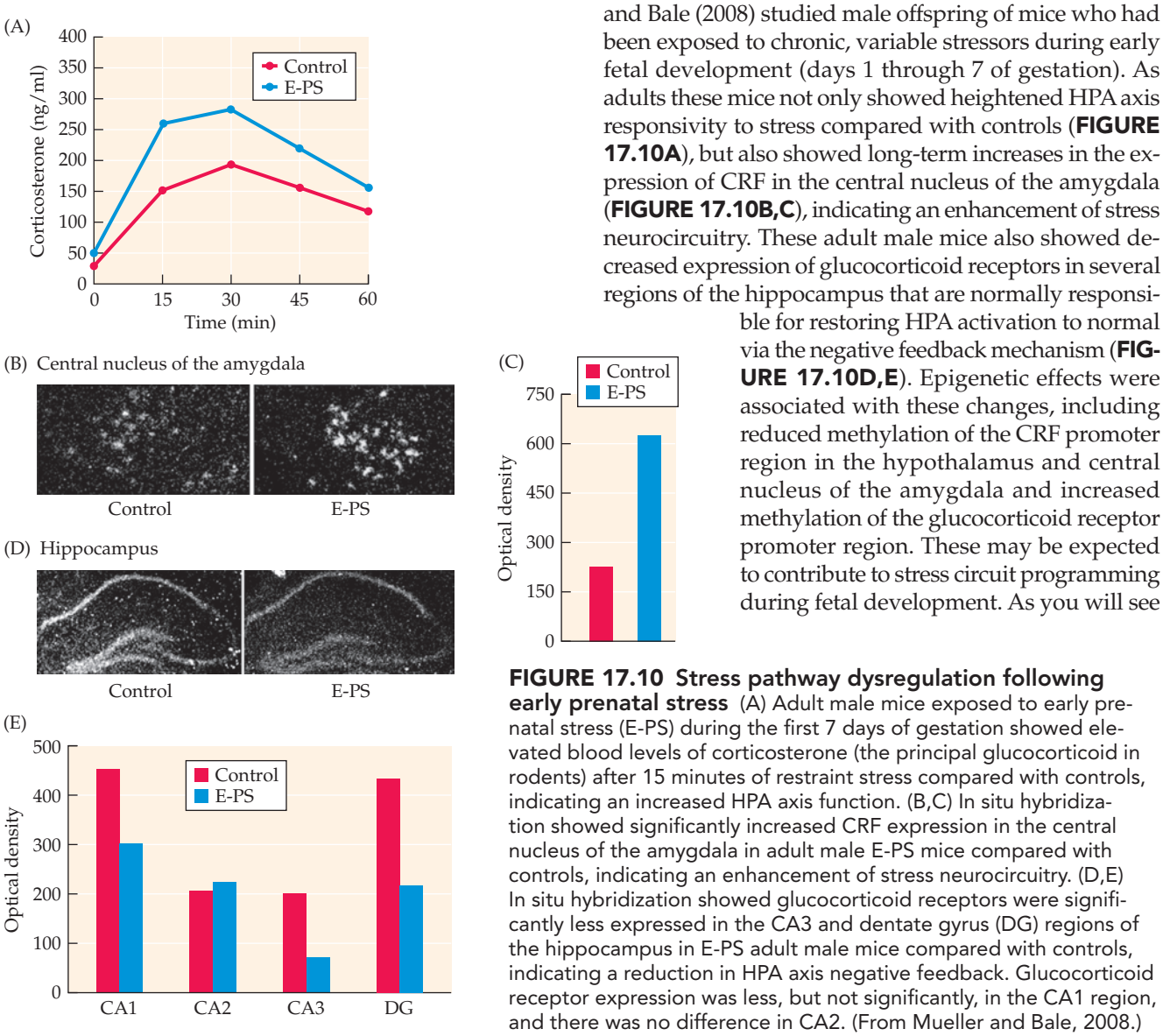


FIGURE 17.10 Stress pathway dysregulation following early prenatal stress (A) Adult male mice exposed to early prenatal stress (E-PS) during the first 7 days of gestation showed elevated blood levels of corticosterone (the principal glucocorticoid in rodents) after 15 minutes of restraint stress compared with controls, indicating an increased HPA axis function. (B,C) In situ hybridization showed significantly increased CRF expression in the central nucleus of the amygdala in adult male E-PS mice compared with controls, indicating an enhancement of stress neurocircuitry. (D,E) In situ hybridization showed glucocorticoid receptors were significantly less expressed in the CA3 and dentate gyrus (DG) regions of the hippocampus in E-PS adult male mice compared with controls, indicating a reduction in HPA axis negative feedback. Glucocorticoid receptor expression was less, but not significantly, in the CA1 region, and there was no difference in CA2. (From Mueller and Bale, 2008.)

later, postnatal stress also is capable of programming the stress responsivity, and this effect is gender specific. **Web Box 17.2** highlights the impact of early maternal neglect in determining the emotional response and hormonal reactivity of offspring to stressful events.

We are now left with the question of how epigenetic changes in the stress response might increase the probability of developing anxiety disorders. The activation of the HPA axis is critical to the survival of an animal, and destruction of the adrenal gland ultimately leads to death. However, excessive HPA activation leads to damaging effects on the body, including changes in brain structure and synaptic transmission. Prolonged stress and the subsequent elevation in glucocorticoids impair the function of the hippocampus by reducing neurogenesis, failing to protect cells against cell death (apoptosis), and preventing the normal dendritic growth and elaboration that enhance synaptic connectivity. Loss of such hippocampal plasticity is reflected in a reduction in cognitive processing and memory consolidation. Glucocorticoids also influence other brain regions that do not support neurogenesis, by preventing synaptic restructuring including dendritic elaboration and an increase in dendritic spines. Most significant to our discussion of anxiety are the changes to limbic structures that comprise the emotion-regulating circuits, including the hippocampus, amygdala, and PFC. Of interest is the finding that prolonged stress apparently produces opposite effects on the hippocampus, where synaptic structures atrophy, and on the amygdala, where dendritic growth, increased arborization, and increased spine connectivity are seen (McEwen, 2008; 2010) (**FIGURE 17.11**). These differences are reflected in behavioral effects: impaired hippocampus-mediated contextual learning, which helps an individual to distinguish between context-appropriate and context-inappropriate emotional responding, and enhanced amygdala-mediated fear conditioning. Furthermore, the medial prefrontal area that is responsible for extinguishing conditioned emotional responses shows a decrease in dendritic connections. The molecular mechanism of these effects is less well studied than in the hippocampus, but as you will see in the next section, abnormal neural activity in these brain regions is characteristic of several clinical disorders.

The effects of early stress are dependent on timing

Not only does stress affect various brain regions in a distinct fashion, but several other variables also help to determine the nature of the effects of early stress. As is true for any other teratogenic agent, an important factor is the timing of the exposure. It is apparent that periods of greatest brain development are the most sensitive to insult, but how the trajectory of brain development

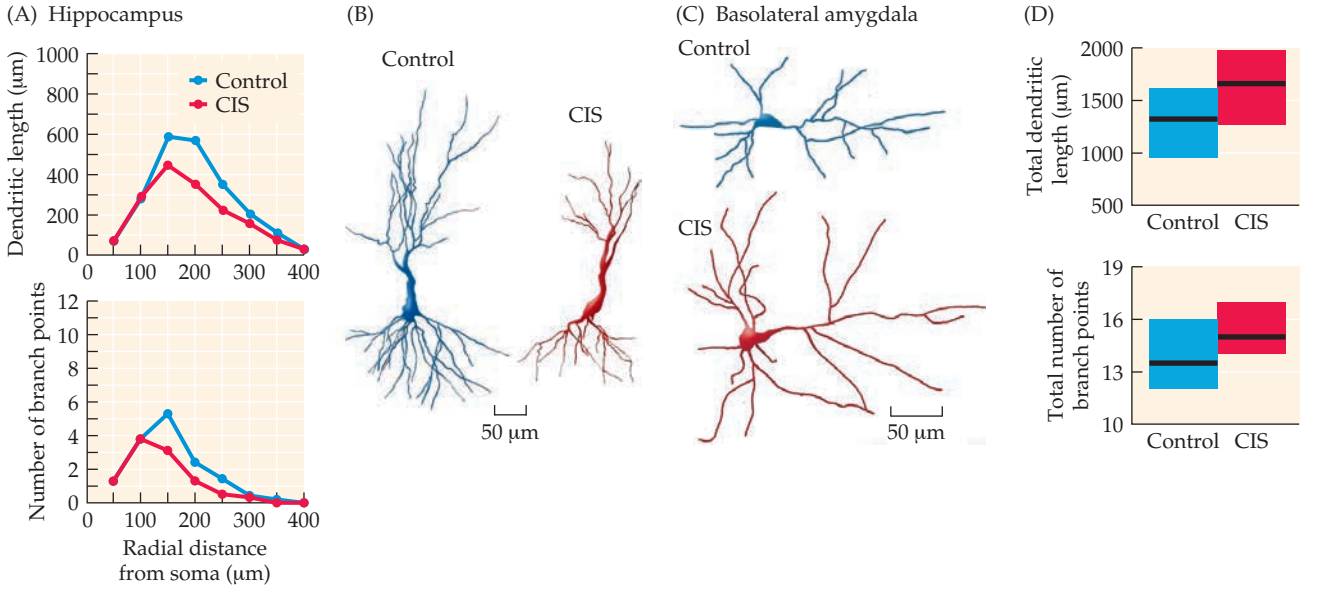


FIGURE 17.11 Effects of 10 days of chronic immobilization stress on dendritic length and complexity in the hippocampus and basolateral amygdala (A) Chronic immobilization stress (CIS) rats compared with controls showed decreased length (top) and number of branch points (bottom) of hippocampal apical dendrites. (B) Drawings of neurons processed with Golgi staining for computerized image analysis show clearly the shrinkage and reduced dendritic complexity in hippocampal neurons of stressed rats compared with controls. (C) Similar drawings of neurons from the basolateral amygdala show the increased length and branching in animals exposed to CIS compared with controls. (D) Plots of median values and ranges for the length (top) and number of branch points (bottom) of basolateral amygdala neurons from control and CIS rats. (After Vyas et al., 2002.)

will be altered depends on the particular developmental stage. Nervous system development continues throughout gestation, and most structures continue to elaborate after birth. In fact, the PFC is not fully mature until adolescence and hence is highly vulnerable throughout this period.

Although most prenatal stress research has been done using rodents, several researchers in a series of studies have provided important data using non-human primates because their stages of gestation and early brain development are more similar to those of humans (see Schneider et al., 2002). In rhesus monkeys, repeated random noise blasts several times a day during pregnancy produced offspring that were lighter in weight at birth and showed impaired motor coordination. In addition to having various motor deficits, when exposed to new stressors, the offspring showed a hyperresponsive HPA axis in the form of elevated ACTH and cortisol, heightened signs of anxiety during social separation, reduced exploration of their environment, limited play behavior, and more clinging to their peers than control monkeys. Additionally, the prenatally stressed animals showed deficits in learning a non-match-to-sample task that requires both working memory and attention shifting as the animals select the object that is different from the one previously presented, to get a food reward. Furthermore, at adolescence, the stressed monkeys demonstrated an increasing preference for ethyl alcohol compared with controls. Both physiological and behavioral effects were most pronounced when the stressor occurred during the most intense period of neuronal migration early in gestation. Since these effects also occurred in the offspring of mothers who were administered ACTH, the elevation of stress hormones, including glucocorticoids, was the likely cause of the altered fetal development. Since such experiments cannot be performed on humans, alternative approaches must be used.

One retrospective study was performed using volumetric magnetic resonance imaging (MRI) of individuals who had been sexually abused during discrete stages of development. The preliminary results showed that several brain regions had unique periods sensitive to the damaging effects of early stress. The findings suggest that hippocampal volume was reduced when childhood sexual abuse occurred during the ages of 3 to 5 and 11 to 15. The corpus callosum was reduced in women abused at 9 to 10 years of age, and the vulnerable period of the slowly developing prefrontal cortex occurred between 14 and 16 years of age (Andersen et al., 2008).

The effects of early stress vary with gender

A second significant variable that determines the effects of stress on the brain is gender. Animal studies have shown that the behavioral and hormonal effects of prenatal stress depend on the sex of the offspring. Although it has been frequently assumed that male and female brains are identical, over the past 15 years, brain imaging has shown significant differences not only in structure but also in neurotransmitter systems and function. Many studies have shown gender differences in hormonal, physiological, and behavioral responses to stress, although the results are frequently contradictory because of the types of stressors used, timing of stress, brain regions examined, hormonal changes during stages of the estrous cycle, and methodological differences. Some have suggested that on the basis of the evolutionary roles of the sexes, it is reasonable to expect differences in the ways that males and females respond to stress. While the males of many species actively respond to acute danger in the environment by fighting or running, females frequently respond by engaging in social interaction such as organizing the herd and protecting the young. These differences are important to study because they may help to explain some of the differences in the incidence and symptomology of psychiatric disorders in men and women. Important to our discussion are the epidemiological studies showing that the male-to-female ratio of lifetime prevalence rates of having any anxiety disorder is 1:1.7. Women have higher rates for each of the disorders, although the literature is not always consistent regarding social anxiety disorder. Also, there are variations in comorbidity and in the overall burden of illness (McLean et al., 2011). Although some of the differences may be explained by sociocultural influences experienced by girls, such as the greater acceptance of women expressing fearfulness, it is apparent that several brain areas in the circuitry modulating emotion respond to stress in different ways. Using fMRI, Goldstein and colleagues (2010) found that brain activity in the stress response circuitry was dissimilar in men and women when they were exposed to negative affect/high arousal pictures, but that difference was highly dependent on the phase of the women's menstrual cycle. This evidence suggests that women have a hormonal regulation of the stress response that is not found in men. Others have shown that glucocorticoid levels fluctuate in synchrony with changes in hormone levels over the menstrual cycle, demonstrating the impact of female hormones on HPA axis function. Both laboratory animal and human studies have found that when estrogen levels are elevated, sensitivity to stress is greater and glucocorticoid release is increased.

MORPHOLOGICAL DIFFERENCES IN RESPONSE TO STRESS In a series of studies, morphological and behavioral differences between the sexes were found in animal tests using chronic restraint stress, chronic foot shock stress, or glucocorticoid administration

(reviewed in McLaughlin et al., 2009). Such stressors cause the previously described reduction in hippocampal neurogenesis, shrinking of the dendritic arbor, and loss of giant dendritic spines in the CA3 region in male rodents. These neurobiological changes are accompanied by spatial memory deficits in the radial arm maze and the Morris water maze. In contrast, similar treatment of females produced neither dendritic atrophy nor memory impairment unless they had had their ovaries removed, in which case dramatic shrinkage occurred. The relative resistance of the female hippocampal cells to stress-induced damage suggests a neuroprotective effect of female hormones. Of particular interest is that in rodents, synaptic complexity in the CA1 region of the hippocampus changes over the 5-day estrous cycle in such a way that synaptic complexity gradually increases over the days when estrogen is elevated and drops when progesterone levels rise. This type of change shows very rapid effects of female hormones on hippocampal plasticity.

Chronic stress also causes shrinking and simplification of dendritic arbors as well as loss of dendritic spines in the PFC of male rats. These changes are associated with the expected deficits in extinction of fear conditioning. In contrast, in females, similar stress causes estrogen-dependent enhancement of dendritic trees of neurons in the PCF that project to the amygdala.

Finally, dendritic arborization in the amygdala of chronically stressed males is enhanced, and this is associated with enhanced conditioned fear acquisition. In humans, this enhanced fear conditioning is correlated with glucocorticoid levels in men but not in women. Although chronic stress impairs fear conditioning in women, the neural basis is not yet clear. In sum, it is quite clear that males and females respond to prenatal and postnatal stress in different ways, and those disparities may help to explain the differences in incidence and characteristics of a variety of clinical disorders, including autism, attention deficit hyperactivity disorder (ADHD), depression, and anxiety. However, it is somewhat puzzling to find that women apparently have a diminished neurobiological response to stress yet show an *increased* incidence of anxiety and mood disorders. One possible resolution to this paradox is to consider male responsiveness as an adaptive mechanism that protects against some psychiatric disorders (Altemus, 2006). For example, stress-induced impairment of hippocampal memory consolidation may serve to limit memories of a trauma and associated stimuli, thereby enhancing recovery. Alternatively, it is perhaps significant that although males have a greater neurobiological response to stress, their receptor affinity for glucocorticoids in the hippocampus is almost twice as great as in females, suggesting that the negative feedback that returns stress hormone levels to normal is more effective. Perhaps the robust response followed by rapid recovery is key to males' relative resilience to some disorders. Finally, one needs to consider that women face many significant reproductive hormonal changes over their lifetimes, including during the prenatal period, puberty, the estrous cycle, pregnancy and lactation, and menopause, each of which may alter neural circuits of emotion regulation. Because of space limitations, we cannot review all the behavioral effects of gonadal hormones that might contribute to vulnerability and resilience to anxiety disorders, but we suggest two review articles for consideration: Altemus, 2006, and Toufexis, 2007.

Section Summary

- Anxiety is a disturbing feeling of concern accompanied by bodily changes including activation of the "fight-or-flight" response that prepares an animal to cope with impending danger.
- Fear and anxiety differ in duration, psychological consequences, and neurobiology.
- Many brain regions (including the insula, cingulate cortex, hypothalamus, and hippocampus) are involved in emotion processing, but the amygdala plays a central role.
- The central nucleus of the amygdala and the BNST project widely and orchestrate the components of the emotional response. The central nucleus organizes the fear response when threatening stimuli appear suddenly and end abruptly. The BNST orchestrates components of emotion to produce sustained preparedness for unclear danger, a state resembling anxiety.
- The amygdala forms emotional memories and enhances semantic memory consolidation by the hippocampus.
- Regions of the prefrontal and cingulate cortices exert inhibitory control over the amygdala and mediate fear extinction.
- Anxiety disorders may arise from an imbalance between emotion generating brain regions and higher cortical control.
- CRF regulates stress hormone secretion and activates neuronal circuits of emotion that produce anxious behaviors in animal models.
- Noradrenergic neurons in the LC are activated by threatening stimuli and produce hypervigilance and fearfulness. Stress-induced CRF release by the amygdala causes LC neurons to fire at a high tonic rate. Other LC cells respond to non-noxious stimuli with phasic bursts of activity.
- NE, aided by adrenal cortisol, mediates the formation and reconsolidation of traumatic memories.

Elevated adrenergic function is found in some anxiety disorders. Several therapeutic drugs modulate LC firing by several different mechanisms.

- Drugs that enhance GABA function (particularly in the amygdala) indirectly via modulatory sites on the GABA receptor for barbiturates, BDZs, and neuroactive steroids reduce anxiety and seizures and produce sedation. Manipulation of neurosteroid synthesis and manipulation of degradation represent potential new drug targets.
- Low levels of BDZ modulatory sites are associated with elevated anxiety in rodents and with panic disorder, PTSD, and GAD in human patients.
- Anxiety is modulated by 5-HT in a complex fashion. 5-HT_{1A} agonists binding to somatodendritic receptors in the raphe nucleus inhibit firing and release of 5-HT at projection sites causing anxiolysis. Local injection of 5-HT_{1A} agonists into the amygdala acting at postsynaptic receptors increases anxiety. Individuals with panic disorder or social anxiety disorder show reduced 5-HT_{1A} binding in the raphe.
- Exposure to uncontrollable (compared with controllable) stress leads to increased anxious behavior that can be prevented by lesioning the raphe nucleus. A high extracellular level of 5-HT during uncontrollable stress desensitizes the somatodendritic autoreceptors in the raphe nucleus and subsequently increases the firing of projection neurons and release of 5-HT in limbic regions.
- The anxiolytic drugs that block 5-HT reuptake (SSRIs) acutely increase synaptic 5-HT and may initially increase anxiety. Chronic administration over several weeks produces neuronal adaptations that are required for clinical effectiveness.
- The neurotrophic effect of 5-HT during fetal development is needed for normal development of the anxiety circuitry. People with a polymorphism of the 5-HT transporter gene who have higher prenatal 5-HT show increased emotionality. They also have reduced volume of the amygdala and ACC and weaker connections between these structures as adults.
- Dopaminergic projections to the amygdala that are activated by threatening stimuli reduce the inhibitory control from the mPFC and increase emotional responses.
- Stress increases phasic burst firing of select cells in VTA, causing increased release of DA, particularly in the nucleus accumbens. The acute increase in DA enhances the salience of the threat, but chronic activation in the absence of threat is associated with dysfunctional behavior.
- The tendency to express anxiety is determined by both genes and environmental events. Prenatal and early postnatal exposure to stress cause epigenetic changes that alter the stress circuitry and increase the behavioral and hormonal response to stressors in the adult. Stress-induced glucocorticoids damage the hippocampus and the PFC but increase synaptic connectivity in the amygdala.
- Stress-induced brain abnormalities depend on the timing and the developmental period. Significant gender differences in stress response are found in neural activity of the anxiety circuit, in HPA response, and in morphological differences after chronic stress.

Characteristics of Anxiety Disorders

Among the disorders that are recognized as anxiety syndromes by the American Psychiatric Association, this chapter will consider the principal categories: generalized anxiety disorder, panic disorder, and several types of phobias. Posttraumatic stress disorder and obsessive-compulsive disorder, now recognized as separate categories by the *DSM-5*, are included here because of similarities in neurobiology and the significance of stress in their presentation. These disorders vary significantly in the constellation of symptoms, the precipitating stimulus, and the time course, but all include high levels of anxiety that significantly impair the quality of life for those suffering with the disorder. The maladaptive emotional responses that characterize these disorders may be a function of inadequate regulation of a hyperactive amygdala by attenuated top-down control by the PFC. Because of space limitations, this discussion will include only an introduction to each of the disorders. Students are directed to Martin et al., 2010, for an excellent review of neuroimaging, associated neurotransmitter signaling, and genetic contributions to each disorder.

GENERALIZED ANXIETY DISORDER Although acute anxiety may at times need to be treated, it generally is not long-lasting and is not considered a clinical disorder. In contrast, for some people the symptoms of anxiety have no real focus, and they can be present for much of the day and can persist for months or years. These individuals suffer from **generalized anxiety disorder** (**GAD**). Individuals with GAD show signs of constant worry and continuously predict, anticipate, or imagine dreadful events. For them, life is generally stressful, and even minor events provoke worry. Being late for an appointment, not completing a task, and making a minor mistake are all causes of worry. The most common physical symptoms include muscle tension and agitation that lead to fatigue, poor concentration, irritability, and sleep difficulties. As you might expect, the

chronic anxiety reduces the individual's performance on many tasks and decreases the pleasure derived from her efforts. GAD is one of the more common anxiety disorders, afflicting an estimated 5% of the general population between the ages of 15 and 45 (Wittchen et al., 1994). Most cases begin gradually, usually in the teens or early adulthood, and persist throughout life.

Although some genetic contribution is suggested by the fact that GAD tends to run in families, twin studies are not consistent in supporting the role of heritability. It is perhaps not surprising that patients with GAD demonstrate an increased volume of the amygdala, which resembles the hypertrophy of that structure in laboratory animals exposed to repeated stressors. In addition, during PET scans, exposure to stimuli evoking negative emotions increases amygdala and insula activity to a greater extent in individuals with GAD than in healthy individuals. It is not entirely clear whether amygdala hyperactivity is secondary to too little inhibitory control by the prefrontal cortex, but the significant reduction in temporal cortical $GABA_A$ receptors demonstrated in imaging studies would suggest that this is the case. Furthermore, other imaging studies showed reduced functional connectivity between the amygdala and PFC, and the extent of connectivity was inversely related to anxiety ratings. The weaker connectivity in combination with elevated PFC activation found in most studies may reflect enhanced attempts to cope but in a less efficient manner (reviewed by Hilbert et al., 2014). Furthermore, symptoms of GAD are reduced by drugs that enhance GABA function, such as the benzodiazepines.

FIGURE 17.12 The word panic comes from Pan
The Greek god of pastures and shepherds is represented as having the legs, horns, and ears of a goat. It was believed that if he were awakened from his nap by travelers, he would let out a blood-curdling scream that would often scare them to death.

PANIC ATTACKS AND PANIC DISORDER WITH ANTICIPATORY ANXIETY In contrast to anxiety, which is the anticipation of potential danger, fear is the physiological reaction to immediate danger that prepares us to fight or run away. When an individual experiences all the effects of a fear reaction without a threatening stimulus, he is having a **panic attack**. The sudden intense fearfulness is accompanied by strong arousal of the sympathetic ANS. The symptoms associated with panic include heart pounding or chest pain, sweating, shortness of breath, faintness, choking, and fear of losing control or dying (**FIGURE 17.12**). These symptoms, which last minutes or even hours, may occur (1) in response to a particular environmental cue (producing a phobia); (2) totally without warning in unexpected fashion; or (3) in a situation where an attack occurred previously, thus making it more likely to occur again. The latter two cases are the basis for **panic disorder**. Panic disorder usually begins in the late 20s and may last for many years, with attacks occurring at different frequencies and intensities over that time. In panic disorder, the individual experiences both panic (in the form of individual attacks) and anxiety (called **anticipatory anxiety**) over the possibility that she may have an attack in a place that is not safe, for example, in the middle of a movie theater or during a church service, where it would be embarrassing or perhaps impossible to escape. The anxiety associated with being in an "unsafe" place leads to **agoraphobia**, a fear of public places, and subsequent avoidance of many common situations (**TABLE 17.1**). Individuals with agoraphobia often lead very limited lives because they never leave the safety of their own homes. You were introduced to such an individual in the chapter opener.

Unlike some of the other anxiety disorders, a genetic predisposition for panic is well documented. The concordance rate is significantly higher in monozygotic than in dizygotic twins. Furthermore, a significant number of patients with panic disorder have parents with the same diagnosis.

It has been suggested that panic attacks represent a normal physiological response that is not regulated by appropriate feedback. It is also possible that the anxiety response is triggered too easily and may be initiated by environmental events that are not consciously processed. It is not entirely clear whether people with panic disorder have a more reactive ANS, but panic attacks can be triggered in individuals with the disorder

TABLE 17.1 Typical Situations Avoided by a Person with Agoraphobia

Shopping malls	Being far from home
Cars (as driver or passenger)	Staying at home alone
Buses	Waiting in line
Trains	Supermarkets
Subways	Stores
Wide streets	Crowds
Tunnels	Planes
Restaurants	Elevators
Theaters	Escalators

Source: After Barlow and Durand, 1995.

by a variety of stimuli that activate the ANS. These include injected lactic acid (a product of muscle exertion), caffeine, or yohimbine (an α_2-adrenergic autoreceptor antagonist) and increased amounts of carbon dioxide in the air they breathe. Because these same techniques do not elicit panic in individuals without panic disorder, they have been useful in studying the disorder in the laboratory (see Nutt et al., 1998). The ability of these agents to induce ANS arousal suggests that dysregulation of noradrenergic function may occur in the disorder. During a panic attack, blood and urine levels of both NE and epinephrine are elevated. Additionally, not only does increasing the release of NE with the α_2-adrenergic autoreceptor antagonist yohimbine induce panic in these individuals, but reducing NE release with the α_2-adrenergic autoreceptor agonist clonidine produces an anxiolytic effect. Preliminary research also suggests that genetic variation in the norepinephrine transporter gene may increase susceptibility to panic disorder. Adrenergic dysfunction in the neurons originating in the locus coeruleus would produce widespread effects including arousal and vigilance.

Anatomically, the most consistent findings suggest that individuals suffering from panic disorder have abnormalities such as small white matter lesions in the temporal lobes and enlargement of the lateral ventricles, which suggests tissue reduction. The temporal lobes are overall smaller in a significant number of patients with panic disorder, which may be accounted for by a reduction in amygdala volume and in some cases reduced hippocampal volume. As is frequently true in anxiety disorders, despite the reduced volume of the amygdala, it shows increased activity under challenge conditions. For example, during an induced panic attack, patients showed a significantly greater increase in activity of the amygdala, cingulate cortex, and insula compared with controls. Projections from the amygdala would contribute to the many bodily signs of panic, and the insula may make the individual more aware of the bodily sensations. In these panicked individuals, widespread areas of cerebral cortex, including the PFC, showed reduced function, indicating a lack of top-down control and failure of appropriate cognitive evaluation of the situation.

PHOBIAS **Phobias** involve fears that the individual recognizes as irrational. Fears may focus on specific objects or situations such as high places, closed-in spaces, water, mice, or snakes, or they may relate to social or interpersonal situations such as speaking in public (see the social anxiety disorder section below). Phobias can affect the individual's daily existence and can reduce his quality of life. Although many of us have irrational fears of things like spiders, usually we can avoid those things with little modification of lifestyle (**FIGURE 17.13**). However, for some people, an irrational fear significantly alters their daily activities. One example of such an individual is John Madden, the well-known

FIGURE 17.13 Cynophobia Individuals with cynophobia suffer signs of anxiety in the presence of dogs or even in response to items closely associated with the animals. Other animals such as snakes, mice, or cats are also frequent bases for phobias and phobic avoidance. (Courtesy of Amy Bedell.)

American sports announcer and former football coach. He suffers from claustrophobia and is overwhelmed with anxiety when traveling within the confined space of an airplane. Although he maintains a busy schedule of cross-country appearances for television, he travels only by train or on a bus designed for his use.

Although there is an almost infinite list of items that can elicit phobic anxiety (**TABLE 17.2**), what people fear is at least partially determined by culture. For instance, in the Chinese culture, *pa-leng* is a morbid fear of the cold and loss of body heat. This fear is based on the Chinese belief that yin represents the cold, dark, and energy-draining parts of life, which optimally should be balanced with yang, the warm, light, and sustaining elements. People with *pa-leng* often wear several layers of clothing even on extremely hot days.

Fortunately, phobias can usually be effectively treated with behavior therapy that involves presenting the fear-inducing stimulus in gradual increments, allowing the individual to maintain a relaxed state while confronting the source of her fear. This technique, called **behavioral desensitization**, is a common modern treatment method but may reflect an ancient Chinese proverb: "Go straight to the heart of danger, for there you will find safety." A more contemporary version utilizes exposure therapy in a virtual reality setting, which is easier than reproducing real-world situations and is more realistic than having the patient imagine the danger (see chapter opener photo). Medication for phobias is rarely needed. Regardless of the method, effective treatment reduces the hyperactivity of the amygdala, bed nucleus of the stria terminalis, anterior cingulate cortex, and insula.

TABLE **17.2** Some Common and Less Common Phobias

Phobia	Fear of
Acrophobia*	Heights
Aichmophobia	Sharp, pointed objects; knives
Ailurophobia	Cats
Algophobia	Pain
Astraphobia*	Storms, thunder, lightning
Claustrophobia*	Tight enclosures
Hematophobia*	Blood
Monophobia*	Being alone
Nyctophobia	Darkness, night
Ochlophobia	Crowds
Pyrophobia	Fire
Thanatophobia*	Death
Xenophobia*	Strangers

*Common

SOCIAL ANXIETY DISORDER Social anxiety disorder (SAD), or social phobia, is among the most common anxiety disorders, with an estimated lifetime prevalence of approximately 12%. It is characterized by extreme fear of being evaluated or criticized by others. Other symptoms are provided in **TABLE 17.3**. Those with social anxiety disorder tend to avoid most interpersonal situations or suffer extreme anxiety when these situations are unavoidable. The extreme anxiety may take the form of a panic attack. The disorder restricts many activities such as public speaking, attending parties, meeting new people, dating, using a public restroom, going to work or school, and even eating in public places. Onset is typically at a young age with almost half of affected individuals developing symptoms by age 11. The disorder is slightly more common in women and in those individuals with low self-esteem and high levels of self-criticism. Evidence suggests that cognitive therapy that modifies negative thoughts, such as the likelihood of looking foolish, plus social skills training is frequently highly beneficial. The serotonin reuptake inhibitors (SSRIs) may be prescribed along with cognitive behavior therapy for persistent symptoms. Benzodiazepines and β-adrenergic blockers that reduce autonomic nervous system arousal can be effective for controlling symptoms for a particular situation, such as giving a speech, but they are not used as an overall treatment strategy.

The amygdala is once again central to this anxiety disorder, and numerous studies have shown that the level of activation of the amygdala in response to pictures of emotional faces correlates with the severity of symptoms. There is also a significantly greater elevation in blood flow in the amygdala during public speaking compared with controls, which is normalized after successful treatment. The neurobiology of social anxiety disorder is highly reminiscent of other anxiety disorders that show increased activity not only in the amygdala, but also in other limbic areas such as the insula and hippocampus, which in this case would lead to the anticipatory anxiety and autonomic response.

POST-TRAUMATIC STRESS DISORDER Severe and chronic emotional disorders can occur after traumatic events such as war, natural disasters like hurricanes or earthquakes, terrorist attacks such as 9/11, physical assault, or auto accidents. In each case, the individual involved feels not only fear but also a sense of helplessness and horror. As many as 10,000 individuals who witnessed the terrorist attack on the World Trade Center in New York City have developed **post-traumatic stress disorder** (**PTSD**), and the soldiers returning from the wars in Iraq and Afghanistan show a particularly high rate of PTSD. Individuals with PTSD frequently experience nightmares and memories that may occur as sudden flashbacks of the traumatic event. In addition, they show increased physiological reactivity to reminders

TABLE 17.3 Emotional and Behavioral Symptoms of Social Anxiety Disorder

Fear of situations in which you may be judged
Worrying about embarrassing yourself
Concern that you'll offend someone
Intense fear of talking with strangers
Worry that others will notice that you look anxious
Fear that sweating, trembling, or having a shaky voice may cause you embarrassment
Fearing events where you might be the center of attention
Being anxious anticipating a feared activity
Spending time after a social situation finding flaws in your performance

of the trauma, sleep disturbances, avoidance of stimuli associated with the trauma, and a numbing of emotional responses for many years after the original event. Many exhibit sudden outbursts of irritability that can emotionally injure family and friends who are making an effort to be supportive. The individuals often feel detached from others and fail to experience the full range of emotions, which leads to diminished interest in life activities. In addition, the probability of attempting suicide is significantly greater in these individuals, as is the incidence of substance abuse and of marital problems, depression, and feelings of guilt and anger. Readers may want to check the Companion Website for a video interview of an individual with PTSD. Children also develop PTSD following trauma, although their symptoms are somewhat different.

Although statistics tell us that lifetime prevalence of PTSD ranges from 1% to 10% in the United States, the occurrence varies widely depending on the trauma. For example, approximately 3% of people who have experienced a personal attack, 4% to 16% surviving a natural disaster, 30% of war veterans, as many as 50% of those who have experienced rape, and 50% to 75% of prisoners of war who were torture victims develop PTSD (Yehuda et al., 1998). Given the frequent occurrence of war, starvation, forced immigration, terrorist activities, and ethnic and religious conflict occurring globally, it is painful to think of the number of cases of PTSD around the world.

Although PTSD is clearly related to the intensity of the traumatic event, some individuals seem far more susceptible than others. Clearly, not all war veterans or those in active combat develop PTSD, nor do all women who experience rape. Family studies of individuals who develop PTSD after trauma show that as many as 74% had a family history of psychopathology (PTSD, anxiety, depression, or antisocial behavior). The significantly higher concordance among monozygotic twins than dizygotic twins further supports the genetic vulnerability model. The interaction of family history and the magnitude of trauma is suggested by the fact that under conditions of high stress, people with a family history of PTSD may be only slightly more vulnerable to PTSD. However, when the magnitude of trauma is less intense, biologically vulnerable individuals are significantly more likely to show signs of the disorder. One possibility is that vulnerable individuals may perceive events as more traumatic than other individuals.

Gender also increases vulnerability, because women show an increased incidence of PTSD compared with men. An additional factor that increases the risk of not only PTSD but also anxiety disorders and associated depression is a history of chronic stress, abuse, or trauma.

Because of the central role of trauma in the etiology of PTSD, early research investigated the function of the major neuroendocrine stress pathway: the HPA axis. Daskalakis and colleagues (2015) provide a recent review of the endocrine aspects of PTSD. Many, but not all, studies found that patients with PTSD had *lower* than normal levels of blood cortisol that was associated with increases in corticotropin-releasing factor (CRF) and NE. The fact that the magnitude of the cortisol response to a stressor can predict the development of PTSD in the future suggests that low cortisol levels represent a vulnerability factor. For instance, the victims of serious automobile accidents who had low levels of cortisol after the accident were more likely to develop PTSD during the next 6 months. Unfortunately the data did not reach significance, because cortisol levels fluctuate with a circadian rhythm, and the time of the accident and subsequent blood sampling could not be controlled. Galatzer-Levy and coworkers (2014) measured the acute cortisol response of a group of police officer recruits immediately following the viewing of a video of police officers exposed to real-life trauma. This measure of cortisol was made during the officers' academic training. The researchers followed this group over their subsequent 4 years of active duty in an urban setting where they would routinely be exposed to multiple stressors and potentially highly traumatic events. The study results showed that a weak cortisol response to the trauma video predicted those individuals who would subsequently develop more chronic, nonremitting distress responses over the 4 years while those with stronger cortisol responses were resilient and demonstrated healthy adaptations to subsequent stress. The practical application of these results is that blunted cortisol response may represent a risk factor for poor adaptation to stress and may help to predict the likely outcome for individuals who may be exposed to work-related trauma, such as police, firefighters, first responders, and soldiers.

Although on the surface it seems odd to have low stress hormones associated with a stress disorder, in individuals with PTSD the normal feedback mechanism

that turns off cortisol and ACTH secretion is hypersensitive. The increased sensitivity is related to the higher density of glucocorticoid receptors, as was found in both combat veterans and civilians with PTSD compared with combat veterans and civilians without PTSD. The premature attenuation of the cortisol response apparently enhances the sympathetic nervous system activation, which is responsible for the high circulating levels of NE found in individuals with PTSD. As described earlier in the chapter (see Joels et al., 2011), the slower genomic effects of cortisol not only return the adrenergic activity of the basolateral amygdala to normal, but also makes that brain region less sensitive to further stress. Without the cortisol, the individual with PTSD has no control over the intense anxiety that occurs during the re-experiencing of the trauma.

Children who have parents with PTSD have an increased risk for PTSD and also tend to have lower than normal blood cortisol. Yehuda and colleagues (2000) showed that in the high-risk population of Holocaust survivor offspring, those who both developed PTSD themselves and had a parent with PTSD had the lowest levels of cortisol (**TABLE 17.4**). Those whose parents had PTSD but who did not themselves show PTSD had intermediate levels, and those who neither had a family history of PTSD nor had symptoms themselves had cortisol levels equal to controls. More recent work by this group (Lehrner et al., 2014) showed that not only was the cortisol response reduced intergenerationally, but the offspring also showed enhanced glucocorticoid receptor sensitivity resulting in enhanced cortisol suppression. These differences were associated with maternal PTSD, rather than paternal PTSD, in which case *reduced* receptor sensitivity has been found in offspring. These gender-based opposing outcomes argue against a genetic explanation for the transmission. The intergenerational transmission of stress response could be explained by parental modeling of behaviors or exposure of the offspring to vicarious parental trauma. However, another possible explanation for these results is that epigenetic programming may be involved in the transgenerational transmission of the trauma-related effects, even when the stress occurred years before conception. Nevertheless, these potential epigenetic modifications of glucocorticoid receptor functioning and HPA axis activity could be due to several factors. First, parenting behavior modified by symptoms of PTSD would impact the offspring's environment significantly, producing epigenetic markers. Earlier we described the role of early maternal neglect in rodents in determining the emotional response and hormonal reactivity of offspring to stressful events (see **Web Box 17.2**). Second, the maternal transmission of altered stress responsivity could also have occurred in utero if the mother exposed the fetus to high levels of glucocorticoids due to postwar symptoms. (The effects of early prenatal exposure to stress are demonstrated in Figure 17.10). Finally, the intergenerational transmission of glucocorticoid receptor functioning could have occurred from preconception maternal stress-induced epigenetic methylation of the glucocorticoid receptor gene and been passed on via gametes (eggs, or in the case of paternal transmission, the sperm or seminal fluid). The reader may want to return to the section on Transgenerational Epigenetic Transmission in Chapter 2 for further discussion.

The abnormal glucocorticoid functioning found in individuals with PTSD apparently has clinical significance because several of the biomarkers that predicted symptom improvement after 12 weeks of exposure therapy included higher bedtime salivary cortisol, lower 24-hour urinary cortisol excretion, and a particular polymorphism of the glucocorticoid receptor gene. Exposure therapy is a cognitive behavior therapy characterized by gradually approaching trauma-related memories and feelings. Furthermore, for those individuals whose symptoms improved, glucocorticoid sensitivity decreased. There was a significant correlation between the pre- and post-treatment glucocorticoid sensitivity and the pre- and post-treatment symptom scores (Yehuda et al., 2014).

Many neuroimaging studies have used patients with PTSD as participants. The most consistent finding is a reduction in the volume of the hippocampus, although differences regarding laterality of the reduction have been reported. A smaller hippocampus might explain some of the cognitive symptoms of PTSD, including deficits in short-term memory, flashbacks, and amnesia for the traumatic events in those individuals with dissociative PTSD. Since the hippocampus plays a part in contextual learning, dysfunction might also explain why individuals with PTSD respond physiologically to cues that are not directly related to trauma. Situations resembling the trauma stimuli activate the amygdala and elicit emotional responses. Normally, the hippocampus would assist in determining whether the present context is safe or resembles the original dangerous context and would inhibit the emotional response of the amygdala in the safe context. Since reduced hippocampal volume

TABLE **17.4** Average Blood Cortisol Levels in the Children of Holocaust Survivors

	Blood cortisol level
Controls	65 μg/day
Children of Holocaust survivors	
No parental PTSD; no resulting PTSD	65 μg/day
Parental PTSD; no resulting PTSD	45 μg/day
Parental PTSD; PTSD present	32 μg/day

Source: After Yehuda et al., 2000.

is found so frequently and is associated with more severe symptoms, it is tempting to assume that it is a component of PTSD. However, several pieces of evidence suggest otherwise. First, reduced hippocampal volume has been reported in traumatized burn victims who do not have PTSD, suggesting that the brain change may be a consequence of trauma, not PTSD. Second, researchers have found that individuals with PTSD have lower than average hippocampal volume very soon after traumatic events, which may mean that the smaller hippocampus was present before the trauma. A more direct evaluation utilized monozygotic twins, only one of whom suffered combat trauma and developed PTSD. The twin with PTSD had a smaller hippocampus than other traumatized GIs with no PTSD, but the identical twin with no trauma-induced PTSD also had a hippocampus with reduced volume, suggesting that the abnormality represents a vulnerability factor for PTSD (Gilbertson et al., 2002).

Since the symptoms of PTSD resemble an unregulated activation of emotional memories, the increased neural activity of the amygdala to trauma cues would be expected along with reduced inhibitory control by the PFC. In fact, both structural imaging studies and functional studies show smaller, less active anterior cingulate cortices and medial prefrontal cortices, which normally inhibit the amygdala and establish extinction of conditioned emotional responses.

OBSESSIVE-COMPULSIVE DISORDER Have you ever made an attempt to forget some peculiar, sexual, or aggressive thought and found the thought recurring over and over? Have you ever checked your alarm clock before you got ready for bed and then felt compelled to check it again and again before you climbed into the sack even though you know you set it correctly? Having experienced those normal events will help you begin to understand **obsessive-compulsive disorder** (**OCD**). However, while these examples are trivial ones, OCD is anything but trivial. It is a severe, chronic psychiatric problem that may require hospitalization, or in the most extreme cases psychosurgery, to control the symptoms. The disorder is characterized by recurring, persistent, intrusive, and troublesome thoughts of contamination, violence, sex, or religion (**obsessions**) that the individual tries to resist but that cause a great deal of anxiety, guilt, and shame. **Compulsions** are repetitive rituals considered attempts to relieve the tremendous anxiety generated by the obsessive thoughts, although they may be directly related or totally unrelated to the obsessive ideas. In the first instance, an individual may wash his hands hundreds of times a day until the skin is raw and bleeding because of an obsession about contracting a fatal disease. Other compulsions are unrelated to obsessions; they may involve meaningless repetitive acts like counting each crack in the sidewalk, jumping through doorways, or chewing each bite of food 100 times because of the belief that a family member may otherwise become fatally ill. Regardless of the compulsion, the individuals are convinced that unless their compulsive rituals are completed, disastrous consequences will occur, and they experience extreme anxiety unless they perform their compulsive behaviors. These activities are recognized by sufferers as inappropriate or irrational and consume most of their waking hours, yet they feel forced to do them against their will. The disorder causes intense emotional distress but is often left untreated because the individual is so ashamed of the symptoms that he recognizes as irrational or bizarre. Only when the symptoms become extreme is help sought. Although OCD was once considered rare, its lifetime prevalence is now estimated at 2% to 3%. A YouTube video from Johnson & Johnson interviews one young boy with OCD and discusses his cognitive behavior therapy (Johnson & Johnson, 2011).

The caudate nucleus (part of the striatum) is believed to have a central role in the neurobiology responsible for the characteristic repetitive and ritualistic thoughts and actions of OCD. Although there are disparities among studies examining the volume of the caudate nucleus in OCD, functional imaging more consistently shows increased metabolic activity in response to provocative stimuli. More significantly, imaging shows coordinated activity of the caudate with several cortical regions. These results led to the formulation of the cortico-striatal-thalamic-cortical loop (see Saxena and Rauch, 2000) as central to OCD symptomology. However, expanded circuitry models have been developed to explain some of the secondary cognitive symptoms such as selective deficits in attention and nonverbal memory. In addition, symptoms associated with subtypes of OCD (e.g., checking rituals, washing rituals, or hoarding disorder) call for dimensional models of the disorder. Nakao and coworkers (2014) review recent neuropsychological and neuroimaging studies of OCD that provide the basis for several of those neural models. **Web Box 17.3** explains more about the neurobiology of OCD.

In addition to research with humans, an increasing number of animal models are being developed to further refine our understanding of the neural circuits responsible for compulsive behavior with the hope of defining potential therapeutic drug targets (reviewed in Szechtman et al., 2017). A variety of animals apparently spontaneously engage in repetitious and meaningless behaviors such as repeated hair biting, paw licking that produces wounds, and repeated pacing of caged zoo animals, all of which model compulsive behaviors. Perhaps the best studied naturalistic model for OCD is the compulsive behavior demonstrated by a fraction of deer mice in a given population. These animals spontaneously develop purposeless, stereotyped behaviors, including running in fixed patterns, backward somersaulting, and repetitive jumping. Since the stereotypic

behaviors are not induced by pharmacological or genetic manipulations, it is likely that there is a genetic basis for their development. Hence this naturally occurring compulsive behavior provides the opportunity to study the genetics of OCD as well as the neurochemical basis of the behavior. One example of the model's usefulness in directing future research is the discovery by researchers, using in vivo microdialysis, that glutamate release increased in the striatum just before the compulsive behaviors were initiated. That finding suggests that glutamate dysfunction may be a component of OCD pathophysiology.

In addition to naturalistic models, several transgenic models have been developed. Of particular interest are SAPAP3 knockout mice that lack a postsynaptic protein normally heavily concentrated in corticostriatal synapses. These mice not only show intense, repetitive grooming activity that leads to bloody facial wounds, but also show increased anxiety in the elevated plus-maze. Electrophysiological evidence further suggests that both of these behaviors are linked to abnormal glutamate NMDA receptor function in corticostriatal, but not thalamostriatal, synapses. Transgenic models are important to drug discovery because a large number of animals with predictable abnormalities can be produced quickly and easily.

Animal models can also be used in conjunction with optogenetic or chemogenetic techniques to clarify the underlying pathophysiology of OCD-like symptoms. By optogenetically stimulating the axonal terminals in the ventromedial striatum of cells originating in the medial orbitofrontal cortex, researchers hoped to mimic the hyperactivity of a portion of the OCD loop to generate the repetitive, stereotyped behaviors characteristic of OCD. To their surprise, the anticipated increase in compulsive grooming behavior did not occur acutely. However, by stimulating the cells with laser light for 5 minutes a day for 5 to 7 days, they saw a gradual increase in the behaviors as well as a parallel increase in light-evoked firing rate. Additionally, after that series of daily stimulations, the OCD-like behaviors persisted without further optogenetic stimulation. The researchers suggested that OCD in humans may be due to even short periods of abnormal circuitry activity if repeated over time. Because chronic activation was needed to elicit the persistent grooming, it is likely that neural plasticity in the OCD loop was responsible for the delay (Ahmari et al., 2013).

Perhaps most significant to the development of effective treatment strategies in humans is the use of animal models that directly mimic performance abnormalities in patients with OCD. For instance, patients with OCD show deficits in the delayed alternation task in which they are required to change their strategy immediately after making a correct response. The nature of the behavioral task can easily be translated to animal testing in order to trace the neural networks involved and evaluate the neurochemical basis, which ultimately may lead to potential treatment that can translate to humans. The animal models described here and many more are discussed more fully and evaluated by Ahmari (2016).

Section Summary

- Anxiety disorders vary in symptoms, incidence, and time course, but all include high levels of anxiety.
- The chronic anxiety experienced in GAD is associated with enlargement and hyperactivity of the amygdala and too little inhibitory control by the PFC. Increasing inhibition with GABA agonists reduces symptoms.
- There is a genetic predisposition to sudden episodes of panic disorder. Dysregulation of adrenergic neurons in the autonomic nervous system and locus coeruleus may be involved. A genetic polymorphism of the NE transporter gene is associated with increased vulnerability to panic.
- In panic disorder, the volumes of the amygdala and the hippocampus are reduced. During a panic attack, neural activity is increased in the amygdala, cingulate cortex, and insula and is reduced in the PFC.
- The individual with panic disorder experiences intense fearfulness with autonomic activation as well as anticipatory anxiety over the concern of being observed having an attack in a public place.
- Phobias involve irrational fears of objects or situations and are best treated with behavioral desensitization.
- Social anxiety disorder involves extreme fear of being evaluated in public and is associated with increased blood flow in the amygdala during challenge that normalizes after treatment.
- Not all trauma victims develop PTSD. Genetic vulnerability factors increase the probability that PTSD will occur following a less intense traumatic event. Other vulnerability factors include female gender, lack of social support after the trauma, and a history of chronic stress or abuse.
- Low blood cortisol is a marker of vulnerability for PTSD and may be due to a hypersensitive negative feedback mechanism.
- Neuroimaging shows a reduction in hippocampal volume in patients with PTSD. It may be a consequence of trauma itself rather than of PTSD. Other reserchers have found that the reduction preceded the trauma-induced PTSD, making it a vulnerability factor.
- In PTSD the amygdala shows increased neural activity, and the anterior cingulate and the medial

PFC are less active and fail to inhibit the limbic structures.

- OCD is a severe, chronic psychiatric problem characterized by recurring, persistent, intrusive thoughts and repetitive rituals. The irrational acts of OCD must be performed to prevent extreme anxiety.
- The caudate nucleus has a central role in the pathophysiology of OCD and is one component in the dysfunctional cortico-striatal-thalamic-cortical loop.
- Animal models of OCD can be naturalistic or produced by genetic manipulations. Optogenetics and chemogenetics provide the opportunity to dissect the neural circuits more precisely than previously. Well-designed behavioral tasks using laboratory animals are needed to ensure that therapeutic drug testing in the lab can translate to clinical application in humans.

Drugs for Treating Anxiety, OCD, and PTSD

Drugs that are used to relieve anxiety are called **anxiolytics**. Many belong to the class of **sedative–hypnotics**, which is part of a still larger category, the **CNS depressants**. CNS depressants include the barbiturates, the benzodiazepines, and alcohol, and all of these drugs reduce neuron excitability. As you may know, the oldest known anxiety-reducing drug is alcohol, and it is still popular as an over-the-counter remedy for stress (see Chapter 10). However, because it is difficult to administer in accurate doses and has a very poor therapeutic index, alcohol has no medical use.

To be considered an anxiolytic, a drug should relieve the feelings of tension and worry and signs of stress that are typical of the anxious individual with minimal side effects such as sedation. As we saw in animal models described in Chapter 4, drugs in this class increase behaviors that are normally suppressed by anxiety or punishment. **FIGURE 17.14** shows the strong correlation between the effectiveness of anxiolytic drugs from several classes in a conflict procedure and the potency of these drugs in clinical trials with human patients.

Drugs that relieve anxiety often also produce a calm and relaxed state, with drowsiness and mental clouding, incoordination, and prolonged reaction time. At higher doses, these drugs also induce sleep, and they are therefore sometimes called hypnotics. At the highest doses, CNS depressants induce coma and death, although even at therapeutic doses they can be fatal if combined with other drugs (**FIGURE 17.15**). Selected sedative–hypnotics also reduce seizures and may be used to treat epilepsy. Others produce muscle relaxation as needed to treat muscle spasms, for example, following an auto accident.

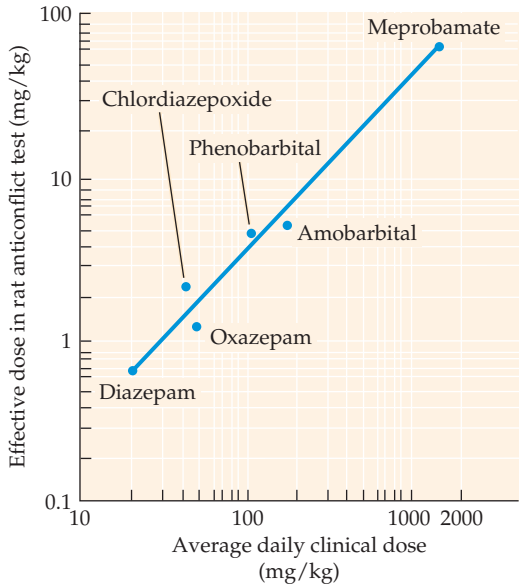


FIGURE 17.14 Correlation of drug potency in rat anticonflict test with clinical potency Drugs that increase punished behaviors in the conflict procedure test at low doses are also clinically useful for treating anxiety in patients at low doses. Less potent anticonflict drugs also require higher doses to be effective for human anxiety. The drugs tested include benzodiazepines (diazepam, oxazepam, chlordiazepoxide), barbiturates (phenobarbital, amobarbital), and a barbiturate-like anxiolytic (meprobamate). (After Cook and Sepinwall, 1975.)

Although the classic sedative–hypnotic drugs affect the functioning of many types of neurons in the CNS, their primary mechanism of action involves enhancing GABA transmission. Since GABA is the major inhibitory neurotransmitter in the nervous system, it has receptors on most cells in the CNS to exert

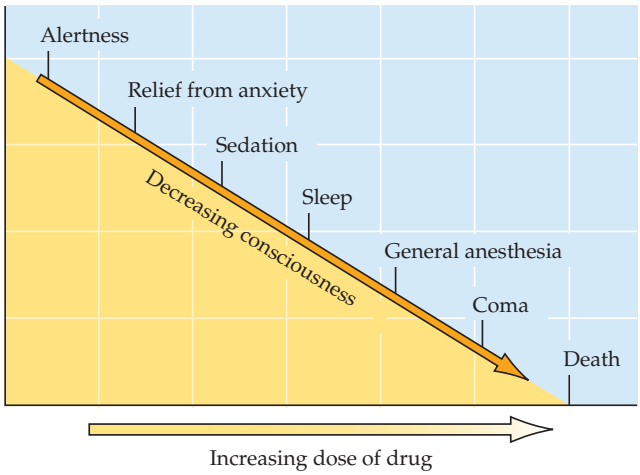


FIGURE 17.15 Dose-dependent effects of CNS depressants on levels of consciousness With increasing doses, the level of awareness and arousability gradually decreases along a continuum until death occurs.

widespread inhibitory effects. You may recall that the $GABA_A$ receptor complex regulates a Cl^- channel that increases Cl^- current into the cell to move the membrane potential farther away from the threshold for firing. Therefore, GABA agonists produce a local hyperpolarization, or inhibitory postsynaptic potential, and inhibit cell firing. As you learned earlier, both barbiturates and benzodiazepines have binding sites as part of the $GABA_A$ receptor complex and enhance the inhibitory effects of GABA. **FIGURE 17.16A** shows the hyperpolarization caused by GABA and its enhancement by diazepam.

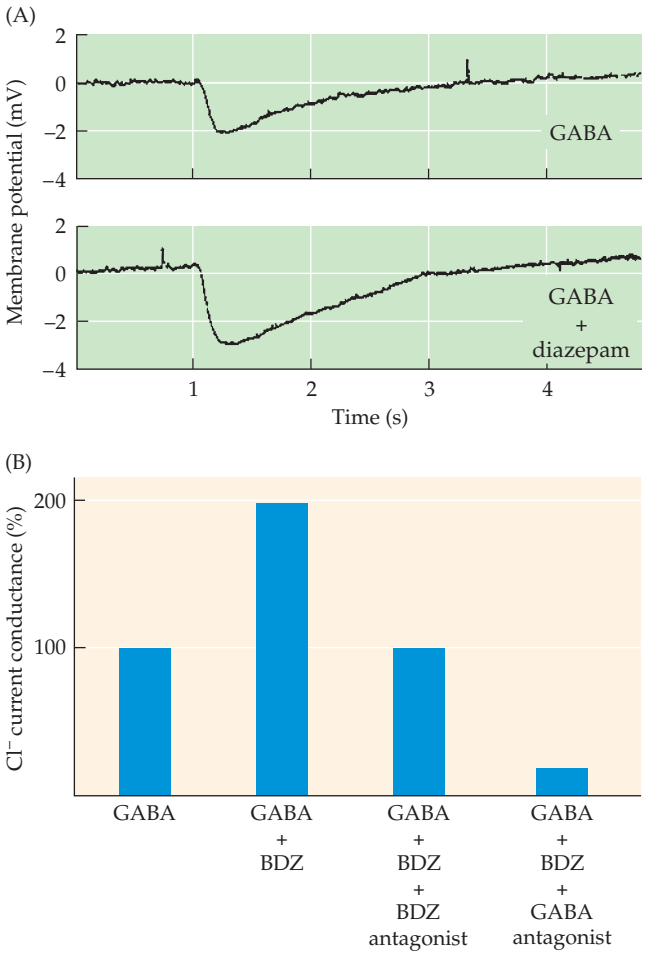


FIGURE 17.16 Effects of GABA and diazepam on membrane potentials and chloride (Cl^-) flux
(A) Electrical recording of the hyperpolarizing effect of GABA and the enhanced hyperpolarization caused by the addition of diazepam to a mouse spinal cord neuron. Diazepam alone would produce little or no hyperpolarization. (B) GABA alone increases the conductance of Cl^- through its channel. Adding a BDZ enhances the amount of Cl^- movement into the cell. Blocking the BDZ binding site prevents the drug's enhancement of GABA but not the effect of GABA itself. A GABA antagonist prevents GABA from opening the Cl^- channel, and the presence of BDZ has no effect. (After Kandel, 2000.)

When BDZs bind to their modulatory sites on the $GABA_A$ complex, they enhance the effect of GABA by increasing the number of times the channel opens. However, in the absence of GABA, the benzodiazepines have no effect on Cl^- channel opening. Apparently the presence of a BDZ alters the physical state of the receptors, increasing the receptor affinity for GABA so that GABA opens the channels more easily, shifting the dose–response curve to the left. As you would expect, the addition of the competitive antagonist flumazenil prevents the BDZ-induced enhancement of GABA action but does not affect GABA-induced hyperpolarization (**FIGURE 17.16B**). In contrast, the addition of a GABA antagonist prevents GABA from opening the channel, and the presence of a benzodiazepine has no further effect. It is generally assumed that the fact that BDZs do not enhance the maximum response to GABA is responsible for their high therapeutic index (i.e., clinical safety).

Competition experiments that measured the effectiveness of various BDZs in displacing [^{3}H]diazepam showed a positive correlation between the ability to displace the radioligand and the clinically effective dose for relieving anxiety (**FIGURE 17.17**). This means that the drugs that bind most readily to the BDZ receptor (i.e., require low concentrations to displace [^{3}H]diazepam) are also clinically effective at low doses. Likewise, BDZs that bind less easily need higher doses to be effective.

Barbiturates also increase the affinity of the $GABA_A$ receptor for GABA; however, they increase the duration of the opening of GABA-activated Cl^- channels rather than the number of openings. In addition to enhancing GABA's action at the receptor, barbiturates directly open the Cl^- channel without GABA. This additional action may explain why barbiturates can be lethal but benzodiazepines are not.

Barbiturates are the oldest sedative–hypnotics

Sodium amytal was the first barbiturate, but as many as 50 others with different profiles of bioavailability were developed. The pharmacokinetic factors of absorption, distribution, and metabolism described in Chapter 1 determine each drug's onset of effect and duration of action, and it is on this basis that the drugs are classified into three groups: ultrashort-acting, short/intermediate-acting, and long-acting. Because the barbiturates have essentially been replaced by the benzodiazepines in the treatment of anxiety disorders, our discussion of these drugs will be brief.

PHARMACOKINETICS All of the barbiturates have a similar basic ring structure but vary in the length and complexity of the side chain attached to the ring. These molecular differences are responsible for their differences in lipid solubility and determine rate of onset

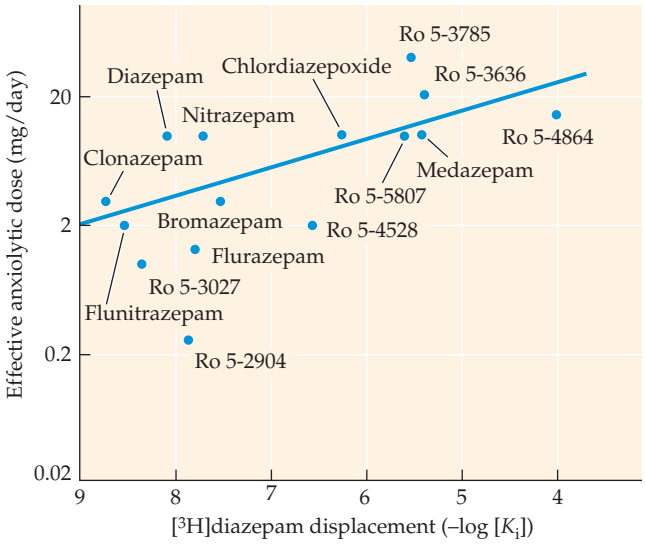


FIGURE 17.17 Correlation of benzodiazepine binding and antianxiety effect The ability of the benzodiazepines (including several experimental compounds having the "Ro" designation) to displace [^{3}H]diazepam from the BDZ binding site is correlated with the doses required for anxiolytic action. The negative log scale on the *x*-axis reflects increasing concentrations from left to right. Therefore, the benzodiazepines that displace labeled diazepam at low concentrations (e.g., clonazepam and flunitrazepam) also tend to be those that require lower doses to reduce anxiety. Those that are less effective at binding (needing higher concentrations) to the diazepam site (e.g., Ro 5-4864) also need higher doses for antianxiety effects. (After Braestrup and Squires, 1978.)

and duration of action. These pharmacokinetic factors determine their clinical uses. **TABLE 17.5** summarizes these characteristics and provides examples.

SIDE EFFECTS First, although barbiturates readily induce sleep, it is not a normal, restful sleep. The drugs alter sleep architecture by reducing the amount of REM (rapid-eye-movement) sleep and causing a rebound in REM after withdrawal.

Second, the anxiolytic effects of these drugs are accompanied by pronounced cognitive side effects including mental clouding, loss of judgment, and slowed reflexes, making driving particularly dangerous. High doses also lead to gross intoxication, staggering, jumbled speech, and impaired thinking. Coma and death due to respiratory depression occur at 10 to 20 times the normal therapeutic dose. These drugs are extremely dangerous when combined with alcohol.

Third, when used repeatedly, barbiturates increase the number of liver microsomal enzymes. This increase enhances drug metabolism, producing lower blood levels (metabolic tolerance) and reduced effectiveness. Since the same liver enzymes metabolize many other drugs, cross-tolerance diminishes the effectiveness of other drugs as well. Further, pharmacodynamic tolerance occurs when CNS neurons adapt to the presence of the drug and become less responsive with chronic drug use. Mood changes and sedation seem to show the greatest and most rapid tolerance, but the lethal respiratory depressant action of the drug does not show tolerance at all. Therefore as one gradually increases the dose of the drug needed to achieve a desired effect, the margin of safety (therapeutic index) becomes less (**FIGURE 17.18**).

Fourth, barbiturates produce significant physical dependence and potential for abuse. Terminating drug use after extended treatment produces a potentially fatal rebound hyperexcitability withdrawal syndrome similar to that for alcohol. The potent reinforcing effect of barbiturates is demonstrated by the high rate

TABLE **17.5** Duration of Action and Uses of Major Barbiturates

Duration of action	Lipid solubility	Onset	Duration	Use
Ultrashort	High	10–20 s	20–30 min	IV anesthesia
Thiopental (Pentothal)				
Methohexital (Brevital)				
Short/intermediate	Moderate	20–40 min	5–8 h	Surgical anesthesia and sleep induction
Amobarbital (Amytal)				
Secobarbital (Seconal)				
Pentobarbital (Nembutal)				
Long	Low	Over 1 h	10–12 h	Prolonged sedation and seizure control
Phenobarbital (Luminal)				
Mephobarbital (Mebaral)				

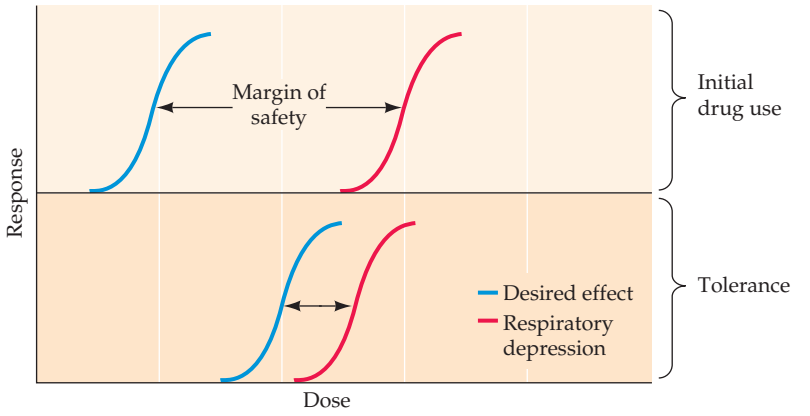


FIGURE 17.18 Margin of safety
Dose–response curves for the barbiturate-induced desired effect (mood change or sedation) and lethal respiratory depression. The top panel shows that with early drug use (nontolerant), the individual experiences mood effects without significant respiratory depression. However, as tolerance develops with repeated use (bottom panel), larger amounts of drug are needed to experience the sedation (the curve shifts to the right), but no change in the dose causing depression of respiration occurs. The margin of safety shrinks dramatically in the tolerant individual.

of self-administration found in rats and monkeys in an operant chamber, which predicts significant abuse potential in humans. It was the concern about abuse potential among patients, as well as the diversion of the prescription medications to street use along with the high incidence of side effects, potential lethality, and rapid tolerance of barbiturates, that prompted the search for a novel anxiolytic drug without these undesirable characteristics. The benzodiazepines were introduced in 1960 and in general have replaced the prescription of barbiturates. The decline in prescriptions for barbiturates over the years has made these drugs less available and has caused a parallel decline in abuse.

Benzodiazepines are highly effective for anxiety reduction

The first benzodiazepine (BDZ) to be introduced was chlordiazepoxide (Librium). It represented the first true anxiolytic that targeted anxiety without producing excessive sedation. It has a low incidence of metabolic tolerance, a less severe withdrawal syndrome than barbiturates, and a very safe therapeutic index. Within a few years, diazepam (Valium), oxazepam (Serax), flurazepam (Dalmane), and at least a dozen other chemically related drugs were developed.

PHARMACOKINETICS All BDZs have a common molecular ring structure but vary in the complexity of the side chains (**FIGURE 17.19**). Additionally, they all have a similar mechanism of action. The choice of a particular benzodiazepine for a given therapeutic situation depends primarily on the speed of onset and the duration of drug action. The onset of action is determined by the drug's lipid solubility; the most soluble are quickest to be absorbed and moved through the blood–brain barrier to initiate the drug effect, but they also readily move out of the brain and redistribute to other body tissues, producing a short duration of action. Those with moderate lipid solubility take longer to reach significant brain levels. Termination depends more on liver metabolism than on redistribution. The long-acting BDZs undergo several metabolic steps to produce multiple active metabolites that may have half-lives of 60 hours or longer (**FIGURE 17.20**). These can be problematic for elderly individuals, who may rapidly accumulate drug in the body because of their reduced metabolic capacity, increasing the probability of side effects. The short-acting BDZs, such as temazepam (Restoril) and lorazepam (Ativan), are metabolized in one step into inactive metabolites by conjugation with glucuronide. Redistribution to other body tissues reduces CNS levels of drug and also contributes to the short duration of action. The slow release from inert depots back into circulation is responsible for any drug hangover effects that might occur.

THERAPEUTIC EFFECTS Unlike barbiturates, the BDZs cannot be used for deep anesthesia, but they are useful as **presurgical anesthesics**, during which the patient is conscious but is less aware of his surroundings and is quite relaxed. They are also commonly used before major dental work as well as for a wide range of stressful diagnostic procedures. One of the newer BDZs is midazolam (Versed), used for rapid onset of relaxation and deep sleep during brief surgical procedures done with local anesthetics. Because it has a short half-life, recovery takes only a few hours without hangover. It also induces an anterograde amnesia that creates an illusion of anesthesia in some patients, which is considered a beneficial drug effect.

In other cases, however, the drug-induced amnesia is highly undesirable. Since the 1990s a BDZ that is marketed outside the United States as a sleep aid has been illegally imported and used as a "date rape" drug. Flunitrazepam (Rohypnol) is quite potent and, when combined with alcohol, impairs judgment and causes amnesia along with significant sedation. There have been a limited number of reports of women who were sexually assaulted and then found themselves in unfamiliar locations with no memory of the events surrounding the attack. Although such situations produced serious concern on college campuses and at social establishments serving

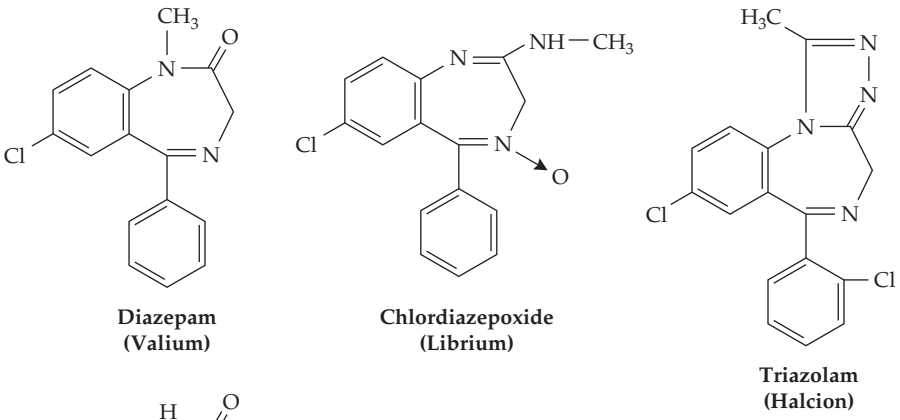


FIGURE 17.19 Molecular structure of several benzodiazepines and the benzodiazepine binding site antagonist flumazenil

alcohol, the number of documented cases is quite small and the risk is low. However, because of the illicit use, the U.S. Drug Enforcement Administration (DEA) has classified flunitrazepam as a Schedule I drug (i.e., a drug with high potential for abuse and no medical use).

The most popular use for BDZs is as an anxiolytic. Benzodiazepines relieve the sense of worry and fearfulness, as well as the physical symptoms associated with anxiety, with less mental clouding, loss of judgment, and motor incoordination than is typical of other sedative–hypnotics. Empirical evidence shows BDZ efficacy in treating the somatic symptoms associated with GAD, panic disorder, OCD, and social anxiety disorder. The mild sedation that accompanies use of some of the BDZs decreases with repeated use over a week to 10 days, but little or no tolerance occurs for the antianxiety effects. Nevertheless, in older individuals with slower drug metabolism, excessive confusion and reduced cognitive function may be quite serious and may resemble senile dementia.

Several of the longer-acting benzodiazepines are useful **hypnotics**. BDZs shorten the time needed to fall asleep and increase the duration of sleep time, as well as reduce the number of nighttime awakenings. Despite their relative safety, all sleep medications pose potential problems, such as causing reduced alertness the next day and rebound withdrawal insomnia after prolonged use (see **Web Box 17.4**). Some BDZs are useful **muscle relaxants**, and others are **anticonvulsants** for the management of particular forms of epilepsy. Intravenous diazepam is the treatment of choice for status epilepticus, a period of severe and persistent

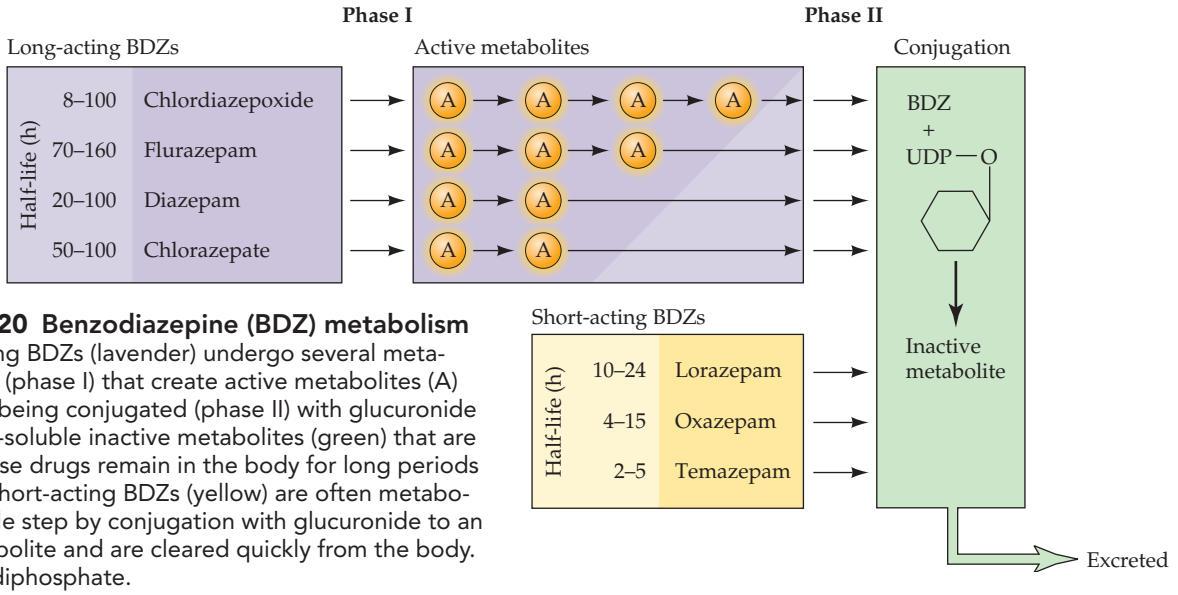


FIGURE 17.20 Benzodiazepine (BDZ) metabolism
The long-acting BDZs (lavender) undergo several metabolic changes (phase I) that create active metabolites (A) before finally being conjugated (phase II) with glucuronide to form water-soluble inactive metabolites (green) that are excreted. These drugs remain in the body for long periods of time. The short-acting BDZs (yellow) are often metabolized in a single step by conjugation with glucuronide to an inactive metabolite and are cleared quickly from the body. UDP, uridine diphosphate.

seizures that can be life threatening. BDZs are also the drugs of choice in preventing acute **alcohol** or **barbiturate withdrawal** symptoms, including seizures. Alcohol, barbiturates, and benzodiazepines are cross dependent, so withdrawal from any one of them can be terminated by administration of any of the others. Since withdrawal from heavy alcohol or barbiturate use can produce a life-threatening situation, the treatment of choice is to substitute a long-acting benzodiazepine (usually Valium) to stop the abstinence syndrome and then to gradually lower the dose of the BDZ over several weeks to minimize the withdrawal.

ADVANTAGES OVER OTHER SEDATIVE–HYPNOTICS BDZs were originally developed to be safer and more effective than the drugs available at the time, such as chloral hydrate, meprobamate, glutethimide, methaqualone, and the barbiturates. Benzodiazepines have several clear advantages over the older drugs. Overall they are more effective, but the most notable advantage is the high therapeutic index. Extremely high doses produce disorientation, cognitive impairment, and amnesia and in some cases, a paradoxical increase in aggressiveness, irritability, and anxiety (Hobbs et al., 1996). However, since they have almost no effect on the respiratory center in the medulla, lethal overdose is extremely rare unless the drugs are taken in combination with other CNS depressants such as alcohol. Unfortunately, recreational use of BDZs is often combined with alcohol, opioids such as methadone, or other CNS depressants, which can produce highly toxic interactions. Although no specific antagonist is available for alcohol or barbiturate overdose, flumazenil (Romazicon) is a competitive antagonist for the BDZ receptor. Individuals brought to the emergency room unconscious can be treated with flumazenil, which quickly reverses the effects of the BDZ while the non-BDZ depressant is gradually eliminated from the body through normal metabolic processes.

Benzodiazepines are also safer because they do not increase the number of liver microsomal enzymes that normally metabolize the drugs. The lack of enzyme induction means there is reduced metabolic tolerance during repeated drug administration and also fewer drug interactions.

PHYSICAL DEPENDENCE AND ABUSE Benzodiazepines have a reputation for lower probability of physical dependence and abuse when taken as prescribed. Nevertheless, chronic use (as recreational use or patients' misuse) and physical dependence do occur. The abstinence syndrome, which is milder than that of the barbiturates and is not life threatening, develops gradually over several weeks, especially for those drugs with very long half-lives. Symptoms may include insomnia, restlessness, headache, anxiety, mild depression, subtle perceptual distortions, muscle pain, and muscle twitches (Carvey, 1998). The most severe symptoms, which resemble those of other CNS depressants, include panic, delirium, and seizures. These occur in individuals who are abusing the drugs at high doses for prolonged periods, often in combination with other drugs, although prolonged use (defined as daily use for at least 3 months) at therapeutic doses also can produce dependence in some vulnerable individuals. Withdrawal is more severe for those long-term users if the drug is withdrawn abruptly or if the dose is rapidly reduced rather than tapered off gradually. Withdrawal can occur even after low therapeutic doses if it is abruptly precipitated by administration of flumazenil.

While physical dependence is manifested by the development of the abstinence syndrome, potential abuse is best predicted by the magnitude of reinforcing properties, which have been evaluated by drug self-administration experiments (see Chapter 4). Abuse of BDZs is not new. In the 1960s and 1970s prescriptions of BDZs to manage stress were extremely popular and the drugs were freely utilized by many, from businessmen to housewives. In fact, middle-class women were the heaviest users. For years, Valium was the number one prescription drug sold in the United States, and it was the first to reach $1 billion in sales (Cooper, 2013). Ultimately the DEA categorized several of the BDZs as Schedule IV drugs (i.e., drugs having a medical use and a real but low potential for abuse or dependence), limited the number of refills, and imposed sanctions for illegal sales. Congressional hearings were held to deal with the BDZ "epidemic," and there was increased media attention following the death of Elvis Presley in 1977 due to massive quantities of Valium combined with other drugs. The next year First Lady Betty Ford openly discussed her addiction to Valium and alcohol. In response to government involvement and high-profile exposure, Valium prescriptions dropped 50% from 1975–1980 (Cooper, 2013). Although concern about the abuse potential of these drugs has led to fewer prescriptions, their misuse seems to be increasing, based on data from drug-related emergency department visits, especially among recreational polydrug users and individuals abusing alcohol. Additionally, the number of sedative-related admissions to treatment programs suggests the problem is serious. Sources for recreational use of BDZs include doctor shopping, forged prescriptions, diversion of drugs from the legal supply by unethical doctors and pharmacists, and unregulated purchases via the internet.

In initial research, laboratory animals did not readily self-administer benzodiazepines in an operant chamber, suggesting that the drugs have little reinforcement value and low abuse potential in humans. However, BDZs with more rapid onset were more likely to be self-administered by animals compared with placebo. Although animals will self-administer BDZs, in breaking point experiments (see Chapter 4) BDZs were weaker reinforcers than other drugs of abuse. In these experiments, the schedule of

reinforcement is progressively increased, requiring more lever presses for each reinforcement. The point at which the effort required exceeds the reinforcing value is the "breaking point." The breaking point for the BDZ midazolam was seen to be significantly lower than for an opioid or cocaine, indicating lower reinforcement value and hence less abuse potential in humans (reviewed in Licata and Rowlett, 2008). It is of interest that in animals that are first trained to self-administer a barbiturate, the reinforcing effects of BDZs are more apparent. This animal behavior models that of humans, for whom the reinforcing effects of BDZs are relatively low in general but are higher in individuals who have previously self-administered other sedative drugs or alcohol. In drug discrimination tests (see Chapter 4) in which rats are taught to discriminate between barbiturates and saline by pressing a lever for reinforcement, BDZs will substitute for barbiturates. However, rats can also be trained to discriminate chlordiazepoxide from barbiturates and alcohol. These results indicate that the subjective drug-induced states of the three drugs must be similar if they substitute for each other in the discrimination test yet have some qualitative differences that can be distinguished. Overall, animal studies suggest that the reinforcement value of BDZs is much less than that of barbiturates and other drugs of abuse (Licata and Rowlett, 2008; Griffiths and Weerts, 1997; Griffiths et al., 1991).

Studies with humans show that nonanxious volunteers prefer to take a placebo over diazepam and that anxious individuals also choose the placebo unless they are seeking treatment for anxiety. In laboratory studies BDZs are not consistently reinforcing in humans except in those with a history of drug or alcohol abuse or who suffer from anxiety or sleep disorders. However, individuals who are experiencing withdrawal after termination of chronic diazepam or other sedative–hypnotics do tend to self-administer a BDZ rather than placebo. These results suggest that BDZs have a relatively low risk of abuse but that physical dependence and withdrawal may encourage continued use. The probability of abuse is almost always associated with polydrug use; that is, individuals who have a history of drug or alcohol abuse are those who most likely will abuse benzodiazepines (Licata and Rowlett, 2008; Woods et al., 1995). Nevertheless, because of the risk of abuse, most clinical guidelines suggest restricting BDZ use to short-term treatment. As you might expect, there is some disagreement among clinicians and researchers. Some clinicians suggest that guidelines should be reassessed for the use of BDZs as first-line, long-term pharmacological treatment for panic disorder, generalized anxiety disorder, and social anxiety disorder (Starcevic, 2014). Evidence to support the reconsideration includes clinical trials showing that the BDZs are equal or superior to the current first-line treatment, the SSRIs. In addition, BDZs act immediately, making them ideal for quick symptomatic relief and use on an as-needed basis. They are also useful in combination with SSRIs during the early course of treatment when the SSRIs may actually increase anxiety and during the time lapse before antidepressant-induced anxiety relief occurs. The BDZs are superior to the antidepressants when there are symptoms of autonomic hyperarousal, prominent muscle tension, and sleep disturbance. They are also generally tolerated better than the antidepressants. Despite these arguments, most clinicians continue to prescribe the safer antidepressants.

SUBUNIT-SELECTIVE DRUG DEVELOPMENT Studies using knockout mice suggest that drugs may be developed to act selectively on $GABA_A$ receptors with distinct α subunit isoforms (see Chapter 8 and Figure 8.20 for a review of GABA receptor subunits and isoforms). Such selectivity would permit targeting of a specific GABA-associated symptom with minimal side effects. The first clinically useful subunit-selective $GABA_A$ receptor modulators are zolpidem (Ambien) and zaleplon (Sonesta), which bind preferentially to $GABA_A$ receptors with the α1 subunit (also called α1 $GABA_A$ receptors). These drugs are useful in treating insomnia while having little anxiolytic, muscle relaxant, or anticonvulsant effects except at high doses.

Ever since the discovery of the subunit-selective modulators of $GABA_A$ function, researchers have made valiant attempts to screen drugs that have the potential to be nonsedating anxiolytics (reviewed by Farb and Ratner, 2014, and Rudolph and Knoflach, 2011). More than a dozen agents showing preference for the α2 $GABA_A$ receptor and perhaps the α3 have been identified (e.g., TPA023, ocinaplon, alpidem) and shown to have anxiolytic effects in animal tests such as the elevated plus-maze and various conflict procedures without impairing motor function or producing sedation or memory impairment. These results demonstrate that it is possible to develop a nonsedating anxiolytic. Unfortunately, in many instances in human clinical trials the drugs were not anxioselective and produced concomitant fatigue, drowsiness, and muscle incoordination. The failure to show selectivity has caused some to question whether sedation is associated with subunits other than the α1 at least in humans (Skolnick, 2012). In addition, in most cases the clinical trials of these agents have been terminated because of a variety of dangerous developments including liver toxicity, prolonged elevated liver enzyme levels that did not resolve after drug termination, severe hepatitis, cataract development, and poor pharmacokinetic properties.

Another goal of research has been to develop BDZ-like drugs without the potential for abuse. To do that it is necessary to understand which $GABA_A$ receptor

subtype is responsible for the reinforcing effects of the drug, the precursor of substance abuse. Immunohistochemical analysis has shown $GABA_A$ receptors with the α1 subunit are located on interneurons that inhibit mesolimbic DA neurons, cells responsible for reinforcement. Hence BDZ-induced inhibition of an inhibitory neuron activates the DA cells, which subsequently increases synaptic DA in the nucleus accumbens, an event common to all abused drugs. Genetically modified mice with their α1 subunits made insensitive failed to self-administer midazolam, while wild-type mice and those with altered α3 subunits readily consumed the BDZ, suggsting that α1 subunits are necessary for reinforcement. In breaking point experiments, drugs that modulate α1 receptors, such as midazolam and zolpidem, had higher breaking points (meaning the animals were more willing to work for reinforcement) than the experimental compound L-838,417. This compound is a partial agonist at $GABA_A$ receptors with α2, α3, or α5 subunits, but is an antagonist at receptors with the α1 subunit. However, animals that were first trained to self-administer a short-acting barbiturate readily self-administered L-838,417, although its breaking point was lower than that of the nonselective BDZs midazolam and diazepam. The nonselective BDZs in turn had lower breaking points than the α1 $GABA_A$ receptor modulator zolpidem. Based on these results, one would have to conclude that α1 subunit modulation may be important for reinforcement but is not necessary.

More recent work using a different approach also suggests that the α1 $GABA_A$ receptor is responsible for the BDZ potential for abuse (Fischer et al., 2016). After a dose–response curve for self-administration of triazolam was established, a variety of selective $GABA_A$ subunit antagonists were administered. The nonselective BDZ site antagonist flumazenil shifted the dose–response curve to the right in a dose-dependent fashion. That shift indicates that higher doses of triazolam were needed to achieve the same level of self-administration because it needed to compete with the antagonist. In a similar fashion, the α1 subunit–preferring antagonists βCCT and 3-PCB caused a right shift in the triazolam dose–response curve, demonstrating the importance of the α1 subunit for drug self-administration, while the α5 subunit antagonist had no effect. Unfortunately, there are no α2 or α3 receptor antagonists currently available. These results demonstrate the importance of the α1 subunit in the reinforcing effects of triazolam. However, when they compared the binding of the antagonists to receptors with the α1 subunit, they found that the rank order for binding to the receptor did not match the rank order of potencies for blocking triazolam self-administration. Their conclusion was that while the α1 $GABA_A$ receptors have a significant modulatory effect on BDZ reinforcement, they do not have the sole role in self-administration. These results all indicate that a designed BDZ that acts effectively at the α2 $GABA_A$ receptor with minimal activity at the α1 $GABA_A$ receptor could be anxiolytic with reduced abuse potential.

MODULATION OF NEUROSTEROIDS Another way to enhance $GABA_A$ receptor function is to act at the neurosteroid modulatory site, described previously. The use of allopregnanolone itself is limited by its poor bioavailability and the fact that it is metabolized to active hormonal steroids that act at peripheral receptors to cause endocrine side effects. Alternatively, synthetic allopregnanolone-like agents could be designed to reduce their metabolism to other active steroids and lengthen their half-lives. Additionally, these agents could be made to cross the blood–brain barrier more readily. One such allopregnanolone analog is ganaxolone. Using a mouse model of PTSD, Pinna and Rasmusson (2014) showed that subcutaneously administered ganaxolone reduced anxious behavior in the elevated plus-maze and also reduced the exaggerated contextual fear conditioning that is normally demonstrated by these socially isolated mice. Further, when the animals' conditioned fear was extinguished, ganaxolone prevented the spontaneous reemergence of the contextual fear responses 7 days later, indicating that the fear extinction was maintained. The research suggests the drug may be effective in treating those with PTSD, who are known to have significantly lower allopregnanalone levels in CSF. Since the drug was shown to be effective with minimal side effects in phase 2 clinical trials for partial onset seizures, it holds promise for continued development not just for seizures but for PTSD as well. Presently the SSRIs are approved for PTSD treatment but have a very low success rate for the complex symptoms of the disorder.

Another option for drug development includes drugs that increase synthesis of allopregnanolone. The synthesis of neurosteroids occurs in both neurons and glial cells in the CNS and is regulated by the mitochondrial translocator protein (TSPO). TSPO transports the precursor cholesterol into mitochondria where neurosteroid synthesis occurs (**FIGURE 17.21**). Because the transport is the rate-limiting step in synthesis, TSPO is an important therapeutic target for enhancing $GABA_A$ inhibition (see Nothdurfter et al., 2012a, or Schule et al., 2014).

Drugs that target TSPO and induce a significant increase in neurosteroids include etifoxine as well as many experimental compounds such as XBD-173 (emapunil) and FGIN-127. These compounds bind to TSPO and significantly increase brain levels of allopregnanolone and other neurosteroids and also produce anxiolytic effects in a variety of animal tests. The reduction in anxiety can be attenuated by finasteride, an inhibitor of neurosteroid

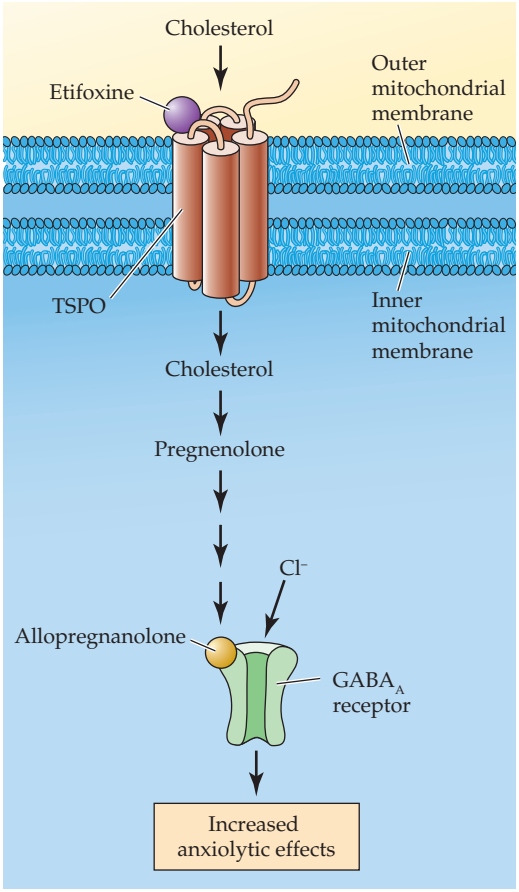


FIGURE 17.21 Neurosteroid synthesis TSPO is a translocator protein that is located on the outer mitochondrial membrane and transports cholesterol through the inner mitochondrial membrane. The conversion of cholesterol to pregnenolone is the rate-limiting step in neurosteroid synthesis. Pregnenolone is further modified in several steps to allopregnanolone, which binds to the $GABA_A$ receptor modulatory site to enhance GABA-induced chloride influx, producing anxiolytic effects. Etifoxine is one drug that targets TSPO to increase neurosteroids and reduces anxiety. (After Schule et al., 2014 and Rupprecht et al., 2010.)

synthesis. Etifoxine is used clinically in some parts of the world but has not received FDA approval in the United States. Etifoxine is a nonbenzodiazepine drug that reduces seizures and anxiety as effectively as the BDZ lorazepam, and reduces neuropathic pain without some of the adverse effects of BDZs such as sedation, memory impairment, and withdrawal upon cessation. It does, however, potentiate the action of other CNS depressants, including ethyl alcohol. In addition to stimulating allopregnanolone synthesis, it has modulatory effects on $GABA_A$ receptors, but it does not act at either the neurosteroid or BDZ modulatory sites. What seems clear is that the β subunit of the $GABA_A$ receptor complex is necessary for the etifoxine-enhanced Cl^- influx produced by GABA binding. In particular the binding to $GABA_A$ receptors with the β2 or β3 subunits produces the greatest enhancement of the chloride-induced hyperpolarization and provides selectivity for those brain regions high in those subunits. Hence the anxiolytic effects of etifoxine are likely due to direct modulation of the $GABA_A$ receptors, as well as the enhanced synthesis of neurosteroids (see Choi and Kim, 2015; Nuss, 2015). Etifoxine and other enhancers of neurosteroid function hold promise for the development of anxiolytics that have fewer side effects than BDZs and work more quickly than the antidepressants, which take 4 to 6 weeks to be effective. However, a report that acute hepatitis occurred in some patients after several weeks of treatment needs further evaluation. An in-depth review of the multiple approaches for targeting neurosteroid synthesis and the potential utility of these drugs in treating not only anxiety but also alcohol use disorders and degenerative diseases such as Alzheimer's disease is provided by Porcu and colleagues (2016).

Second-generation anxiolytics produce distinctive clinical effects

The drugs in this group were developed to provide anxiety reduction without some of the side effects of the benzodiazepines. The best known is **buspirone** (**BuSpar**), which has a novel structure and mechanism of action compared with the sedative–hypnotics. It is also unusual in that it does not necessarily increase punished behaviors, as in the water-lick suppression test. Furthermore, in drug discrimination tests, it does not substitute for either barbiturates or BDZs. Clearly, buspirone has distinctive subjective effects as well as a distinctive mechanism of action.

In clinical tests, buspirone has measurable anxiolytic actions, although it is much less effective in reducing the physical symptoms of anxiety than the cognitive aspects of worry and poor concentration. It has been shown to be effective in treating GAD, but effectiveness for other anxiety disorders and OCD is less clear. Some suggest that it may be best used in combination with other pharmacotherapies, such as the SSRIs, or along with cognitive behavioral therapy (Harvey and Balon, 1995).

Buspirone has several advantages over the benzodiazepines, including its usefulness in treating depression that often accompanies anxiety. In addition, its anxiety reduction is not accompanied by sedation, confusion, or mental clouding. Buspirone does not enhance the CNS-depressing effects of alcohol or other CNS depressants, so it is still safer than the BDZs. It also has a minimum of severe side effects, and fatalities have not been reported. Further, it has little or no potential for recreational use or dependence. In fact, some patients report a dysphoric effect, described as a

feeling of restlessness and malaise. Finally, no rebound withdrawal syndrome has been reported for buspirone. **FIGURE 17.22** compares the effects of buspirone and diazepam in the light–dark exploration test. Mice treated for 14 days with either drug spent more time in the lighted box than saline-treated controls, demonstrating antianxiety effects. When the drugs were abruptly stopped, the mice that had been treated with buspirone showed a slow gradual return of anxiety (the time in the white box decreased) to control values, with no rebound in anxiety (see Figure 17.22A). In contrast, mice treated with diazepam showed an abstinence-induced rebound to less than control levels of exploration (suggesting increased anxiety) followed by a slow recovery (see Figure 17.22B).

One downside of buspirone use is that its onset of effectiveness in humans is quite long and its effectiveness in relieving anxiety is less than that of BDZs. In general, several weeks of daily use are required for significant anxiolytic effects to be seen. This characteristic makes it less desirable for individuals who are accustomed to the immediate relief induced by BDZs. Also, its delayed action makes it less useful for patients who take the drug only when needed for situational anxiety. Second, buspirone has a rather short half-life, so dosing must occur twice daily. Third, buspirone, as well as other structurally related drugs (gepirone and ipsapirone), does not show cross-tolerance or cross dependence with BDZs or sedative–hypnotics. This feature makes it inappropriate for use as a substitution in cases of alcohol or barbiturate withdrawal. Finally, it lacks the hypnotic effects necessary to treat insomnia, has no muscle relaxant effects, and does not control seizures.

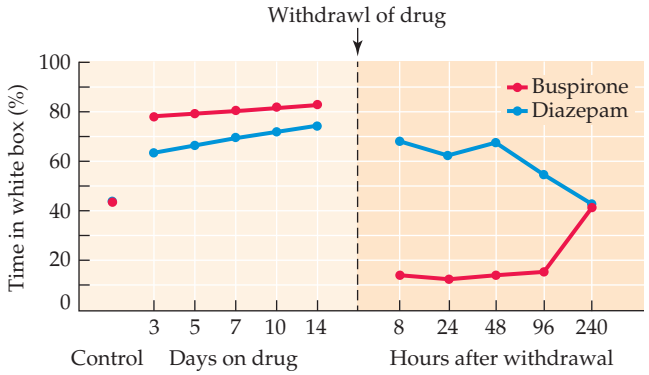


FIGURE 17.22 Abstinence effect after chronic diazepam but not buspirone Mice treated with buspirone (A) or diazepam (B) for 14 consecutive days and placed in the light–dark test on days 3, 7, and 14 showed reduced anxiety by exploring the bright box for a longer time than control mice. Neither drug produced tolerance over the 14 days. When the test was repeated at various times after drug withdrawal, the buspirone-treated mice showed a gradual return of anxiety. In contrast, mice treated with diazepam showed an abstinence-induced rebound in anxiety. This effect is shown by levels of exploration significantly less than control levels, followed by a slow recovery. (After Costall and Naylor, 1991.)

Buspirone has unusual characteristics because, unlike the sedative–hypnotics, it does not enhance GABA function but instead acts as a partial agonist at serotonergic 5-HT_{1A} receptors. These receptors are in heavy concentration in the limbic system, raphe nucleus, and frontal and entorhinal cortices. Although some of these receptors are located postsynaptically, autoradiographic and immunohistochemical studies also show 5-HT_{1A} somatodendritic autoreceptors in the nucleus of the raphe. The neurochemical basis of the anxiolytic action of buspirone is not fully understood, but its partial agonist action at 5-HT_{1A} receptors is the likely mediator of the drugs' effectiveness. Its delayed onset of action would lead one to believe synaptic plasticity is necessary.

Antidepressants relieve anxiety and depression

Several of the disorders described in earlier sections are effectively treated with antidepressant drugs. In fact because of fear of abuse of BDZs, the SSRIs are considered the first line of treatment. In all cases it is worth noting that individual drug treatment response is quite variable among patients with anxiety and stress disorders, and symptoms are lessened but not eliminated for many individuals. Furthermore, in contrast to the immediate effectiveness of the BDZs, the antidepressants take 4 to 6 weeks to become effective. In some cases, the optimum treatment is cognitive behavior therapy with or without adjunctive pharmacotherapy (e.g., see Ivarsson et al., 2015). The antidepressants are important because anxiety and depression very often occur in the same individual and a single drug can be used to treat both conditions. However, several antidepressants have beneficial effects in treating anxiety apart from their antidepressant action. For example, in OCD, the SSRIs clomipramine (Anafranil), fluoxetine (Prozac), fluvoxamine (Luvox), and sertraline (Zoloft) have been found somewhat effective in reducing some symptoms, and the benefits are apparently unrelated to the antidepressant action of the SSRIs. Keep in mind that although the antidepressants initially enhance monoamine function by blocking reuptake from the synapse, the prolonged treatment needed for clinical effectiveness may be causing neuronal adaptations that reverse the stress-induced atrophy of cells in brain regions such as the hippocampus and PFC and enhance synaptic restructuring and dendritic elaboration. Further discussion of the neurotrophic effects of the antidepressants will be found in the chapter on affective disorders (see Chapter 18).

Tricyclic antidepressants such as imipramine (Tofranil) and MAOIs (e.g., phenelzine [Nardil] and

tranylcypromine [Parnate]) are also often effective in treating some anxiety disorders, including panic, phobic disorders, and GAD, although side effects are often troublesome (see Chapter 18). Since the side effects of SSRIs are sometimes less disturbing to patients and they have a more favorable therapeutic index, the serotonergic drugs are more often prescribed as a first choice than the other antidepressants. However, as you would expect, the SSRIs are not without side effects, including increased anxiety, restlessness, movement disorders, muscle rigidity, nausea, headache, insomnia, and sexual dysfunction. Also, when taken in combination with other 5-HT agonists, a potentially fatal event called the serotonin syndrome can occur. Because abuse potential of the SSRIs is low, they may be used on occasions when benzodiazepine dependence is a concern. Although there is low abuse potential, the SSRIs cause physical dependence, and as many as 60% of patients show signs of withdrawal on terminating the drug. More detail on the SSRIs and other classes of antidepressants is provided in Chapter 18.

Because buspirone (a partial agonist at 5-HT_{1A} receptors) is frequently used as an adjunctive therapy with SSRIs when the latter fail to produce adequate relief from symptoms, a relatively new drug (approved by the FDA in 2011) has been developed that combines 5-HT reuptake inhibition with potent partial agonist activity at 5-HT_{1A} receptors. In rodent experiments vilazodone reduced anxiety, including predator-induced stress and ultrasonic vocalizations of rat pups separated from their mothers. The drug also showed antidepressant action in the forced swim test (see Chapter 4). These results translated to positive clinical outcomes in several double-blind, placebo-controlled trials in patients with GAD and also in patients with anxious depression. Side effects over 8 weeks were generally mild, and vilazodone did not impair sexual functioning, as is common with SSRIs. Long-term safety evaluation is still necessary, but vilazodone looks promising at this point (Sahli et al., 2016). It represents one more potential tool in the pharmacological arsenal to optimize treatment for a given individual. **TABLE 17.6** summarizes a variety of treatment options for anxiety disorders. Keep in mind that behavioral therapy is the principal way of treating simple phobias and, along with cognitive therapy, is a significant approach in treating GAD, social phobia, OCD, and PTSD.

Many novel approaches to treating anxiety are being developed

Over the years, GABA and 5-HT have been the primary focus of drug development in the treatment of anxiety. However, more recently, a wide variety of new approaches based on increasing knowledge of the neurobiology of anxiety have been developed. It is important to keep in mind that each of the anxiety disorders is likely to have distinct neural substrates. Although they all involve fear or anxiety, each has other quite distinct symptoms, so the same drugs are not likely to be effective in treating all the disorders and most drugs will relieve only selective symptoms of a given disorder. The fact that most animal models measure levels of fear or anxiety rather than modeling specific symptoms of the anxiety disorders means that preclinical effectiveness in the laboratory does not necessarily translate into clinical effectiveness for a particular disorder. New drug research into anxiolytics is further complicated by the high rate of comorbidity with clinical depression. There are numerous drugs in the development pipeline at various stages. Because of space limitations, we cannot review all the new developments; however, various neuropharmacological studies suggest that anxiolytic effects may be associated with modulation of CRF, glutamate, oxytocin, endocannabinoids, substance P, neuropeptide Y, galanin, orexin, and others. **Web Box 17.1** describes just a few with potential promise. Mathew et al. (2008) provide an excellent overview, and several articles on potential new drug targets are provided in Recommended Readings on the Companion Website.

TABLE **17.6** Drugs Used to Treat Various Anxiety Disorders

Drug class	Trade name	Anxiety disorders
Benzodiazepines	Valium, Xanax	GAD, panic disorder, OCD, social phobia, alcohol withdrawal, acute situational anxiety
Tricyclic antidepressants	Tofranil, Aventil	Panic disorder, GAD, OCD, PTSD
Monoamine oxidase inhibitors	Nardil, Parnate	Social phobia, panic disorder
Selective serotonin reuptake inhibitors	Prozac, Zoloft, Paxil	Social phobia, panic disorder, OCD, PTSD
Buspirone	BuSpar	GAD, panic disorder

Section Summary

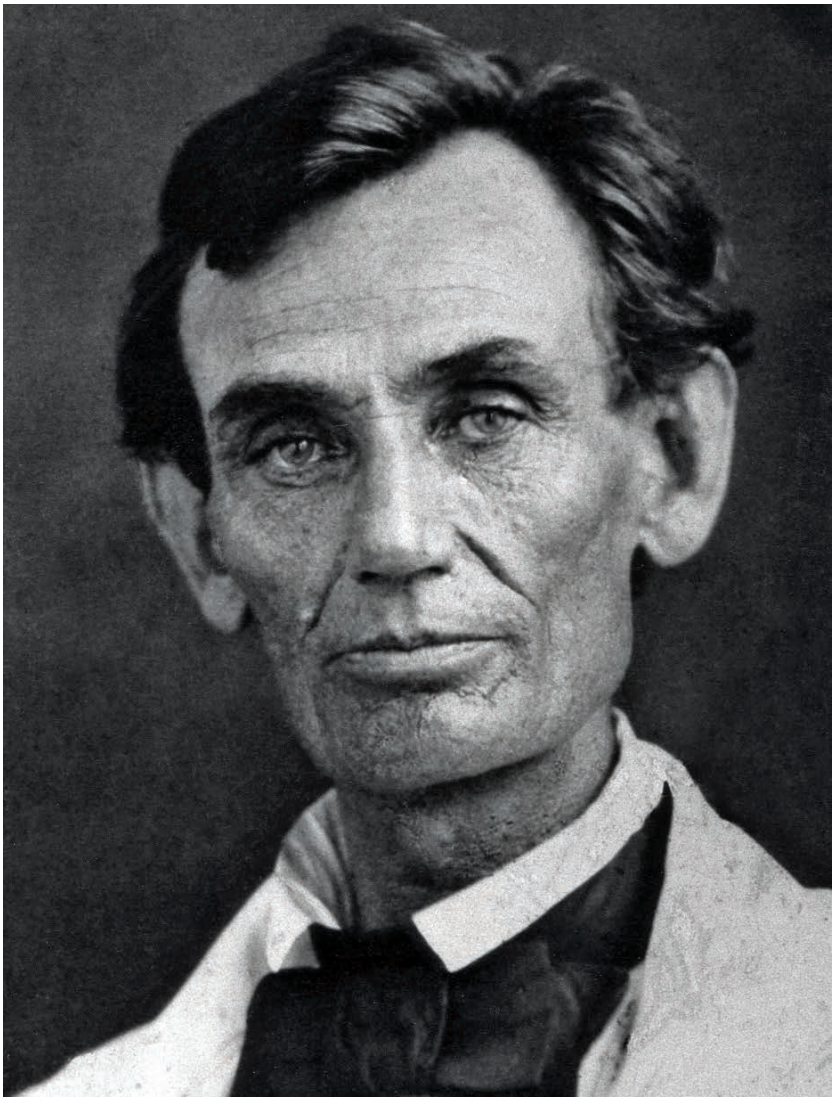
- Anxiolytics are sedative–hypnotics that belong to the larger class of CNS depressants.
- Dose-dependent effects of sedative–hypnotics begin with reduction in anxiety and progress through stages of increasing sedation, incoordination, sleep, coma, and death.
- Sedative–hypnotics increase GABA-induced Cl^- current into the cell, causing enhanced hyperpolarization and inhibition of many cells.
- BDZs enhance GABA inhibition but have no effect of their own on chloride conductance. Flumazenil is a BDZ receptor competitive antagonist that reduces BDZ effects but has no effect on GABA-induced hyperpolarization. Since BDZs shift the GABA dose–response curve to the left but do not increase the maximum GABA response, they are safer than barbiturates and other sedative–hypnotics.
- Barbiturates increase GABA-induced Cl^- conductance and directly open the Cl^- channel without GABA.
- The barbiturates are ultrashort-acting, short/intermediate-acting, and long-acting drugs, depending on their lipid solubility, which determines the rate of penetration into the brain and the extent of redistribution to drug depots and liver metabolism. Duration of action determines their clinical uses.
- Side effects of barbiturates include altered sleep architecture, mental clouding and cognitive impairment, low therapeutic index, rapid tolerance and cross-tolerance, physical dependence, and dangerous withdrawal.
- BDZs are prescribed on the basis of onset and duration. Lipid solubility determines onset. Redistribution to depots and metabolism determine duration. Many have active metabolites, making them long acting.
- Therapeutic uses of BDZs include presurgical anesthesia, anxiolysis, sleep induction, muscle relaxation, seizure control, and termination of alcohol withdrawal.
- Advantages of BDZs compared with other sedative–hypnotics include high therapeutic index, availability of a competitive antagonist to reverse overdose, reduced tolerance and drug interactions, less physical dependence and milder withdrawal, less reinforcement value, and lower abuse potential. Fatalities do not occur unless a BDZ is combined with another sedative–hypnotic.
- Because of the risk of abuse, most clinical guidelines recommend BDZ use only for short-term treatment.
- Multiple forms of the $GABA_A$ receptor subunits provide targets in drug development aimed at increasing therapeutic selectivity and reducing side effects. Drugs selective for the $\alpha 2$ $GABA_A$ receptor show anxiolysis without sedation in animal studies, but not in clinical trials. The $\alpha 1$ $GABA_A$ receptor is likely to play a significant role in sedation, as well as BDZ reinforcement, predicting abuse potential. New drugs targeting the $\alpha 2$ $GABA_A$ receptor with minimal activity at $\alpha 1$ $GABA_A$ receptors may be anxiolytic with less risk of abuse.
- Allopregnanolone and other similar neurosteroids bind to the neurosteroid modulatory site on $GABA_A$ receptors and enhance the function of GABA, causing antianxiety effects. Synthetic allopregnanolone analogs reduce anxiety without sedation. Drugs that increase the synthesis of allopregnanolone by targeting the rate-limiting step (i.e., the transport of cholesterol into mitochondria) have anxiolytic properties without some of the usual adverse effects of anxiolytics, such as sedation, memory impairment, and signs of withdrawal.
- Buspirone is an anxiolytic that does not enhance GABA action but is a partial agonist at $5\text{-}HT_{1A}$ receptors. Its advantages over BDZs are that it reduces both anxiety and depression without sedation or mental clouding, it does not enhance other sedative–hypnotics, and it has no withdrawal syndrome or abuse potential.
- The disadvantages of buspirone are its slow onset of anxiolytic effects; its ineffectiveness for relieving alcohol or barbiturate withdrawal, insomnia, or seizures; and its lack of muscle relaxant effects.
- Antidepressants, including tricyclic antidepressants, MAOIs, and SSRIs, may be used to reduce the anxiety accompanying depression. Some antidepressants relieve symptoms of specific anxiety disorders.
- SSRIs are a drug class of first choice for anxiety because they have a high therapeutic index, and low abuse potential. However, SSRIs take 4 to 6 weeks to show effectiveness following neural adaptations to chronic use. Side effects that are problematic include increased anxiety and insomnia and sexual dysfunction. Withdrawal symptoms are common following drug cessation.

STUDY QUESTIONS

1. Identify the most important brain regions in the emotion-processing circuits.
2. Describe the important role of the central nucleus of the amygdala in orchestrating emotion. How does that compare with the role of the bed nucleus of the stria terminalis?
3. How does the amygdala form emotional memories? Explain why that is important.
4. Describe the HPA axis.
5. Provide several pieces of evidence that demonstrate the role of CRF in extrahypothalamic neural circuits involved in anxiety and the response to stress.
6. Distinguish among the three types of neurons found in the locus coeruleus.
7. Describe four lines of evidence showing that NE has an important role in anxiety.
8. Compare the effects of a BDZ, flumazenil, and β-carboline on the BDZ modulatory site on $GABA_A$ receptors.
9. What are the behavioral effects of neurosteroid modulation of GABA function? Which brain area is important for these effects?
10. Describe the complex effects of $5\text{-}HT_{1A}$ agonists on anxiety.
11. In what ways does uncontrollable stress alter serotonergic function?
12. Describe the neurotrophic effect of 5-HT during fetal development.
13. Discuss the effects of stress on mesocortical and mesolimbic pathways as well as on the VTA.
14. How does early exposure to aversive childhood experiences predispose the adult to medical and psychiatric disorders? Include a discussion of glucocorticoid-induced neural plasticity in the hippocampus, amygdala, and medial PFC.
15. Compare the effects of stress on males and females.
16. Briefly summarize the symptoms of GAD, panic disorder, phobias, social anxiety disorder, PTSD, and OCD. Provide at least one neurobiological correlate for each.
17. Describe the $GABA_A$ receptor and its function. How do BDZs and barbiturates modify the receptor function?
18. What are the significant side effects of barbiturates that prompted the development of new sedative–hypnotics?
19. How do pharmacokinetic factors determine the duration of BDZ action?
20. What are the principal therapeutic uses of BDZs?
21. What are the advantages of BDZs compared with other sedative–hypnotics? What are the drugs' side effects?
22. How do $GABA_A$ receptor subunit–selective drugs target specific symptoms such as insomnia? Anxiety? Reinforcement?
23. Describe two ways that neurosteroids can be used to enhance $GABA_A$ receptor function. How is neurosteroid synthesis enhanced by drugs that act at TSPO? What is the mechanism of action of etifoxine, and what are its clinical effects?
24. Describe the advantages and disadvantages of buspirone.
25. Discuss the advantages and disadvantages of SSRIs, including potential side effects

Go to the *Psychopharmacology* Companion Website at oup-arc.com/access/meyer-3e for animations, web boxes, flashcards, and other study aids.

CHAPTER 18



Our revered sixteenth president struggled with clinical depression at various times throughout his life. (Abraham Byers, 1858. Wikimedia.)

Affective Disorders: Antidepressants and Mood Stabilizers

CLINICAL DEPRESSION CAN BE TOTALLY DEBILITATING, leaving the individual in absolute despair and feeling empty and worthless, unable to function, and preoccupied with his own death. But sometimes during breaks in their depression, people can accomplish important things. Consider Abraham Lincoln. Biographers have described him as the classic image of gloom. He often wept in public and cited maudlin poetry. He told jokes at odd times—he needed laughs, he said, for his survival. As a young man, he talked of suicide, and as he grew older, he said he saw the world as hard and grim. His law partner once said about Lincoln, "His melancholy dripped from him as he walked." There were times when the depression overwhelmed him and he remained in bed. On his wedding day, suffering from a severe depressive episode, he never showed up and left his bride and wedding party waiting for him at the ceremony. To quote Lincoln, "I am now the most miserable man living." Nevertheless, his lifelong illness shaped his character and helped him avoid the pitfalls of inappropriate optimism, making him a realist with vision. His troubles gave him wisdom and deeper humanity and prepared him for the painful tasks of his presidency. He possessed insight, fortitude, and moral will. His depression made him stronger, graced him with dignity, and gave him the courage and confidence to take the risks that made him one of our greatest and most beloved presidents (for more on Lincoln's story, readers can go to *Lincoln's Melancholy*, by Joshua Wolf Shenk, 2005). ■

Characteristics of Affective Disorders

The *Diagnostic and Statistical Manual of Mental Disorders*, fifth edition, (*DSM-5*) describes two principal types of affective disorder: **major depression** and **bipolar disorder**. Both of these are characterized by extreme and inappropriate exaggeration of mood (or affect). Major depression, also called unipolar depression, is characterized by recurring episodes of dysphoria and negative thinking that are also reflected in behavior. Bipolar disorder (also called bipolar depression) is also cyclical, but moods swing from depression to mania over time. The thinking and behavior of individuals with affective disorders are consistent with the exaggerated mood, but the mood does not reflect a realistic appraisal of the environment. Mood disorders are among the most common forms of mental illness today and were described as early as 400 BCE by Hippocrates. The Greeks called depression *melancholia*, meaning "black bile," and recognized that it was associated with anxiety and heavy alcohol use. However, only in the past 150 years has it been recognized as a disorder of brain function.

Major depression damages the quality of life

We are all familiar with the essential feelings associated with depression: feeling down and blue, feeling listless, and lacking energy to do even the fun things we normally enjoy. The state of sadness that occurs in response to situations such as the loss of a loved one, failure to achieve goals, or disappointment in love is called **reactive depression** and does not constitute mental illness unless symptoms are disproportionate to the event or are significantly prolonged. The fact that we all have experienced depression does not make the clinical condition any easier to understand. In clinical depression, the mood disorder is so severe that the individual withdraws from life and from all social interactions. The intense pain and loneliness may make suicide seem like the only option. Pathological depression resembles the emotional state that we have all experienced but differs significantly in both intensity and duration.

The dysphoric mood is characterized by a loss of interest in almost everything and an inability to experience pleasure in anything (anhedonia). Most depressed patients express feelings of hopelessness, worthlessness, sadness, guilt, and desperation. Frequently, patients exhibit loss of appetite, insomnia (characterized particularly by early morning awakening), crying, diminished sexual desire, loss of ambition, fatigue, and either motor retardation or agitation. Self-devaluation and loss of self-esteem are very common and are combined with a complete sense of hopelessness about the future. Individuals may stop eating or caring for themselves physically, sometimes remaining in bed for prolonged periods. Other physical symptoms may include localized pain, severe digestive disturbances, and difficulty breathing. Thoughts of suicide are common; one estimate of suicide rates suggests that 7% to 15% of depressed individuals commit suicide, in contrast to a rate of 1% to 1.5% of the overall population. **TABLE 18.1** summarizes the *DSM-5* criteria for manic episodes and major depression.

Although there are some common features of clinical depression, symptom clusters do vary with the individual. Furthermore, particular patterns of symptoms suggest that there are depression subtypes that may be associated with distinct pathophysiologies and distinct causes. What has been well recognized since the time of Hippocrates (around 400 BCE) is that there is an extensive overlap of depression with anxiety and alcohol dependence (see Chapter 10). Confirmation of the relationship has been shown by many epidemiological surveys that estimate comorbidity (i.e., when two or more disorders occur in the same individual) at almost 60%. When the disorders are comorbid, it is usually the anxiety disorder, particularly generalized anxiety disorder or social anxiety disorder (see Chapter 17), that precedes the onset of depression. Comorbidity of the disorders predicts more *severe* symptoms, causing impaired daily function in work or while attending school or social events. Comorbidity also predicts more *persistent* symptoms that are more difficult to treat. Further discussion of the relationship between anxiety and depression follows in the sections on the roles of heredity and stress in depression etiology. Belzer and Schneier (2004) provide more information on diagnosis and treatment and theoretical explanatory models for the comorbidity of anxiety and depression.

If left untreated, most episodes of unipolar depression improve in about 6 to 9 months. However, the episodes usually recur throughout life, often increasing in frequency and intensity in later years. Although stress often precedes the first episodes of depression, later episodes are more likely to occur without the influence of psychosocial stress. Estimates of the incidence of depression vary significantly, but it is generally believed that 15% to 20% of the population experience depressive symptoms at any given time. The lifetime risk for a first episode of unipolar depression is between 3% and 4% for men and from 5% to 9% for women. The gender difference in the risk for depression is a topic of considerable interest and debate. The mean age of onset for depression is 27 years. This figure has decreased in recent years: **FIGURE 18.1** shows that among Americans born before 1905, only 1% developed depression by age 75, whereas among those born since 1955, 6% had become depressed by age 24.

In bipolar disorder moods alternate from mania to depression

The second type of exaggerated mood is mania. Mania rarely occurs alone but rather alternates with periods

Table 18.1 Symptoms of Manic Episodes and Major Depression

Diagnosis	Symptom
Manic episode	A. A distinct period of abnormally and persistently elevated, expansive, or irritable mood and abnormally and persistently increased goal-directed activity or energy, lasting at least 1 week and present most of the day, nearly every day (or any duration if hospitalization is necessary). B. During the period of mood disturbance and increased energy or activity, three (or more) of the following symptoms (four if the mood is only irritable) are present to a significant degree and represent a noticeable change from usual behavior: 1. Inflated self-esteem or grandiosity 2. Decreased need for sleep (e.g., feels rested after only 3 hours of sleep) 3. More talkative than usual or pressure to keep talking 4. Flight of ideas or subjective experience that thoughts are racing 5. Distractibility (i.e., attention too easily drawn to unimportant or irrelevant external stimuli), as reported or observed 6. Increase in goal-directed activity (either socially, at work or school, or sexually) or psychomotor agitation (i.e., purposeless non-goal-directed activity) 7. Excessive involvement in activities that have a high potential for painful consequences (e.g., engaging in unrestrained buying sprees, sexual indiscretions, or foolish business investments) C. The mood disturbance is sufficiently severe to cause marked impairment in social or occupational functioning or to necessitate hospitalization to prevent harm to self or others, or there are psychotic features. D. The episode is not attributable to the physiological effects of a substance (e.g., a drug of abuse, a medication, other treatment) or to another medical condition.
Major depressive episode	A. Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure. 1. Depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad, empty, hopeless) or observation made by others (e.g., appears tearful). (**Note**: In children and adolescents, can be irritable mood.) 2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation) 3. Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day. (**Note**: In children, consider failure to make expected weight gain.) 4. Insomnia or hypersomnia nearly every day 5. Psychomotor agitation or retardation nearly every day (observable by others; not merely subjective feelings of restlessness or being slowed down) 6. Fatigue or loss of energy nearly every day 7. Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick) 8. Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others) 9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide B. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning. C. The episode is not attributable to the physiological effects of a substance or another medical condition.

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of depression to form bipolar disorder. The primary symptom of mania is elation. Manic individuals feel faultless, full of fun, and bursting with energy. Their need for sleep is significantly reduced. They tend to be more talkative than usual and experience racing thoughts and ideas. In some individuals, the predominant mood is characterized by irritability, belligerence, and impatience because the rest of us are just too slow.

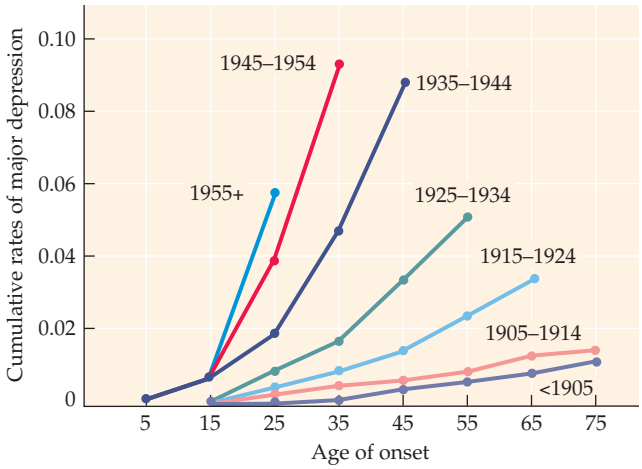


FIGURE 18.1 Age of onset of major depression
Americans are developing major depression at higher rates and younger ages than previously, according to this analysis of data. The study evaluated 18,244 people at five sites, grouped in cohorts on the basis of year of birth. Cross-cultural surveys indicate a similar phenomenon worldwide. (After Barlow and Durand, 1995.)

They tend to make impulsive decisions of the grandiose sort and have unlimited confidence in themselves. The manic individual becomes involved in activities that have a high potential for negative consequences that often go unrecognized by the individual, such as foolish business investments, reckless driving, buying sprees, or sexual indiscretions. You may be interested in watching a short video from the University of Nottingham in which a manic patient is being interviewed by his psychiatrist (University of Nottingham, 2012). Despite the potential danger, some individuals during a manic phase are capable of highly productive efforts when channeled appropriately. A high proportion of creative individuals in the arts and sciences have experienced bipolar disorder and find that during the manic periods, their thought processes quicken and they feel both creative and productive. Is creativity linked to mental illness? **Web Box 18.1** considers that possibility.

The incidence of bipolar disorder is the same in men and women: it occurs in approximately 1% of the population. The time of onset for bipolar illness is typically between 20 and 30 years of age, and episodes continue throughout the life span.

Risk factors for mood disorders are biological and environmental

Most scientists agree that psychiatric disorders develop in a given individual because of the interaction of genes and environmental events. Individuals with particular clusters of genes inherit the tendency to express certain traits or behaviors that increase their vulnerability to specific disorders. Having those genes does not mean that you will develop the disorder, but exposure to particular environmental events is more likely to trigger the disorder in the vulnerable individual. Heredity, environmental stress, and altered biological rhythms are risk factors for affective disorders.

ROLE OF HEREDITY Evidence for a genetic contribution to affective disorders comes from several sources. **Adoption studies** help to clarify the roles of genetics and family environment. In these studies, individuals with a firm diagnosis who were adopted at an early age are the focus of the research. If a heritable component exists, one would expect to see that compared with controls, the individual with an affective disorder would have a greater number of biological relatives with the same disorder, despite being raised in a different environment. Although many adoption studies have found such a relationship and suggest a role for genetics, the results have not always been consistent.

The best evidence for a heritable component of affective illness comes from **twin studies**, which show a significant difference between monozygotic (identical) and dizygotic (fraternal) twins in the rate of concordance for the disorders. The data in **FIGURE 18.2** show that if one twin has a mood disorder, the concordance rate (i.e., the likelihood of the other twin sharing the trait) for a monozygotic twin is approximately 65%. This means that if one of the pair of identical twins (having the same genes) experiences affective illness, the probability that the other twin will also experience

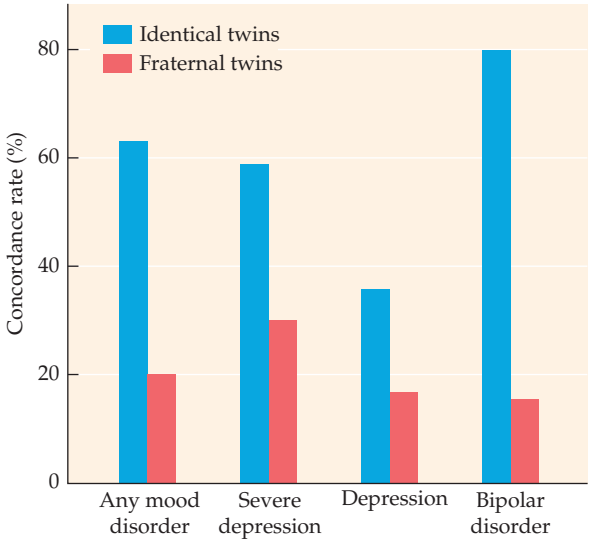


FIGURE 18.2 Concordance rates for mood disorders among identical and fraternal twins
Identical twins are far more likely to share mood disorders than are fraternal twins, especially in the case of bipolar disorder. Concordance for clinical depression depends to some extent on severity. In this case, severe depression is defined as three or more episodes of depression. Data were derived from 110 pairs of twins. (After Bertelsen et al., 1977.)

some affective disorder is 65%. In contrast, the concordance rate for dizygotic twins (who are genetically no more similar than other siblings) is 20%. The difference in these two rates suggests the extent to which genetics contributes to the disorder (estimated at between 40% and 50%) and that a family history of clinical depression is the strongest predictor of vulnerability to the disorder. Keep in mind that if genetics were the only determining factor, the concordance rate in identical twins would be 100%. The genetics of an individual can certainly make him more vulnerable, but whether or not he actually develops the disorder must also depend on other psychosocial or pathophysiological factors.

If you look again at Figure 18.2, you will see that the concordance rate is also dependent on the severity of clinical depression: more severe mood disorders may have a stronger genetic contribution than less severe disorders. Of additional interest is the finding that there are shared genetic risk factors for clinical depression and anxiety disorders, although the magnitude depends on the particular anxiety disorder. For instance, major depressive disorder is most closely related genetically to generalized anxiety disorder. Figure 18.2 also shows that the genetic contribution to bipolar disorder is significantly greater than that to major depression. Eighty percent concordance in monozygotic twins compared with 16% in dizygotic twins indicates a very strong role for heredity in bipolar disorder.

Despite **linkage studies**, which look for similarities in gene location on chromosomes in families with affected members, and other more sophisticated methods of molecular biology that examine DNA fragments, no single dominant gene for affective disorders is known. We may well find that the genes involved confer a general vulnerability to a host of mood and anxiety disorders. For example, linkage studies for major depressive disorder and associated personality traits like neuroticism have been attempted because high scores on neuroticism are a strong predictor of future depressive disorder and other psychiatric ailments. Individuals who score high on neuroticism experience more negative emotions, including anxiety, anger, guilt, and depressed mood, and are more bothered by psychosocial stress. The particular disorder that is expressed in such an individual may ultimately be determined by developmental or psychosocial factors. Discussion of several candidate genes associated with clinical depression will be found in the section on serotonin dysfunction and in the discussion of brain-derived neurotrophic factor (BDNF). It is important to keep in mind that in any complex psychiatric disorder, there are many vulnerability genes, each of which contributes only a very modest amount.

ROLE OF STRESS Both neurobiological studies and family studies indicate that anxiety and depression are closely related. First, anxiety along with its associated physiological symptoms is a frequent accompaniment to depression. Second, intense environmental stress and anxiety often precede episodes of depression, particularly early on in the course of the disorder. Further, altered patterns of stress hormone levels are frequently found in depressed patients. Chapter 17 has already introduced the relationship between anxiety and depression. Despite the importance of environmental stress, keep in mind that identical life stresses may be perceived very differently by individuals. Many people seem resilient and capable of coping despite extraordinary stresses, while others seem to succumb to relatively minor problems. It is likely that genetics plays a role in determining how one responds physically and behaviorally to daily traumas and stress. The dual importance of nature (genetics) and nurture (environment) can never be ignored.

The importance of stress to the etiology of depression and its mediation by the hypothalamic–pituitary–adrenal (HPA) axis is a significant focus in neuroscience. In response to stress, multiple neurotransmitters (including norepinephrine [NE], acetylcholine [ACh], and γ-aminobutyric acid [GABA]) regulate the secretion of corticotropin-releasing factor (CRF) from hypothalamic cells. CRF controls the release of adrenocorticotropic hormone (ACTH) from the pituitary into the blood. ACTH in turn acts on the adrenal gland to increase secretion of cortisol and other glucocorticoids, all of which play a role in the mobilization of energy to deal with stress (see Box 2.4 for a diagram of the HPA axis). Normally, cortisol feeds back to shut down HPA activation, resulting in transient activity of the system and brief surges in cortisol.

Among the most consistent neuroendocrine abnormalities in depressed individuals is abnormal secretion of cortisol, which is demonstrated in several ways. First, many depressed patients have elevated levels of cortisol (**FIGURE 18.3A**) in response to a greater than normal release of ACTH. Although both the pituitary and adrenal glands are enlarged as a result of hypersecretion, evidence from several sources suggests that the abnormality is not in the glands but in the brain. The hypersecretion is most likely due to abnormal regulation of CRF by the hypothalamus. Numerous studies have found higher than normal levels of CRF in the cerebrospinal fluid (CSF) of depressed patients and increased numbers of CRF-producing cells in the hypothalamus in postmortem brain tissue. This exaggerated HPA axis function may be explained in part by the impact of early life traumas on the vulnerable individual. Early life stress apparently alters the set point of the HPA axis, making it permanently overly responsive and increasing the risk for later depression as well as anxiety disorders and alcohol abuse (**Web Box 18.2**). It is important to note that antidepressant

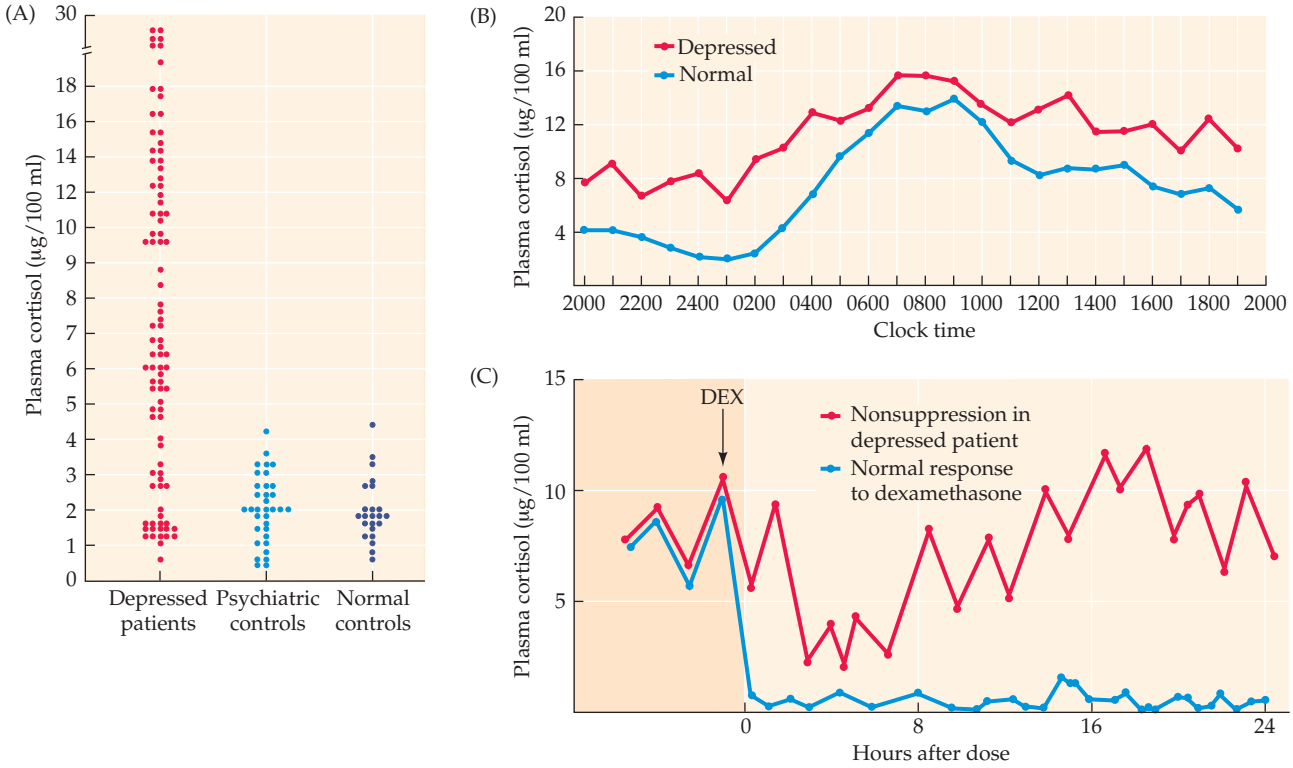


FIGURE 18.3 Abnormalities in glucocorticoids
(A) Many (but not all) depressed patients have elevated cortisol levels compared with controls. Each dot represents a single individual. (B) Differences exist in circadian changes in blood cortisol levels between depressed patients and healthy controls. The measures were made each hour over a 1-day period. The decline in cortisol that normally occurs in the early morning and evening occurs to a lesser extent in depressed patients. (C) Depressed individuals fail to respond with reduced cortisol levels after injection with 1 mg dexamethasone (DEX). The injected glucocorticoid also normally reduces both CRF release from the hypothalamus and pituitary release of ACTH. (B after Kandel, 2000; C after Klein, 2000.)

drug treatment and electroconvulsive therapy reduce CRF levels in depressed patients.

Second, the high level of cortisol found in depressed patients is characterized by an abnormal circadian rhythm in cortisol secretion. The elevated and relatively flat pattern (depicted in **FIGURE 18.3B**) may reflect a more general abnormality in the biological clock, since altered rhythmicity also occurs for body temperature changes and sleep patterns (see later in this chapter). Third, since many depressed individuals have elevated cortisol, it is not surprising that some fail to respond to dexamethasone challenge. **Dexamethasone** is a synthetic glucocorticoid that should act as a negative feedback stimulus to suppress hypothalamic release of CRF and pituitary release of ACTH, resulting in decreased cortisol levels (**FIGURE 18.3C**). Several studies have suggested that patients who remain nonresponders to dexamethasone (i.e., fail to have cortisol release suppressed) after successful antidepressant treatment have a higher probability of relapse than those who show normal response.

Although usually adrenal glucocorticoids (including cortisol) are helpful in preparing an organism for stress, when the levels are persistently elevated, several systems begin to show pathological changes. Besides having damaging effects on immune system and organ function, glucocorticoids are associated with neuronal atrophy in the hippocampus, leading to cognitive impairment, imbalances in the serotonin (5-HT) system correlated with anxiety, and hormonal changes associated with depression (McEwen, 2008). The section on the neurobiological models of depression later in the chapter will provide more detail on the role of glucocorticoids in depression.

ALTERED BIOLOGICAL RHYTHMS Cortisol secretion is not the only biological rhythm that is disturbed in major depression. Altered sleep rhythms are among the most common and persistent symptoms of depression. Circadian rhythm controls the onset, pattern, and termination of sleep. The typical healthy sleep cycle is quite regular, having four stages of non-REM sleep (stages I to IV) lasting a total of 70 to 100 minutes, followed by a 10-to-15-minute period of rapid-eye-movement (REM) sleep, during which time dreaming occurs. This cycle is repeated four or five times a night. Depressed individuals show several distinct abnormalities in their sleep rhythm (**FIGURE 18.4A**). First, there is a long

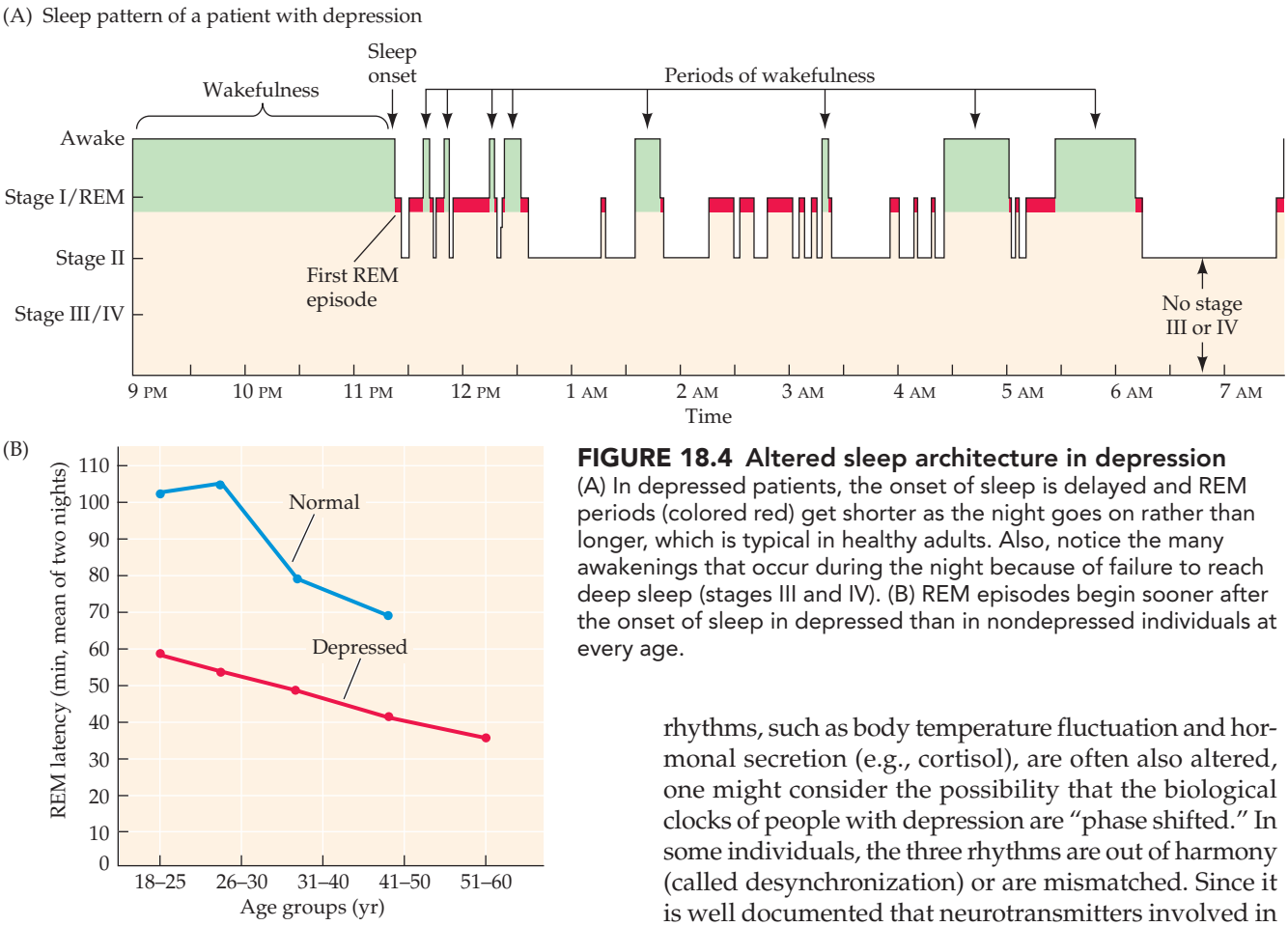


FIGURE 18.4 Altered sleep architecture in depression
(A) In depressed patients, the onset of sleep is delayed and REM periods (colored red) get shorter as the night goes on rather than longer, which is typical in healthy adults. Also, notice the many awakenings that occur during the night because of failure to reach deep sleep (stages III and IV). (B) REM episodes begin sooner after the onset of sleep in depressed than in nondepressed individuals at every age.

period before sleep onset. Second, there is a significant decrease in the time spent in slow-wave sleep, or deep sleep (stages III and IV), which leads to repeated awakenings during the night. Third, the onset of REM sleep occurs much earlier after the onset of sleep. Although REM latency decreases with age, individuals who are depressed show a shorter latency at all ages (**FIGURE 18.4B**). In extreme cases, the individual may enter REM sleep almost immediately after falling asleep. Fourth, REM sleep is significantly increased during the first third of the night in depressed individuals, but nondepressed individuals have proportionately more REM sleep in the final third of sleep. Also, although normal REM periods tend to increase in duration during the night, depressed patients do not show such a pattern. Finally, when ocular movement is measured, depressed individuals show more frequent and vigorous eye movements during REM sleep, which suggests more intense dreaming.

Although we don't know what the altered rhythms mean, the irregularities in sleep patterns found in depressed individuals resemble the sleep patterns of nondepressed individuals who must alter their time of sleep by 12 hours. Since other indicators of biological rhythms, such as body temperature fluctuation and hormonal secretion (e.g., cortisol), are often also altered, one might consider the possibility that the biological clocks of people with depression are "phase shifted." In some individuals, the three rhythms are out of harmony (called desynchronization) or are mismatched. Since it is well documented that neurotransmitters involved in emotion regulation, such as 5-HT and NE, show circadian rhythms in function, the importance of evaluating altered rhythms in mood disorders is clear. The implications of these irregularities in sleep cycles have led to several novel treatment strategies, including the use of melatonin agonists, described in **Web Box 18.3**, and sleep deprivation therapy, discussed in **Web Box 18.4**.

It is probably no surprise that patients with bipolar disorder also show altered sleep rhythms (Plante and Winkelman, 2008). Although their sleep during depressive episodes resembles that of unipolar depression with its shortened REM latency onset, one of the hallmark diagnostic symptoms of mania is the severely reduced need (sometimes almost absence) of sleep with no loss of energy. It is particularly interesting that in the nonsymptomatic bipolar individual, sleep deprivation that is intentional or is associated with environmental events such as experiencing jet lag, bereavement, or late night studying can actually trigger an episode of mania. It is not clear whether the lack of sleep causes the onset of mania or represents an early-occurring symptom. However, when manic patients are treated with a sleep-inducing benzodiazepine, the manic symptoms subside. Such sedatives are used as a common additive treatment along with a mood stabilizer such as lithium (see the last section of this chapter).

The sleep–wake cycle is not the only disturbed circadian rhythm found in patients with bipolar disorder. In addition, feeding, activity patterns, body temperature, blood pressure, plasma cortisol, thyroid-stimulating hormone, and melatonin rhythms are irregular. All of these are controlled by the molecular biological clock in the suprachiasmatic nucleus of the hypothalamus. Further, as was described for clinical depression, circadian rhythms, determined by the molecular clock, also control NE, 5-HT, and dopamine (DA) synthesis, degradation, levels, release, and some of their receptors. Abnormalities in these systems may be the basis for mood dysfunction, so the genes that are responsible for the molecular clock proteins are being extensively investigated. One such circadian gene is the *CLOCK* gene. A particular single nucleotide polymorphism of the gene has been associated with several bipolar symptoms, such as the extent of insomnia during the depressive stage, evening activity, and measures of neuropsychological performance as well as violent suicide attempts (Dallaspezia and Benedetti, 2015; Logan and McClung, 2016). Why different genes involved with modulating circadian rhythms are associated with quite different psychiatric outcomes is not clear at this point, but Landgraf and colleagues (2014b) propose that because the circadian biological clock and the HPA axis stress response are so closely interconnected, the circadian rhythm abnormalities may affect the individual's response to environmental stressors. That in turn would alter the expression of genetic vulnerability to distinct psychiatric disorders. Given the role of psychosocial stress on numerous psychiatric disorders, modulation by circadian rhythms should be investigated more fully.

Animal Models of Affective Disorders

Although the affective symptoms of depression, such as feelings of worthlessness and guilt, can really be described only in human terms, animal models are desperately needed as important tools with which to evaluate drugs and neurobiology of depression. In Chapter 4 you read that if a screening test predicts clinical effectiveness of a drug, its face validity is less important because it has predictive validity. Unfortunately even many of the available tests that predict effective antidepressant treatment in rodents do not translate to the human condition. More important for neurobiological studies of mental illness is construct validity, in which the tool or model actually reflects and measures the neural and behavioral features of the disorder. Modeling poses a particular challenge in such a complex disorder, since the neurobiology underlying depression is not clear and there is no neurochemical or neuroendocrine abnormality in depression that is sufficiently consistent to use as a diagnostic tool or to validate an animal model. For example, although several models are based on altering the HPA axis function, such dysfunction is found in only a subset of depressed individuals, so construct and face validity may be questioned. Additionally, in depressed individuals many genetic variants have been identified that contribute only a small amount to the depression phenotype, which limits genetic modeling. The variable and complex symptom presentation in depression means that most often models reflect only one or a few behavioral symptoms. However, there is no reason to expect a model to reflect all the symptoms of depression, since patients vary in which symptoms they experience. Because there is a tremendous effort to both understand the neurobiology of depression and identify medications that translate to human efficacy, many models continue to be developed. Although there are too many to evaluate here, several reviews can be found that describe and provide guidelines to evaluate those models, as does Chapter 4 (Abelaira et al., 2013; Nestler and Hyman, 2010).

Models of bipolar disorder

In general, researchers rely on the tests for screening antidepressants to model the depression phase of bipolar disorder and have focused more recently on developing models for mania. Attempts to develop models eliciting spontaneously cycling episodes of mania and depression are even more limited. Several models are based on the significance of the altered circadian rhythms observed in bipolar individuals and the fact that circadian rhythm disruption and sleep disturbances can trigger manic episodes. In one environmentally induced model, the animals are exposed to **sleep deprivation** for an extended period. At the end of the deprivation period, the rat does not immediately fall asleep but instead remains awake for about 30 minutes and demonstrates multiple manic-like symptoms including insomnia, hyperactivity, irritability, aggressiveness, hypersexuality, and stereotypical behaviors. This sleep deprivation–induced mania resembles that observed in individuals with bipolar disorder. If lithium (a drug commonly used to treat bipolar disorder) is added to the animal's food during the sleep deprivation, some but not all of the manic behaviors are reduced. Although it has face validity, this test is time-consuming and provides only a 30-minute window of opportunity for investigation.

A second approach utilizes **mutant mice** and targets genes of the circadian clock. Altered diurnal patterns of the biological clock genes have been found in patients with bipolar disorder. Even more interesting is that the clinical state (mania or depression) seems to alter the molecular clock gene transcription. The most well-studied gene is the *CLOCK* gene. In patients with bipolar disorder, a polymorphism of *CLOCK* has been associated with more frequent manic episodes, insomnia, early morning waking, and reduced need for sleep

(see Logan and McClung, 2016). Mice lacking the Clock protein show manic-like behaviors such as less depression-like behavior in the forced swim test, reduced anxiety, hyperactivity, disrupted circadian rhythms, decreased sleep, and increased risk taking. Logan and McClung (2016) describe a translational behavioral task to evaluate activity and goal-directed behavior that can be used with both human participants and rodent subjects. *Clock* mutant mice show sensorimotor deficits and hyperactivity similar to those seen in manic individuals. However, the amount, pattern, and organization of activity as measured in the behavioral pattern monitor (BPM) are different from those of human patients evaluated in a similar manner. The BPM is an open field containing novel and familiar objects available to explore and interact with. Manic patients, in addition to showing high levels of activity, spend large amounts of time exploring the objects and showing linear, purposeful, goal-directed behavior. The rodents, in contrast, show more short, repetitive movements, and their patterns of activity are distinctly different. Further research may identify the neurobiological mechanisms of the similarities and differences in this translational paradigm.

One further similarity between acute manic patients and *Clock* mutant mice is their heightened sensitivity to reward. Their enhanced reward seeking has been demonstrated in multiple behavioral tasks. For instance, the animals show high levels of cocaine self-administration, greater sucrose preference, and higher consumption of alcohol than control animals in a two-bottle choice design, and they have lower reward thresholds during intracranial self-stimulation (i.e., lower amounts of electrical current are reinforcing). These behaviors are reminiscent of individuals with bipolar disorder, who frequently develop substance abuse disorders. Chronic lithium treatment, a common therapeutic approach in patients, restores most manic-like behaviors in the mice to control levels. *Clock* mutant mice provide an enticing model of bipolar disorder, and more investigation into these behaviors and into pharmacological treatments that are effective and ineffective in altering manic-like behaviors will be forthcoming.

At least some of these manic behaviors in the knockout mice have been linked to increased dopaminergic cell firing and bursting, which supports the substantial evidence of a link between the mesolimbic DA pathway and mania. In addition there are increases in DA levels, greater sensitivity to DA shown by higher firing rates, and increased numbers of DA receptors in the striatum. One elegant study demonstrated that a viral-mediated *Clock* gene transferred into the ventral tegmental area (VTA) of *Clock* mutant mice restored locomotor activity and anxiety levels to the levels of wild-type mice. Additionally, knockout of *VTA-specific Clock* gene transcription produced multiple manic-type behaviors. Furthermore, chronic lithium is known to reduce VTA function. Hence at least some manic-like behaviors are a result of *Clock* function loss in the VTA. *Clock* expression in other brain areas is likely to modulate other aspects of bipolar symptoms, so there is a need to manipulate gene expression in specific mood-regulating brain areas (Landgraf et al., 2014a; Roybal et al., 2007).

A second genetic model of bipolar disorder, also associated with circadian cycling, is transgenic mice overexpressing the enzyme glycogen synthase kinase-3 (GSK-3). The gene in individuals with bipolar disorder is associated with impulsivity and suicide risk, age of onset of the disorder, and response to treatment. In animals, the genetic manipulation produces multiple mania-like behaviors including hyperactivity, impulsivity, and enhanced reward seeking. Perhaps most significant is that lithium inhibits GSK-3 and that its action alters circadian rhythms, which may be responsible for its mood-stabilizing effects (for further discussion see the section below on drugs for treating bipolar disorder). However, there are also distinct differences between the mice and patients with bipolar disorder. Those differences include an increased startle response and a normal, rather than blunted, corticosterone response to acute stress. For the interested reader, Logan and McClung (2016) and Young and colleagues (2011) provide reviews and assessment of pharmacological, environmental, and genetic models of bipolar disorder.

Section Summary

- Affective disorders, including major depression and bipolar disorder, are chronic disorders that recur in episodes over the life span. Symptoms are listed in Table 18.1.
- The incidence of depression is approximately 15% to 20% of the population at any one time. Depression is twice as common in women and is highly comorbid with anxiety and alcohol abuse.
- Bipolar disorder constitutes episodes of depression alternating with mania and occurs in about 1% of the population.
- On the basis of twin studies and adoption studies, genetic contribution to the occurrence of major depression is estimated at 45%, and family history is the strongest predictor of vulnerability. Genetic contribution to bipolar disorder is significantly greater than that for depression.
- Depression is associated with abnormalities of HPA axis function: high plasma ACTH and cortisol, hypersecretion of CRF, flat circadian rhythm of cortisol, and failure of dexamethasone-induced negative feedback.
- In depression, altered sleep architecture is seen in the following conditions: onset insomnia, reduced

slow-wave sleep, early onset of REM sleep, more frequent and longer REM episodes early in the night, and more vigorous eye movement during REM.

- Individuals with mania sleep very little without loss of energy. In symptom-free patients, sleep deprivation initiates a manic episode. Because many rhythms are irregular, research into the *CLOCK* gene has increased.
- Modeling complex psychiatric disorders poses multiple challenges, and each model focuses on one or a few symptoms. Each varies in face, predictive, and construct validity.
- Exposing rodents to sleep deprivation and creating mutant mice with genes that affect the biological clock and circadian cycling are two means used to produce models of mania in rodents.

Neurochemical Basis of Mood Disorders

The earliest attempt to develop a cohesive theory of the neurochemical basis of affective disorders was the **monoamine hypothesis**. The monoamine hypothesis originated with the observation that reserpine, a drug effective in reducing high blood pressure, induces depression as a side effect in a significant number of patients. The drug prevents the packaging of neurotransmitters into vesicles, leaving the molecules in the cytoplasm, where monoamine oxidase (MAO) degrades them. In this way, reserpine treatment produces empty vesicles and reduces the levels of DA, NE, and 5-HT (all monoamines). Early researchers wondered if it could be that the reduced level of monoamines in the central nervous system (CNS) is responsible for the depressed mood. This possibility seemed increasingly likely when the mechanism of action of two types of antidepressants, monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants (TCAs), was considered. Despite their varied synaptic action, the antidepressant drugs acutely increase the function of NE or 5-HT or both. In addition, drugs in both classes reverse reserpine-induced reduction in motor activity—a popular animal model at the time for screening antidepressant agents (**FIGURE 18.5**). The drug studies were combined with early data showing reduced levels of the NE metabolite 3-methoxy-4-hydroxy-phenylglycol (MHPG), suggesting lowered NE synaptic activity, and reduced levels of 5-hydroxyindoleacetic acid (5-HIAA), suggesting lowered 5-HT synaptic activity, in the CSF, plasma, or urine of some depressed patients. These measures suggest low utilization of the monoamines; however, these differences were not consistently found. Nevertheless this type of evidence formed the basis of the early monoamine hypothesis of affective disorders (Schildkraut, 1965).

Although many new questions have challenged the original hypothesis, when it was first proposed, the best evidence supported the idea that depression is associated with low levels of monoamines, whereas mania coincides with excess monoamine activity. Because reserpine acts on all monoamines and the early antidepressants also were nonselective in increasing NE and 5-HT, it really was not clear which of the neurotransmitters was most important in the etiology of depression. Unfortunately, we have not yet resolved this issue, and more and more researchers are coming to the conclusion that both of these amines, as well as DA, are likely to play some role in clinical depression, and other neurotransmitters may also contribute to the complex pattern of symptoms. Increasing evidence suggests that there is anatomical and functional interaction between noradrenergic neurons in the locus coeruleus and serotonergic neurons originating in the midbrain raphe. Each of the two transmitter systems seems to be capable of modulating the other. In the meantime, it is important to remember that neurotransmitter systems should be considered not in isolation but instead as a part of a complex network of interacting neurons.

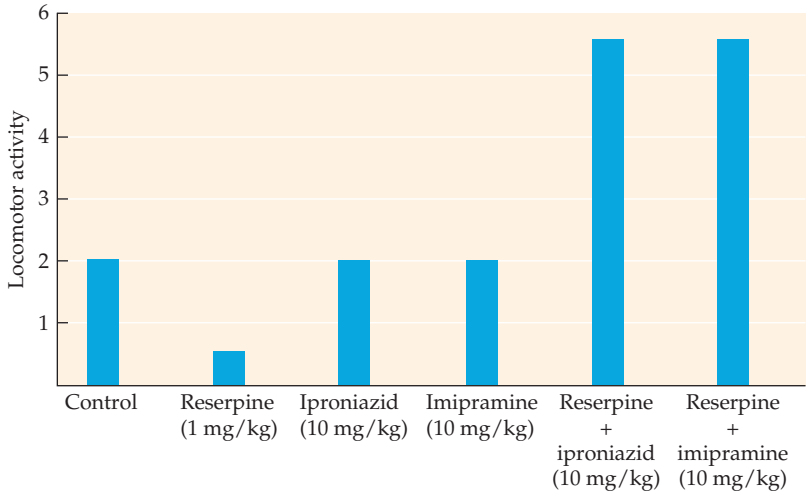


FIGURE 18.5 Effects of reserpine, the MAOI iproniazid, and the TCA imipramine on rat locomotor activity Reserpine clearly reduces motor activity of rats compared with control treatment. Neither of the antidepressants has any effect on motor activity when given alone. However, a dramatic increase in motor behavior occurs when reserpine is given in combination with either of the antidepressants. The reversal of reserpine-induced depression parallels the changes in brain amines and contributes to the monoamine hypothesis of affective disorders. (After Snyder, 1996.)

Although we now know that the monoamine hypothesis is overly simple, it provided an important theoretical model that was the focus of enormous amounts of research over many years. It provided the basis for new drug development, for the creation and testing of new animal models, and for the formulation of new questions that could not be answered within the old theory. Keep in mind that although our thinking about depression and antidepressant drugs has evolved since 1965, the most commonly used classical antidepressants still target 5-HT and NE. As is always the case in good science, new and often conflicting evidence must be accounted for, and old theories modified, as more sensitive biochemical and imaging techniques are developed.

The monoamine hypothesis as originally stated was based heavily on acute antidepressant drug effects. It is too simplistic to account for the complex syndrome of affective disorders, and it fails to resolve several discrepancies. The most important of these is the discrepancy in time between the rapid neurochemical actions of antidepressants and the slow onset of clinical effects over several weeks. This disparity in time course clearly demonstrates that the acute enhancement of monoamine function is *not* the neurochemical basis for therapeutic activity and that downstream effects such as changes in synaptic plasticity and synapse remodeling are more central to antidepressant effects (Duman and Duman, 2015). Despite the vast improvements in technology, newer testable models of the neurobiological basis of affective disorders still use three basic approaches: (1) developing animal models including gene manipulation, (2) evaluating the mechanism of action of effective drug treatment, and (3) examining neurobiological and genetic differences in patient populations. It has been and remains a long and difficult process. Nevertheless, clarifying the neurobiological mechanisms of major depression will ultimately pave the way for the discovery of new and more effective treatments (Belmaker and Agam, 2008).

Serotonin dysfunction contributes to mood disorders

Serotonin continues to be a focus of research because it has a significant influence on sensitivity to pain, emotionality, and response to negative consequences as well as to reward. The effects of 5-HT on sleep, eating, and thermoregulation are likewise well documented and intuitively seem to contribute to depressive symptoms. There is no solid evidence of abnormal 5-HT function in depressed patients, but one indication of function is the measurement of the principal synaptic metabolite of serotonin, 5-HIAA. It is generally assumed that high 5-HIAA reflects increased function of serotonergic neurons, and low 5-HIAA the converse. Lower 5-HIAA levels have been found in postmortem brains of depressed individuals, most consistently in the brains of suicide victims. Several studies have also reported lower 5-HIAA levels in the CSF of depressed individuals.

The newest techniques to modify serotonin function include creating knockout mice that lack tryptophan hydroxylase, the rate-limiting step in 5-HT synthesis, which depletes stores of 5-HT. Other manipulations include knocking out specific 5-HT receptor subtypes. Among the behavioral outcomes are irritability and aggression, increased sensitivity to pain, modified anxiety-like behavior, and altered patterns of eating and satiety. Details of these 5-HT manipulations are provided in Chapter 6 as well as in a review by Mosienko and colleagues (2015). Parallels in humans suffering from major affective disorders can be easily seen.

MEASURING 5-HT IN HUMANS Unfortunately there has never been solid evidence of abnormal 5-HT function in depressed patients because, as mentioned previously, there are often inconsistent results due to patient variables in symptoms, duration of illness, history of drug use, and other lifestyle issues. One of the most intriguing ways to investigate the role of serotonin in depressive disorders is the **tryptophan depletion challenge**, in which individuals consume a tryptophan-deficient amino acid cocktail that transiently reduces 5-HT levels in the brain by 70% to 80% because tryptophan is necessary for 5-HT synthesis. It should be pointed out that although brain synthesis and metabolism are reduced, it is not yet clear whether the procedure alters 5-HT release and synaptic signaling. Tryptophan depletion of unmedicated patients in remission causes a relapse of depression symptoms. The same depletion leads to a depressed mood in healthy individuals who have a family history of depression, but not in healthy people without such a family history. These findings together indicate that merely having low levels of brain serotonin does not cause depression, except in vulnerable individuals. Hence sensitivity to reduced brain serotonin represents a *vulnerability factor* and may be considered a trait abnormality in depression. In addition those patients who were medicated with certain classes of antidepressant drugs also showed a relapse following tryptophan depletion, demonstrating that at least some antidepressant drug effects rely on 5-HT availability.

The identification of a relatively common gene variation, a polymorphism of the **serotonin reuptake transporter** (**SERT**) gene, has generated a good deal of interest, particularly because it is a key target of many commonly used antidepressants, that is, the selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine (Prozac). The two forms of the SERT gene are referred to as the long (*l*) and short (*s*) allele. It is apparent that the short allele, whether on one chromosome (s/l) or

both chromosomes (s/s), is associated with significantly reduced level and function of the transporter. Although the short allele has been found by some researchers to be associated with depressive disorder, the relationship occurs only in association with increased stressful life events. In one prominent and highly acclaimed early study by Caspi et al. (2003), the researchers evaluated almost 850 people over a period of 20 years. They found that the likelihood of both depression and suicide attempts increased with the number of significant stressful life events. However, in both cases the effects of stressful events were moderated in those individuals with two long alleles. Those with two short alleles were most likely to develop symptoms, while heterozygous (s/l) individuals were intermediate. The stress–gene interaction may help to explain why not all people experiencing stressful events ultimately develop depression. It also would explain why not all people with the short allele develop depression. Since stress elevates synaptic serotonin, more effective 5-HT reuptake associated with the l/l genotype may improve modulation of the extremes of synaptic function and the individual's sensitivity to stress. Additionally, patients with two long alleles have shown better and longer-lasting treatment outcomes with antidepressant medication than patients with a single or two short alleles. Despite the seemingly convincing evidence for the significance of the SERT genotype in depressive behaviors, at least one meta-analysis that examined 31 data sets found no evidence for an association of the SERT genotype–stress interaction with elevated risk for depression (Culverhouse et al., 2017). Outcomes such as these teach us that despite their difficulty, replication studies are critical to good science. However, despite the failure to replicate the earlier findings, the overall approach of evaluating the interaction of genotype with environmental events to predict mental illness has been embraced because of frustration with earlier lack of progress in gene identification for mental illness.

TABLE 18.2 Effects of Chronic Antidepressant Treatment on Serotonin Neurons

Antidepressant treatment	Effect on 5-HT_2 receptor binding*	Electrophysiological response to 5-HT
Tricyclics		
Amitriptyline	↓ or =	↑
Chlorimipramine	↓	↑
Desmethylimipramine	most ↓	↑
Imipramine	↓ or =	↑
Second-generation		
Fluoxetine	↓ or =	=
Iprindole	↓	↑
Mianserin	↓	↑
Trazodone	↓	↑
Monoamine oxidase inhibitors (MAOIs)		
Tranylcypromine	↓	↓
Clorgyline	↓	↓
Electroconvulsive therapy (ECT)	= or ↑	↑

Source: After Willner, 1985.
*↑,enhancement; =, no change; ↓, reduction.

Receptor binding studies in postmortem brain samples from unmedicated individuals with mood disorders have usually found *increased* density of postsynaptic 5-HT_2 receptors, which may be considered a compensatory response to low serotonergic activity. In accord with this finding, animal studies show that chronic antidepressant treatment leads to a fairly consistent decrease (down-regulation) in 5-HT_2 receptors. **TABLE 18.2** gives you some idea of the variety of antidepressant treatments that produce this down-regulation. Only the clinically effective use of chronic electroconvulsive therapy (ECT) fails to reduce these receptors. The same table indicates that electrophysiological response to the application of 5-HT agonists is enhanced by long-term, but not acute, antidepressant treatment. Although the argument for enhanced sensitivity of serotonergic neurons is among the most consistent, it is somewhat difficult to reconcile with the down-regulation of receptors. However the enhanced sensitivity may be due to differences in pre- and postsynaptic receptors or the up-regulation of intracellular signaling. Further discussion of the role of receptor subtypes and variations based on brain region is beyond the scope of this chapter.

ANTIDEPRESSANT EFFECTS ON 5-HT IN ANIMALS In addition to evaluating depressed patients, we can look at the long-term effects of antidepressant drugs on 5-HT by using animals. Because SSRIs are the most commonly prescribed drugs, research into their cellular and molecular mechanism of action has become a focal point. Much of the research stems from the fact that although they are most often prescribed, not only do they take weeks of chronic treatment to be effective, but 60% to 70% of patients fail to achieve complete remission and 30% to 40% show no significant response. Animal studies have shown that most antidepressants increase 5-HT by blocking reuptake or inhibiting MAO. The increased synaptic 5-HT has postsynaptic action but also acts on 5-HT_{1A} autoreceptors to slow the firing rate of cells and to reduce 5-HT synthesis as well as release. Therefore, the two effects tend to cancel one another out. Overall, lower neuronal activity reduces metabolism of 5-HT to

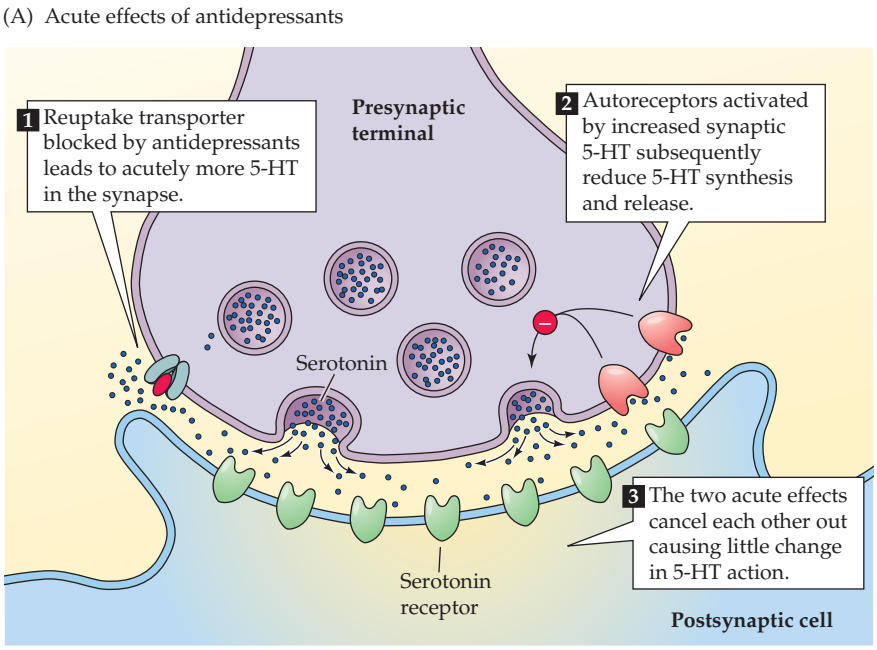


FIGURE 18.6 Effects of antidepressants on serotonergic cells (A) The initial effects of reuptake blockade include increased 5-HT in the synapse and activity at both postsynaptic receptors and autoreceptors. Autoreceptor activation reduces the rate of firing of the cell as well as the rate of synthesis and release of 5-HT and subsequently also reduces the rate of formation of 5-HIAA. (B) With repeated administration, the autoreceptors become less sensitive, and their inhibition of serotonergic neurons decreases, which leads to an increase in 5-HT function and 5-HIAA. However, the increase in 5-HT is perhaps just the initial step, and subsequent changes downstream, postsynaptically, are required, including hippocampal neurogenesis and functional remodeling of corticolimbic circuits.

5-HIAA, indicating reduced turnover (**FIGURE 18.6**). However, chronic treatment results in tolerance and reduces the action of the autoreceptor (down-regulation) and in that way gradually increases the amount of 5-HT in the synapse. The reuptake transporter blockade is still effective, so at this point the two actions both produce an increase in 5-HT. Since the therapeutic effects of antidepressants take several weeks to develop, the delay in autoreceptor desensitization and subsequent enhanced 5-HT activity may be in part responsible for the delayed therapeutic onset or explain the limited efficacy (Blier et al., 1990). Several pieces of evidence suggest the importance of 5-HT_{1A} autoreceptors in depression. For instance, genetic and imaging studies show that individuals with a higher density of presynaptic 5-HT_{1A} receptors are more vulnerable to depression and suicide and tend to show less response to antidepressant drugs. Further evidence for a role of presynaptic 5-HT_{1A} comes from several studies suggesting that reducing the expression of the presynaptic autoreceptors in mice, which would be analogous to drug-induced down-regulation (see Celada et al., 2013), elicits antidepressant-like behavioral effects. Additionally, acute 5-HT_{1A} autoreceptor knockdown (an RNA interference technique that silences the activity of specific genes without totally abolishing the gene in adult mice) did not change basal extracellular 5-HT in the medial prefrontal cortex as measured by microdialysis. However, the animals showed robust antidepressant behaviors that were accompanied by elevations of prefrontal 5-HT and enhanced 5-HT release that may have been caused by the stress of the forced swim test. Apparently silencing the 5-HT_{1A} autoreceptor produces 5-HT–mediated antidepressant effects (Ferres-Coy et al., 2013).

A more recent study suggests that the autoreceptor down-regulation hypothesis of SSRI action may be more complex than originally believed. In their meta-analysis of 42 in vivo microdialysis studies of chronic SSRIs,

Fritze and colleagues (2017) found that the time course of enhanced 5-HT release varied with brain region. In support of the 2-to-3-week time course of down-regulation of autoreceptors, they found that in frontal cortex after an initial reduction in extracellular 5-HT during week 1 of treatment, the levels steadily increased over the subsequent 2 weeks. In contrast, in hippocampus, prefrontal cortex (PFC), VTA, and nucleus accumbens (NAcc) the extracellular 5-HT increased within 3 days, long before autoreceptor desensitization would have occurred. Clearly multiple adaptive mechanisms are needed to explain the slow onset of SSRI clinical effectiveness (see the neurotrophic hypothesis in the section on Neurobiological Models of Depression).

Arguing against the importance of the autoreceptor down-regulation, Richardson-Jones and colleagues (2010), using novel manipulations in adult mice, were able to suppress 5-HT_{1A} presynaptic receptors selectively but leave intact 5-HT_{1A} postsynaptic receptors. Their two groups of mouse subjects were those with high levels of the autoreceptor or low levels of the autoreceptor. During fluoxetine chronic administration, the low-autoreceptor mice showed faster increases (8 days) in raphe firing rate and subsequent increases in 5-HT function in the hippocampus and prefrontal cortex, as would be expected since there were many fewer inhibitory autoreceptors. They also showed an antidepressant response in tests of behavioral despair (see Chapter 4). However, the mice with high autoreceptor densities ultimately reached the same level of serotonin function after 26 days when the autoreceptors were desensitized, but they still did not show a behavioral antidepressant response. The authors conclude that 5-HT_{1A} autoreceptor desensitization cannot be the explanation for fluoxetine's antidepressant effect. Instead they suggest that serotonergic function existing before treatment begins may be the critical factor and that if serotonin can be elevated before SSRI treatment begins, the antidepressants may be more effective for more patients and faster acting.

One recently tested hypothesis is that the outcome of elevating 5-HT with antidepressants is highly dependent on the environment in which it occurs. The researchers induced depression-like behavior in mice through 14 days of stress exposure. For the next 21 days the animals received fluoxetine or vehicle while being maintained in either an enriched environment or a stressful one. There were a number of significant differences among fluoxetine-treated animals, depending on the environment. While fluoxetine treatment in the enriched environment had antidepressant effects, the same treatment administered to animals living in a stressful situation showed a worsening of depression-like behaviors. BDNF levels and several proteins (e.g., p11) implicated in modulating depression were enhanced in the hippocampus of those mice in the enriched environment but remained unchanged under stress. Oddly, fluoxetine did not enhance BDNF-induced neurogenesis in the enriched conditions as would be expected; however, the drug treatment *reduced* proliferation of cells in the stressed animals. Additionally fluoxetine apparently enhanced glucocorticoid feedback on the HPA axis, leading to reduced stress hormones in the enriched environment. In contrast the animals in the stressed condition showed blunted negative feedback by glucocorticoids, leading to sustained stress hormone release. These and other differences reported by the authors demonstrate that the effects of fluoxetine vary depending on the living conditions of the subjects. They interpret their findings by saying that the SSRI-induced 5-HT enhancement leads to plasticity that provides the opportunity for either improvement or deterioration based on the environment. Practically speaking, human living environments are not easy to change, but the authors suggest that cognitive therapy could teach individuals to make a stressful environment less so and in that way improve the effectiveness of the antidepressant therapy (Alboni et al., 2017).

Norepinephrine activity is altered by antidepressants

Norepinephrine also continues to be a focus of research because it has a known role in neuroendocrine function, reward mechanisms, attention and arousal, and response to stress, each of which may contribute to the symptoms of the affective disorders. Regrettably, results of studies with depressed patients are difficult to interpret. Levels of the principal noradrenergic metabolite MHPG in the body fluids of depressed patients have been found to be higher, lower, or no different from those of controls. In general, MHPG is usually found to be elevated in patients undergoing treatment, suggesting an increase in turnover with antidepressant use. Animal studies show chronic antidepressant treatment leads to down-regulation of both β-receptors and α_2-autoreceptors. Unfortunately, when both α_2- and β-receptors are down-regulated, they have opposite effects on adrenergic synapses. Since α_2-autoreceptors acutely reduce noradrenergic cell function by decreasing the rate of firing and reducing NE release, α_2-autoreceptor down-regulation increases both of these cell functions. With the use of α_2-challenge measures, the majority of experiments show that chronic, but not acute, antidepressant treatment produces a reduction in autoreceptor responsiveness that coincides with the increase in turnover described earlier.

One of the most consistent findings regarding catecholamine response to chronic antidepressant treatment is the down-regulation of β-receptors, which requires 7 to 21 days of treatment—a lag that parallels that seen in the onset of therapeutic response in depressed patients. Similar results occur with many of the antidepressant drugs tested, including TCAs, MAOIs, SSRIs, and second-generation antidepressants. ECT, lithium (used to

treat bipolar disorder) under some conditions, and even REM sleep deprivation that has antidepressant action seem to reduce β-receptors. However, not all antidepressants reduce β-receptors, and yohimbine, an α_2-autoreceptor antagonist that enhances the antidepressant-induced down-regulation of β-receptors, does not enhance the antidepressant effects as would be expected.

To help clarify the previous findings by using human brain tissue, Rivero and colleagues (2014) using postmortem prefrontal cortex of depressed nonmedicated individuals showed elevations in both α_2- and β_1-adrenergic receptors, and in agreement with earlier studies, they found significant down-regulation of β_1-receptors in those individuals who had been treated with antidepressant medication. However, contrary to earlier reports, they found that people who had been treated with antidepressant drugs showed little down-regulation of α_2-autoreceptors. Interpretation of their findings is complicated by the fact that the postmortem tissue was from suicide victims who were clinically depressed and that they had been treated with a variety of antidepressant drugs over their lifetimes. Nevertheless, among the most consistent differences in noradrenergic receptor binding is that untreated depressed individuals and those with bipolar disorder had increased density of α_2-autoreceptors.

The importance of NE to the actions of antidepressant drugs can be demonstrated in patients treated with adrenergic antidepressants (i.e., NE reuptake inhibitors), who show relapse of symptoms if NE synthesis is prevented by depletion of the NE precursor tyrosine. Clearly NE is necessary for those drugs to be effective. A similar NE synthesis inhibition does not cause relapse in patients treated with the serotonergic reuptake inhibitors.

Norepinephrine and serotonin modulate one another

Because the most consistent chronic effects of antidepressants are down-regulation of β-receptors and 5-HT_2 receptors and an enhanced physiological response to 5-HT, Sulser (1989) proposed a "serotonin–norepinephrine" hypothesis of depression. Both anatomical and functional interactions exist between the noradrenergic neurons originating in the locus coeruleus and the serotonergic neurons in the raphe nuclei (**FIGURE 18.7**), and each system is capable of modulating the other. Destroying 5-HT terminals with the neurotoxin 5,6-dihydroxytryptamine prevents the down-regulation of β-receptors that follows chronic antidepressant treatment. Others have shown that 5-HT agonists can indirectly stimulate the noradrenergic system, causing β-receptor down-regulation, and that increased noradrenergic function may also increase electrophysiological activity in the raphe nuclei. Sulser suggests that NE function involves multiple feedback loops that use a variety of neurotransmitters, including 5-HT, ACh, DA, GABA, and opioid peptides. For more information on the contribution of these neurotransmitters to the symptoms of depression, refer to several excellent reviews (Maletic and Raison, 2009; Ordway et al., 2002).

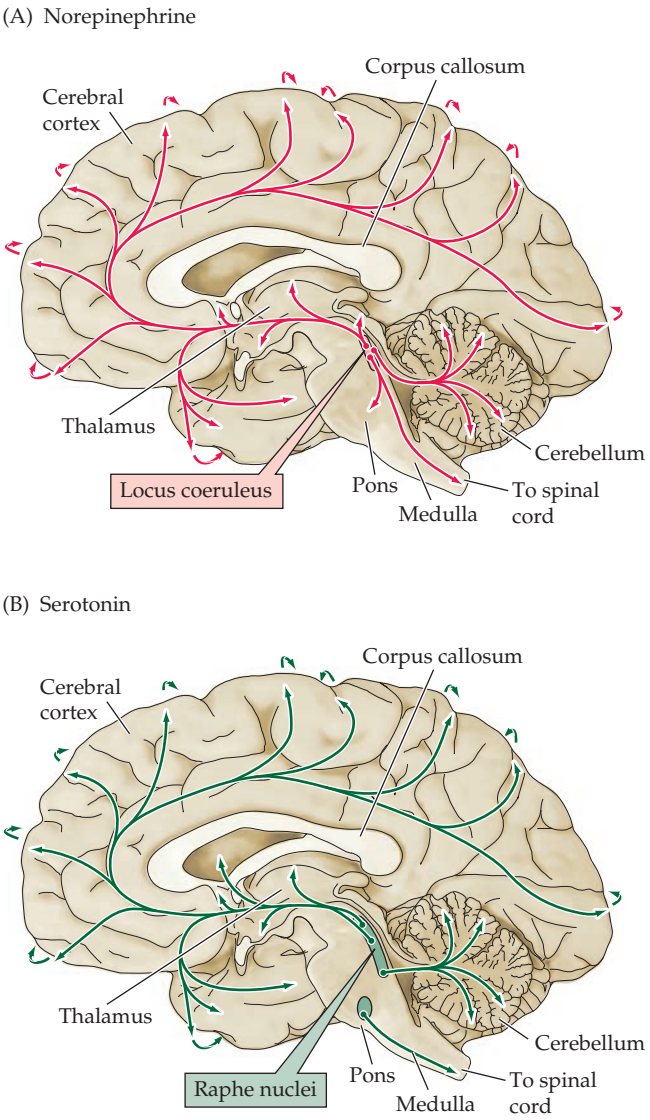


FIGURE 18.7 Two monoamine pathways in the human brain This schematic diagram shows noradrenergic pathways originating in the locus coeruleus (A) and serotonergic pathways originating in the raphe nuclei (B). The overlapping nature and interaction of the two neurotransmitter systems are very apparent.

Neurobiological Models of Depression

In addition to the consideration of neurotransmitter function in depression, other hypotheses pose alternative neurobiological models that are now being tested. For years it has been known that there are multiple structural and functional abnormalities in the brains of clinically depressed individuals (reviewed by aan het

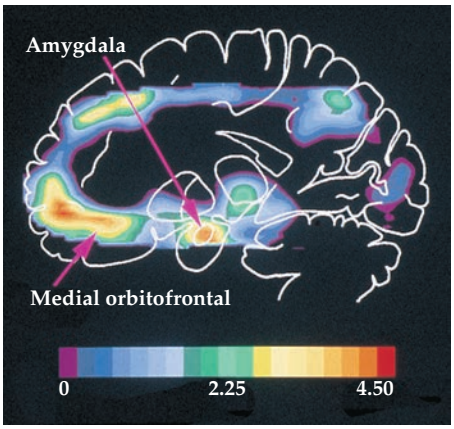


FIGURE 18.8 PET scan of blood flow in the brain of a patient with unipolar depression Increased metabolic activity occurs in the amygdala and the medial orbitofrontal cortex. Activation is shown in red and orange. (Courtesy of Wayne C. Drevets.)

Rot et al., 2009). Neuroimaging studies have focused particularly on reduced hippocampal volume because it has been the most consistent finding, but other brain regions also show reductions, including the amygdala, ventral striatum, anterior cingulate cortex, orbitofrontal cortex, and prefrontal cortex. Some of these regions show decreased metabolic activity as well, but others show increased function, based on PET or fMRI scans. For example, blood flow changes in the brains of patients with depression, compared with normal blood flow in controls, show increased activity in part of the medial orbitofrontal cortex and in the amygdala, brain areas important in emotion regulation (**FIGURE 18.8**). Increased metabolic activity in the amygdala is correlated with the severity of depression and returns to normal after antidepressant drug treatment. Increased activity of the orbitofrontal cortex may reflect the individual's effort to control unpleasant thoughts and emotions (Drevets, 2001). Increasing evidence suggests that visualizing isolated brain structures may not be as important as evaluating the connectivity among the regions of what might be called depression circuits. In fact a common finding has been the lack of cortical control over limbic structures that leaves the individual more vulnerable to stress, impulsivity, cognitive dysfunction, anhedonia, and emotional dysregulation. Also resting-state fMRI (see Chapter 4) has shown that successful treatment is associated with increased connectivity between prefrontal cortex and limbic areas and with restoring the cortical inhibitory control (Dichter et al., 2015).

Reduced connectivity in depression does of course depend on axonal integrity, but of much greater significance are the identified synaptic abnormalities such as altered dendritic complexity and density of dendritic spines. Of importance is the role of stress in producing dendritic atrophy and dendritic spine loss in multiple brain areas that show significant plasticity in response to environmental events. These areas include the hippocampus and prefrontal cortex. However, other brain regions such as the orbitofrontal cortex and amygdala show *enhanced* dendritic arborization. A more detailed discussion of stress-induced synaptic remodeling was presented in Chapter 17. For more details on the role of dendritic arborization in both the onset and treatment of depression, as well as the importance of sex hormones in that plasticity, refer to Duman and Duman (2015) or McEwen (2010). The importance of stress to these brain changes has led to the formulation of the glucocorticoid hypothesis and a related BDNF or neurotrophic hypothesis.

The **glucocorticoid hypothesis** focuses on the stress-related neuroendocrine abnormalities that are frequently found in depressed individuals (see the section on biological rhythms earlier in the chapter). McEwen (2008) provides an excellent discussion of how stress hormones and lifestyle behaviors interact to promote pathophysiology of the brain and the rest of the body. In the depressed individual, the abnormal secretion of CRF from the hypothalamus is apparently responsible for the hypersecretion of ACTH from the pituitary and cortisol from the adrenal cortex. The hypothalamic CRF neurons are normally controlled by other areas of the CNS: the amygdala, which is central to emotional responses, normally stimulates the CRF circuit, and the hippocampus has inhibitory control (see Box 2.4). The hippocampus has receptors that when activated by high levels of glucocorticoids (such as cortisol), help to inhibit CRF release from the hypothalamus, subsequently returning glucocorticoid levels to normal. However, when stress is intense and/or prolonged, glucocorticoid levels remain high, and as shown in animal studies, hippocampal neurons are damaged and no longer respond. The principal damage includes decreases in dendritic branches and loss of dendritic spines, both of which occur in the PFC as well as in the hippocampus. Additionally, the elevated cortisol reduces the formation of new hippocampal cells (neurogenesis). Cell loss in the hippocampus means reduced response to circulating cortisol and loss of inhibition of the HPA axis, inducing further glucocorticoid-mediated hippocampal cell loss.

One might speculate that the elevated cortisol levels found in depressed individuals contribute to cell death and to some of the cognitive symptoms of depression. Small reductions in hippocampal volume are found in magnetic resonance imaging scans of depressed patients. Further, antidepressant drugs and ECT reduce CRF levels in depressed patients. In animals, several types of antidepressant drugs also reverse the loss of dendrites in the hippocampus and other brain areas and increase neurogenesis in the hippocampus. Finally,

intracerebroventricular (ICV) administration of CRF elicits stress-related behavioral and physiological responses in animals, including the expected enhancement of cortisol levels and sympathetic nervous system activity. CRF also elicits behaviors in animals that are closely correlated with symptoms of clinical depression in humans: arousal, insomnia, decreased eating, reduced sexual activity, and anxiety.

The glucocorticoid hypothesis of affective disorders is the basis for the clinical tests of CRF receptor antagonists, which showed early promise as antidepressants. Preliminary clinical studies (Zobel et al., 2000) found significant improvement in both depression and anxiety scores using the CRF receptor antagonist R121919. Minimal side effects were found in this preliminary study; however, subsequent research showed that patients frequently showed elevations in liver enzymes, and further development of the drug was halted.

A second, closely related neurobiological model looks at potential mechanisms underlying the hippocampal cell loss following stress-induced glucocorticoid elevation: deficits in neurotrophic factors such as **BDNF** (**brain-derived neurotrophic factor**). Neurotrophic factors are important proteins that are needed during brain development, but they also regulate changes in synapses and cell survival in adult brains. The **neurotrophic hypothesis** suggests that low BDNF may be responsible for the loss of dendritic branches and spines in the hippocampus and PFC and for reduced neurogenesis in the hippocampus. Furthermore, antidepressants may protect vulnerable cells by preventing the decrease in BDNF. Evidence in support can be briefly summarized as follows: (1) chronic stress reduces BDNF in the hippocampus in rats, (2) chronic but not acute antidepressant treatment increases BDNF in both animals and humans, and (3) antidepressants prevent stress-induced reductions in BDNF and neuronal atrophy (**FIGURE 18.9**).

A *causal* connection between BDNF-enhanced neurogenesis and antidepressant effects is more difficult to determine. However, direct demonstration that enhanced neurogenesis is necessary for antidepressant action was initially provided by Santarelli and colleagues (2003) using mice and several animal models of depression. The 90% reduction in hippocampal cell proliferation following focused irradiation prevented both fluoxetine- and imipramine-induced neurogenesis as well as their antidepressant action in the chronic unpredictable stress model of depression. Although this finding is a critical component of the neurogenesis hypothesis, cautious interpretation is needed because irradiation does not act selectively to interfere with the production of neurons, and other damaging effects occur. Nevertheless, more recent studies supported the earlier work and indicated that antidepressants may impact neurons in several ways: by enhancing proliferation of new cells, by protecting them from apoptosis and atrophy, and by strengthening neuropil development by increasing the number and length of dendrites. Each of these changes is influenced by neurotrophins; however, how the cellular changes contribute to function at the circuit level and how that translates into changes in affect in depressed individuals remain unclear (Balu and Lucki, 2009). If neuroprotective effects are central to antidepressant action, an additional treatment approach might be to use modulation of epigenetic events (**BOX 18.1**). As you learned earlier, environmental events including the stress of early abuse or neglect can cause long-lasting epigenetic changes that alter brain development and increase vulnerability to a variety of disorders. Hence early intervention to reverse the epigenetic effects of stress may enhance resilience.

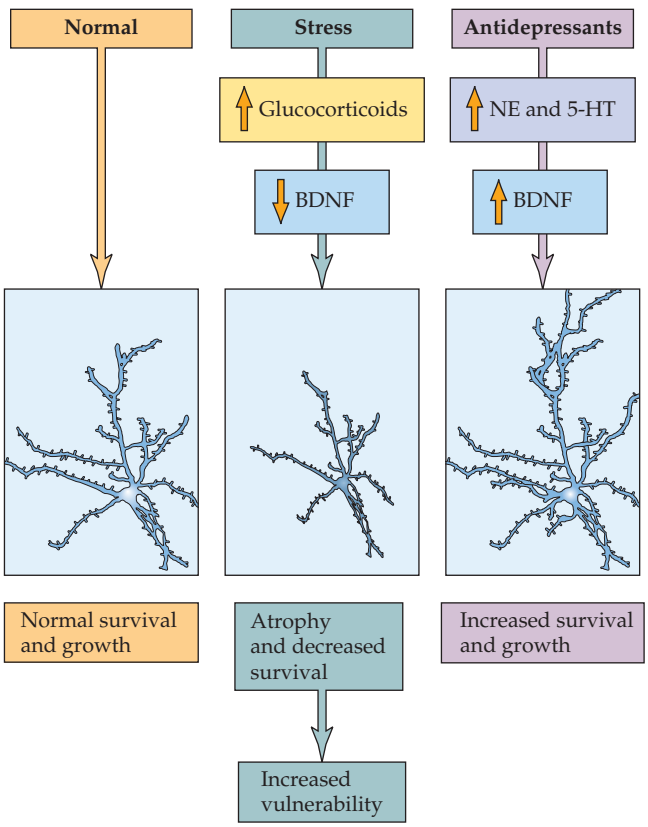


FIGURE 18.9 Effect of stress and antidepressant treatment on BDNF in hippocampal cells The box on the left shows a typical hippocampal cell in the CA3 area. Chronic stress (center) elevates glucocorticoids and decreases BDNF, which may be responsible for the loss of dendritic trees and may make the cells more vulnerable to a variety of detrimental factors. Chronic antidepressant treatment (right) not only alters monoamine transmission but also increases BDNF. BDNF may protect the cells from further damage and may help repair those already damaged. (After Duman et al., 1999.)

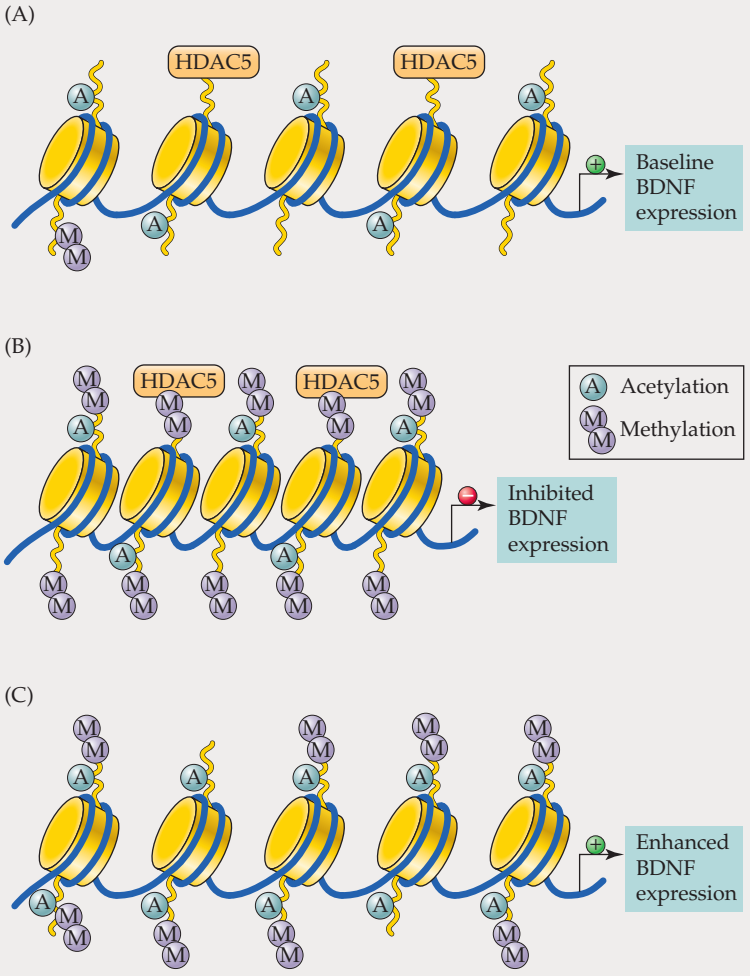
BOX 18.1 The Cutting Edge

Epigenetic Modifications in Psychopathology and Treatment

You learned in Chapter 17 that genes and environment interact to determine the tendency to express anxiety. Environmental stress, including parental abuse or neglect, causes long-lasting epigenetic changes to the genes of several components of the stress circuit, leading to enhanced CRF expression in the amygdala and hypothalamus and decreased glucocorticoid receptors in the hippocampus. These and other changes contribute to stress circuit programming during development, making it more sensitive, and increasing the vulnerability to both anxiety disorders and depression in the adult. Given the close relationship between stress and depression, this outcome is not surprising.

Other genes of significance are also modified by stress-induced chromatin remodeling. Chromatin remodeling or rearrangement occurs when the small proteins known as histones, which are complexed with DNA, are altered by any one of several chemical changes brought about by enzymes. These chemical changes, including methylation, acetylation, phosphorylation, and others less well studied, occur on the amino acid tails of the histone molecules. These changes either make the chromatin more tightly packed, which limits gene expression by physically limiting the access of transcription factors, or loosen the chromatin structure, enhancing transcription.

A delicate balance of epigenetic factors maintains normal cell function. Dysregulation in the form of either too much expression of a gene or too little may be the basis of pathological conditions. Chronic social defeat stress (see Chapter 4) causes long-lasting reductions in BDNF expression by epigenetically increasing histone methylation more than fourfold. The methylation of histone induces a more "closed" chromatin state that reduces transcription of the gene for BDNF (see **Figure**). Chronic, but not acute, antidepressant treatment with imipramine reverses the stress-induced depression-like symptoms but, somewhat surprisingly, does not reverse the stress-induced methylation of histone. Instead, it apparently reverses the suppression of BDNF expression by increasing the level of histone acetylation subsequent to the activation of histone acetylase. The acetylation of histone induces a more "open" chromatin state, resulting in an imipramine-induced increase in BDNF. Nemeroff and colleagues (as reported in Tsankova et al., 2007) further evaluated the underlying mechanism and found that in socially defeated mice, not only does imipramine cause hyperacetylation, but it additionally down-regulates histone deacetylase



Chromatin remodeling (A) Under nonstress conditions, the chromatin of the brain-derived neurotrophic factor (BDNF) gene has low levels of acetylation and virtually no methylation, providing a basal BDNF expression. (B) The histone methylation following prolonged episodes of defeat stress induces a tighter envelope of chromatin proteins and blocks BDNF gene expression. (C) Chronic antidepressants increase acetylation without altering the chronic stress-induced methylation, which produces a "relaxation" of the repressed chromatin, encouraging transcription of the BDNF gene, which may be necessary for the antidepressant effects. HDAC5, histone deacetylase 5. (After Tsankova et al., 2007.)

BOX 18.1 The Cutting Edge (*continued*)

(HDAC), the enzyme that normally removes the acetyl groups. The lack of these effects in nonstressed mice is particularly interesting because antidepressants rarely have any effect in healthy, nondepressed individuals. Furthermore, when the researchers caused overexpression of HDAC in the dentate gyrus of the hippocampus with genetic manipulation, they found total blockade of the antidepressant effects of imipramine.

The importance of down-regulation of HDAC to antidepressant action has been investigated further as a potential approach to treating mood disorders (see Machado-Vieira et al., 2010). If HDAC inhibitors could reverse dysfunctional epigenetic changes, neurotrophic proteins could be elevated and could enhance the neural connectivity that is necessary for behavioral adaptation. Several preclinical studies have now demonstrated antidepressant effects in rodents after intracerebral injection of several different HDAC inhibitors. Further, the HDAC inhibitors have been shown to reverse oxidative stress–induced neuronal injury in vitro. Oxidative stress is characteristic of individuals with bipolar disorder, who often find lithium and valproate, drugs that reduce oxidative stress, to be therapeutic. Clearly this early research has promise, and the role of epigenetic acetylation in both pathophysiology of mood disorders and therapeutics deserves further study.

Since the production of BDNF is dependent on the cyclic adenosine monophosphate (cAMP) second-messenger system, it is significant that *chronic* antidepressant drug treatment up-regulates several components of the system in the hippocampus and frontal cortex. This up-regulation occurs despite the down-regulation of the β-adrenergic receptors and 5-HT receptors that are coupled to the cAMP cascade (**FIGURE 18.10**). Up-regulation occurs in several stages of the cascade, including enhanced coupling between stimulatory G_s protein and adenylyl cyclase, an increase in activated cAMP-dependent protein kinase A (PKA), and an increase in phosphorylation of cAMP response element binding protein (CREB), which is a transcription factor that induces protein synthesis of BDNF and other proteins.

Although at present there is no way to directly inject BDNF into humans as a test for antidepressant activity, intracerebral injection of BDNF or CREB into the hippocampus in rodents produced antidepressant effects in the forced swim and learned helplessness tests (Shirayama et al., 2002). Brain levels of CREB are low in depressed patients and are increased by most antidepressant drugs after several weeks. It is tempting to try to develop therapeutic methods that might rapidly enhance CREB and the neurotrophic factors. One might consider that enhancing any portion of the cAMP cascade could ultimately enhance BDNF production and relieve depression. One approach involves inhibiting **phosphodiesterase** (**PDE**), the enzyme that normally degrades cAMP to 5′-AMP. Inhibition of PDE would up-regulate the cAMP cascade in a prolonged fashion. One phosphodiesterase inhibitor, rolipram, reduced

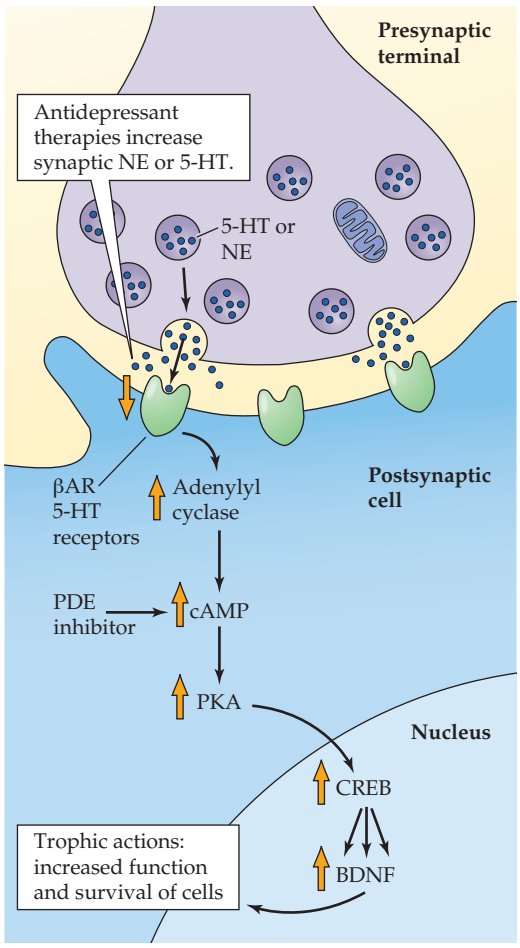


FIGURE 18.10 Up-regulation of second-messenger pathway by chronic antidepressant treatment The increase in NE and 5-HT caused by acute antidepressant treatment produces down-regulation of their receptors when treatment is chronic. In response to the reduction in receptors, the cAMP pathway is up-regulated, producing increases in adenylyl cyclase activity, cAMP, protein kinase A (PKA), and the transcription factor within the nucleus, CREB. CREB increases the synthesis of several proteins, including BDNF. βAR, β-adrenergic receptor; PDE, phosphodiesterase. (After Duman et al., 1997.)

symptoms in a small trial of depressed patients, but side effects prohibit its regular use. More selective inhibitors may prove effective with reduced side effects. Although the possibilities are exciting, application to human therapeutics is clearly a long way off.

Although there is fairly convincing evidence that increased neurogenesis is needed for antidepressant effects, a causal role for neurogenesis in the etiology of depression remains more difficult to demonstrate. The reduction in hippocampal volume and the morphological changes found in depressed patients that are reversed by antidepressant treatment could be related to the reduced levels of BDNF found in the hippocampus and the PFC postmortem, although these findings are not consistent and are best documented in depressed suicides. Also a common polymorphism of the gene that codes for BDNF (*val66met*) has sometimes been associated with mood disorders, although it is not specific to depression but also occurs with higher frequency in Alzheimer's disease, OCD, and others. More convincing are the results of studies finding that *temporary* reduction of BDNF gene expression in *specific* regions of the forebrain produces depression-like behaviors in mice, particularly in females. However, others have found that heterozygous BDNF knockout mice display increased aggressiveness, hyperphagia, deficits in spatial learning, and subtle alterations in sensory systems, but rarely depressive-like behaviors. Whether the differences can be explained by the permanence of the BDNF loss in the knockout studies or the lack of brain specificity is not clear. Further research in this area is ongoing.

These two neurobiological hypotheses, along with a third, which considers the impairment of brain reward pathways, are discussed in detail in an excellent review by Krishnan and Nestler (2010). They provide both an overview and supporting evidence as well as a discussion of future directions for research.

Section Summary

- The monoamine hypothesis was based on pharmacological evidence showing that depression is associated with low levels of monoamines, whereas mania coincides with excess monoamine activity.
- Modifications of the hypothesis were needed to explain the discrepancy in time between the rapid increase in monoamines by antidepressant drugs and the slow onset of clinical effects over several weeks.
- A role for 5-HT in depression is suggested by the following: (1) lower 5-HIAA occurs in depressed individuals, (2) knockout mice show some behaviors analogous to human depression, (3) depletion of tryptophan causes depressed mood in patients in remission, (4) a polymorphism of the SERT gene is associated with depression, and (5) increased postsynaptic 5-HT_2 receptors are found in patients postmortem.
- Chronic antidepressant treatment causes the down-regulation of 5-HT autoreceptors, which increases synaptic 5-HT and subsequent intracellular changes leading to neurogenesis and synaptic remodeling. The effects of fluoxetine on autoreceptor down-regulation and depression may depend on several factors, including pretreatment 5-HT function and the nature of the environment. The electrophysiological response to 5-HT is enhanced by chronic antidepressant treatment.
- In depressed patients, chronic antidepressants increase NE turnover, which leads to down-regulation of β-adrenergic receptors and α_2-autoreceptors.
- Inhibiting the synthesis of 5-HT causes relapse in patients treated with serotonin reuptake inhibitors, but not in those treated with adrenergic antidepressants. Likewise, inhibiting NE synthesis produces relapse in patients treated with NE reuptake inhibitors, but not in those treated with serotonergic agents.
- Prolonged hypersecretion of CRF, ACTH, and glucocorticoids damages dendritic branching and spines in the hippocampus and PFC. It also reduces neurogenesis in the hippocampus, which further diminishes the negative feedback on HPA axis function. Chronic antidepressants reverse these effects.
- ICV CRF elicits stress-related behavior and hormone response and signs of depression in rodents. A clinical trial of a CRF antagonist improved depression and anxiety scores in patients.
- The neurotrophic hypothesis suggests that stress hormones that reduce BDNF may cause neuronal damage, and antidepressants may prevent it by elevating BDNF. Intrahippocampal BDNF has antidepressant action.
- Preventing hippocampal cell proliferation prevents antidepressant-induced neurogenesis and behavioral effects.
- Chronic antidepressants up-regulate the cAMP cascade, leading to increased phosphorylated CREB and subsequent expression of BDNF. Enhancing the cAMP cascade by inhibiting phosphodiesterase produced antidepressant effects in some patients.
- Evidence for BDNF in the etiology of depression includes the following: (1) BDNF is low in the hippocampus and the PFC of depressed patients postmortem, (2) a BDNF gene polymorphism may be associated with mood disorders, and (3) modifying BDNF gene expression in mice leads to depressive behaviors.

Therapies for Affective Disorders

Three major classes of antidepressants have proved effective in reducing symptoms of mood disorders (**TABLE 18.3**). They are the monoamine oxidase inhibitors, the tricyclic antidepressants, and the second-generation antidepressants, which include the selective serotonin reuptake inhibitors. In addition, there are several atypical antidepressants as well as several nondrug therapies, including sleep deprivation (see **Web Box 18.4**), electroconvulsive therapy, transcranial magnetic stimulation, vagal nerve stimulation, and deep brain stimulation. These brain stimulation treatments are discussed in **Web Box 18.5**. Drugs for treating the manic episodes of bipolar disorder will be considered separately.

The availability of a variety of antidepressant drugs means that many clinically depressed individuals can find significant relief. However, double-blind, placebo-controlled trials of antidepressants show that no one specific drug or drug type is more effective than any other, and there is no way to predict which patient will respond to a particular drug. Each drug is effective in reducing, but not necessarily eliminating, symptoms for about two-thirds of the cases of clinical depression. The different pharmacological characteristics of the agents mean that they will reduce different symptoms and will produce distinct but characteristic side effects. Each patient must usually undergo trials to find an antidepressant that optimally balances effectiveness and side effects. Frequently, outcomes are enhanced by the addition of a second drug to the treatment regimen. Every one of the treatment methods currently available requires chronic administration, suggesting that although we understand how each works acutely at the synapse, the clinical effect must depend on compensatory changes in function that require time to develop. Although significant changes in symptoms can occur during the first 1 to 3 weeks of drug treatment, maximum effectiveness may not be achieved until after 4 to 6 weeks of therapy. This time lag is especially worrisome in patients who are severely depressed and suicidal. Some symptoms, including irregularities in sleep and appetite, are the first signs that show improvement, followed over the next few weeks by mood enhancement. Several long-term studies from the National Institute of Mental Health suggest a period of maintenance drug treatment of at least 6 to 8 months after symptoms are reduced. Because maintenance therapy significantly reduces the probability of relapse, treatment is extended indefinitely for some individuals.

Although we are treating antidepressant drugs and drugs used for anxiety (see Chapter 17) as separate entities, we would like to make it clear that the distinction is often more semantic than real. As we noted earlier, stress and anxiety are components of affective disorders, and the trend in drug treatment further blurs the distinction. Antidepressant drugs reduce the anxiety that accompanies depression, and they are increasingly being used to treat anxiety disorders unrelated to depression.

Monoamine oxidase inhibitors are the oldest antidepressant drugs

The first true antidepressant action was discovered quite by accident as the result of a lucky clinical

TABLE **18.3** Major Classes of Antidepressants and Their Most Notable Side Effects

Class	Antidepressants	Side effects
Monoamine oxidase inhibitors	Phenelzine (Nardil) Tranylcypromine (Parnate) Isocarboxazid (Marplan)	Insomnia, weight gain, hypertension, drug interactions, tyramine effect
Classic tricyclics	Imipramine (Tofranil) Amitriptyline (Vanatrip) Desipramine (Norpramine)	Sedation, anticholinergic effects, cardiovascular toxicity
Selective serotonin reuptake inhibitors	Fluoxetine (Prozac) Sertraline (Zoloft) Paroxetine (Paxil)	Insomnia, gastrointestinal disturbances, sexual dysfunction, serotonin syndrome
Atypical antidepressants	Maprotiline (Ludiomil) Bupropion (Wellbutrin) Mirtazapine (Remeron)	Varies with individual mechanism of action
Electroconvulsive shock and transcranial magnetic stimulation		Memory impairment, confusion, amnesia

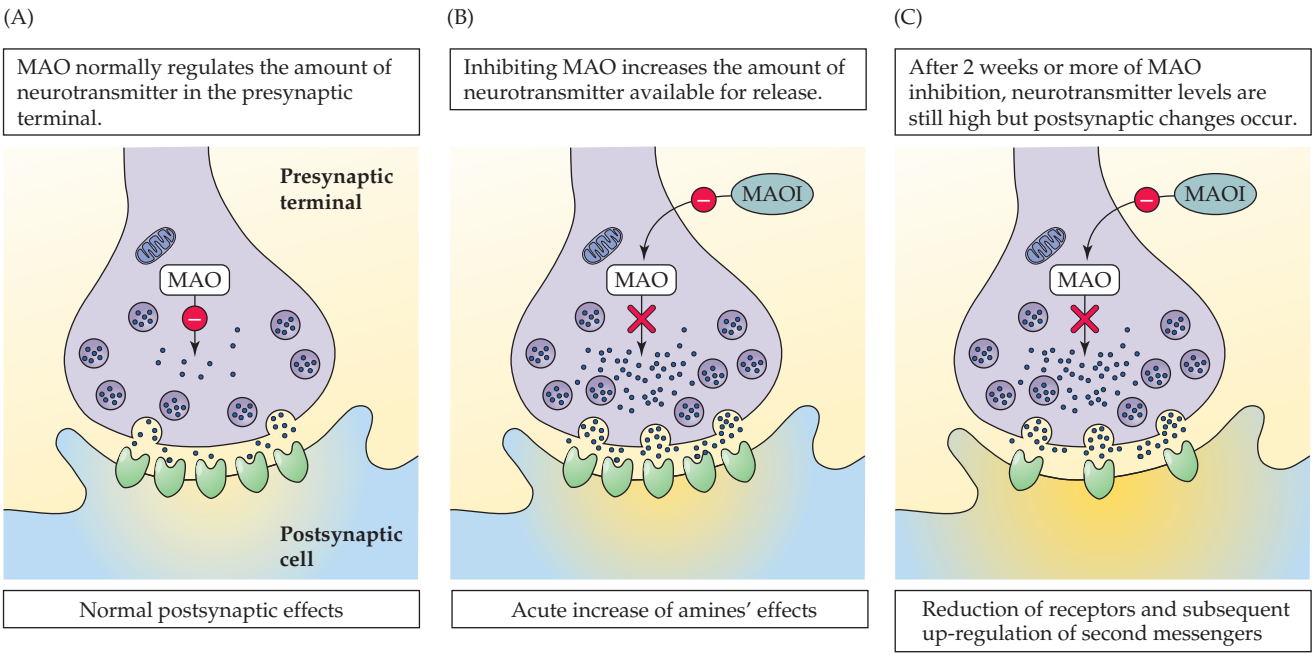


FIGURE 18.11 Acute and long-term effects of MAOIs on synaptic function (A) Presynaptic MAO degrades neurotransmitter molecules that are not in vesicles, to keep amines at "normal" levels. (B) MAOIs inhibit the enzyme, causing an elevation in available neurotransmitter for release, resulting in increased action at receptors. (C) After 10 days to 2 weeks of antidepressant treatment, the amount of neurotransmitter in the synapse is still elevated over control conditions, but neural adaptation has occurred: down-regulation of amine receptors and up-regulation of the cyclic adenosine monophosphate (cAMP) second-messenger system. Other antidepressant drugs produce similar adaptive changes in neurons.

observation. The drug iproniazid was used in the early 1950s to treat tuberculosis but had significant mood-elevating effects unrelated to its effects on the disease. Following that observation, iproniazid was found to inhibit monoamine oxidase (MAO). Although met with enthusiasm following their early introduction as antidepressants, the **MAO inhibitors** (**MAOIs**) fell into disfavor because of their reputation for having severe and dangerous side effects (see side effects section below). However, over the years it has become apparent that, with appropriate dietary restrictions, MAOIs can be used safely and often work well for patients who are treatment-resistant (do not respond to other drugs) and who reject the idea of electroconvulsive therapy. In addition to their use in affective disorders, MAOIs are used in the treatment of several anxiety states and have positive effects on the eating behavior and mood of patients with bulimia and anorexia nervosa. The currently available MAOIs include phenelzine (Nardil), tranylcypromine (Parnate), and isocarboxazid (Marplan).

MECHANISM OF ACTION You will recall from Chapter 5 that MAO is an enzyme found inside the cells of many tissues, including neurons. The normal function of the enzyme is to metabolize the monoamine neurotransmitters in the presynaptic terminals that are not contained in protective synaptic vesicles. The inhibition of MAO increases the amount of neurotransmitter available for release. A single dose of an MAOI increases NE, DA, and 5-HT and thus increases the action of the transmitters at their receptors. It was initially assumed that enhanced neurotransmitter function was responsible for the antidepressant action; however, those biochemical changes occur within hours, but the antidepressant effects require weeks of chronic treatment. It is now apparent that neuron adaptation involving change in receptor density or second-messenger function must play an important part in these drug effects (**FIGURE 18.11**).

SIDE EFFECTS The more common side effects of MAOIs include changes in blood pressure, sleep disturbances including insomnia, and overeating, especially of carbohydrates, which may lead to excessive weight gain. In addition to these side effects, three other types of side effects are significantly more dangerous. First, because inhibition of MAO elevates NE levels in peripheral nerves of the sympathetic branch of the autonomic nervous system as well as in the CNS, any prescription or over-the-counter drug that enhances NE function will have a much greater effect than normal. For example, nasal sprays, cold medications, antiasthma drugs, amphetamine, and cocaine will all have greater than expected effects and will produce elevated blood pressure, sweating, and increased body temperature. Second, some serious side effects are due to the inhibition of MAO in the liver as well as in the brain. The MAO in the liver

is responsible for deaminating tyramine, which is a naturally occurring amine formed as a by-product of fermentation in many foods, including cheeses, certain meats, and pickled products. These foods must be avoided by individuals using MAOIs (**TABLE 18.4**). Elevated tyramine levels release the higher than normal stores of NE at nerve endings, causing a dramatic increase in blood pressure. Blood pressure may reach critical levels, and this is accompanied by headache, sweating, nausea, vomiting, and sometimes stroke. Third, MAOIs also inhibit other liver enzymes such as the cytochrome P450 enzymes (see Chapter 1), which normally degrade such drugs as barbiturates, alcohol, opioids, aspirin, and many others. The effects of these drugs are prolonged and intensified in the presence of MAOIs.

TABLE 18.4 Dietary Restrictions for Patients Taking MAOIs

Food group	Examples
Dairy	Unpasteurized milk and yogurt; aged cheese; other cheeses including blue, Boursault, brick, Brie, Camembert, cheddar, Colby, Emmenthaler, Gouda, Gruyère, mozzarella, Parmesan, provolone, Romano, Roquefort, and Stilton
Meat and meat alternatives	Aged game; liver; canned meats; yeast extracts; salami; dry sausage; salted, dried, smoked, or pickled fish such as herring, cod, and caviar; peanuts
Breads, cereals, and grains	Homemade yeast breads with substantial quantities of yeast; bread or crackers containing cheese
Vegetables and fruits	Italian broad beans, sauerkraut, bananas, red plums, avocados, raspberries
Miscellaneous	Alcoholic beverages including red and white table wines, ale, beer, champagne, sherry, and vermouth; yeast concentrates, soup cubes, commercial gravies, or meat extracts; soups containing items that must be avoided; soy sauce; soy bean curd (hoisin)

Tricyclic antidepressants block the reuptake of norepinephrine and serotonin

This class of antidepressant is named for its characteristic three-ring structure (**FIGURE 18.12**), which is closely related to that of the antipsychotic drugs in the phenothiazine class (see Chapter 19). Although the prototypical **tricyclic antidepressant** (**TCA**) imipramine (Tofranil) failed its original test for antipsychotic effects, it did appear to have mood-elevating actions. Many other tricyclics were subsequently developed.

MECHANISM OF ACTION The drugs in this class act by binding to the presynaptic transporter proteins and inhibiting reuptake of neurotransmitters into the presynaptic terminal. Inhibition of reuptake prolongs the duration of transmitter action at the synapse, ultimately producing changes in both pre- and postsynaptic receptors. Although many of the drugs in this class are equally effective in inhibiting the reuptake of NE and 5-HT, some are more effective on one transmitter than the other. Although this difference does not change their antidepressant action, it does determine the side effects of the drugs. **TABLE 18.5** provides a comparison of the relative NE and 5-HT reuptake-blocking potencies of several TCAs and some second-generation antidepressants as well. You may notice that just as selective reuptake inhibitors for serotonin were developed, so too are there selective norepinephrine reuptake inhibitors. As was true for the MAOIs, the immediate increase in NE and 5-HT function is not correlated with clinical effectiveness, which takes several weeks. Clearly, an acute increase in synaptic activity is only the first step in antidepressant action; neuronal adaptation, occurring over a period of time, also plays an important role. In addition to reuptake blockade, most of the TCAs block ACh, histamine, and α-adrenergic receptors, and this contributes to their side effects.

SIDE EFFECTS Although we assume that TCA action is due to enhancement of monoamine activity followed by compensatory changes in the transmitter systems, the receptor-blocking activity contributes to side effects. Histamine receptor blockade is responsible for the sedation

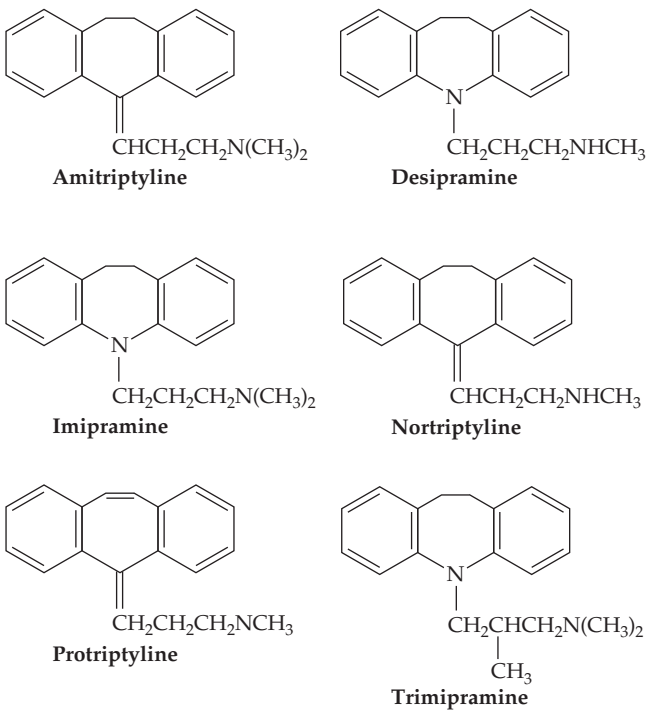


FIGURE 18.12 Tricyclic antidepressants' characteristic three-ring structure

TABLE 18.5 Relative Specificity of Antidepressant Drugs That Block Monoamine Reuptake

Drug name	Extent of reuptake inhibition*	
	Norepinephrine	Serotonin
Tricyclic Compounds		
Desipramine (Norpramine)	+++	+
Protriptyline (Vivactil)	+++	+
Amitriptyline (Vanatrip)	++	++
Imipramine (Tofranil)	++	++
Clomipramine (Anafranil)	++	++++
Selective serotonin reuptake inhibitors		
Fluoxetine (Prozac)	0	++++
Sertraline (Zoloft)	0	++++
Paroxetine (Paxil)	+	++++
Selective norepinephrine reuptake inhibitors		
Reboxetine (Edronax)	++++	0
Atomoxetine (Strattera)	++++	0

*0, no effect; +, mild effect; ++, moderate effect; +++, strong effect; ++++, maximal effect.

and fatigue that are frequent side effects that limit the drugs' usefulness in individuals who must remain alert. On the other hand, for patients who experience agitation, the sedative effects may be welcome. Anticholinergic side effects are troublesome for others and include dry mouth, constipation, urinary retention, dizziness, confusion, impaired memory, and blurred vision. The α_1-blockade in combination with the NE reuptake–blocking effects leads to several potentially dangerous cardiovascular side effects, including orthostatic hypotension, tachycardia, and arrhythmias. This particular set of side effects makes it especially difficult to treat depression in elderly patients with known cardiac disorders.

In addition, toxicity following overdose causes cardiovascular depression, delirium, convulsions, respiratory depression, and coma. Heart arrhythmias may produce cardiac arrest and fatalities. Since the fatalities associated with TCA treatment occur at approximately 10 times the normal dose, these drugs have a relatively low therapeutic index (TD_{50}/ED_{50}), particularly when used by patients demonstrating suicidal tendencies.

Second-generation antidepressants have different side effects

In an attempt to offer drugs with fewer side effects and more rapid onset of action, a host of new antidepressants have been developed. In general, they are designed to be more selective in their action with the hope of eliminating the anticholinergic and cardiovascular effects produced by the older drugs while still elevating levels of NE and/or 5-HT to provide antidepressant action. None are more effective, however, nor do they have a more rapid onset. The most significant difference is seen in the nature of their side effects, which are related to their neurochemical mechanisms of action. Many are considered safer than the older drugs if taken as an overdose.

The **selective serotonin reuptake inhibitors** (**SSRIs**) deserve special mention because they are often the first choice among antidepressants because of their greater relative safety. In addition to major depression, these drugs are also used to treat several distinct disorders: panic and anxiety disorders, obsessive-compulsive disorder, obesity, and alcohol use disorder.

MECHANISM OF ACTION Drugs in this class, which include fluoxetine (Prozac), sertraline (Zoloft), and paroxetine (Paxil), are more selective than TCAs in enhancing serotonin function because they block the presynaptic reuptake transporter for 5-HT to a greater extent than the noradrenergic transporter. As is true for all of the antidepressants discussed, we assume that the antidepressant action requires compensatory changes in neurons that occur over several weeks, as shown in Figure 18.10.

SIDE EFFECTS The side effects of the SSRIs are different from those of the TCAs because the drugs do not alter NE, histamine, or ACh. Hence, the frequent TCA-induced side effects of sedation, cardiovascular toxicity, and anticholinergic effects are absent. Nevertheless, SSRIs produce a different pattern of side effects because they enhance 5-HT function at all serotonergic receptors. Although the antidepressant action may be related to increased 5-HT function at some serotonergic receptors, increased 5-HT activity at other receptors causes side effects: anxiety, restlessness, movement disorders, muscle rigidity, nausea, headache, insomnia, and sexual dysfunction. The sexual dysfunction, which occurs in 40% to 70% of patients, is a frequent reason for terminating therapy, particularly among young male patients.

Although the SSRIs are generally safer than the older drugs, they have potentially life-threatening effects when combined with other serotonergic agonists or with drugs that interfere with the normal metabolism of the SSRIs. These effects, referred to as the **serotonin syndrome**, are characterized by severe agitation, disorientation and confusion, ataxia, muscle spasms, and exaggerated autonomic nervous system functions including fever, shivering, chills, diarrhea, elevated blood pressure, and increased heart rate (Lane and Baldwin, 1997).

One other distinctive characteristic of the SSRIs compared with the older antidepressants is their ability to cause physical dependence. As many as 60%

of patients suffer withdrawal effects following drug termination, particularly with the short-acting SSRIs, unless the dose is tapered off gradually (Zajecka et al., 1997). These withdrawal symptoms, which can last for several weeks, include dizziness and ataxia, nausea, vomiting and diarrhea, fatigue, chills, sensory disturbances, insomnia, vivid dreams and increased anxiety, agitation, and irritability. Although the SSRIs avoid many of the dangerous side effects of the older drugs, caution in their use is still warranted.

Although the SSRIs are second-generation antidepressants, some of the newer antidepressants are once again **dual NE/5-HT modulators** because the most current thinking suggests that enhancing both NE and 5-HT function is more beneficial than acting on a single monoamine. The reuptake blocker duloxetine (Cymbalta) and mirtazapine (Remeron) are two such drugs. Mirtazapine is an antidepressant with a unique mechanism of action. It blocks α_2-autoreceptors, which increases synaptic NE and α_2-heteroreceptors on serotonergic cells, which increases 5-HT release. Additionally, to reduce side effects, the drug specifically blocks selected 5-HT receptors. Early mirtazapine trials showed clear clinical benefit in a broad range of patients when compared with placebo, and equal effectiveness compared with the TCA amitriptyline, but it had somewhat fewer (65%) adverse side effects compared with placebo (70%) or amitriptyline (87%).

Third-generation antidepressants have distinctive mechanisms of action

Third-generation antidepressants are currently in the development and testing stages. The goals for the newest drugs will be to continue to minimize side effects and toxicity as well as speed up the onset of effectiveness. Despite our best attempts, it is evident that the neuropharmacology is still unclear with respect to the cellular changes that produce effective antidepressant action, but it is clear that a series of molecular changes underlie the therapies. The two newest approaches—CRF receptor antagonism and enhancement of the cAMP intracellular second-messenger system and subsequent synaptic plasticity—were discussed earlier in the chapter, in the section on neurobiological models of depression. In addition to these approaches, regulation of circadian dysfunctions by agomelatine (as discussed in **Web Box 18.3**) holds great promise. It has passed both phase 2 and phase 3 clinical trials and is already approved for use in Europe. Several others that also attack depression symptoms from a novel direction include intravenous ketamine and galanin agonists.

INTRAVENOUS KETAMINE Among the more troublesome problems in depression therapy is finding a way to reduce symptoms in the large number of treatment-resistant patients (i.e., those who do not respond to available medications or cannot tolerate the side effects). One new approach has been the use of the *N*-methyl-D-aspartate (NMDA) receptor antagonist ketamine, a dissociative anesthetic (reviewed by Murrough, 2012). This glutamate receptor subtype has received new attention because there is evidence of abnormal NMDA receptor binding in suicidal patients. Also, chronic stress alters NMDA receptor binding in animal studies, and NMDA receptor antagonists have shown antidepressant properties in a wide variety of animal models (see Chapter 4), including learned helplessness, forced swim test, sucrose preference tests, chronic mild stress, and others (reviewed by Browne and Lucki 2013).

When administered intravenously at subanesthetic doses, ketamine has been shown in multiple small clinical trials to produce a rapid (generally within an hour) reduction in depression symptoms for 65% to 70% of *treatment-resistant* patients. In some cases it has led to total remission of all symptoms. Although the antidepressant effect has a rapid onset, the duration of action varies widely. A recent meta-analysis of 21 studies showed antidepressant effects lasted a week for most patients, although a few studies showed effects for some individuals lasting as long as 12 to 14 days after injection (Coyle and Laws, 2015). However, for patients with bipolar disorder the antidepressant effects last on average only a few days. Follow-up studies have evaluated the efficacy and safety of *multiple* ketamine infusions in treatment-resistant patients; however, the majority of patients (almost 90%) relapsed in an average of 19 days after the last administration. Interestingly a very few individuals showed more prolonged effects, lasting up to 3 months. It could be important to identify characteristics of those individuals that differed from the majority of patients, to identify a subtype of responders. Based on the data, it would seem that multiple administrations do not show benefits for a given individual beyond a single infusion. One important discovery was that gender differences in outcome may have affected the statistical results, since men seem to have greater symptom reduction than women. This was a surprise because in rodents, females seem to be more sensitive to the behavioral effects of ketamine. The rapid onset of effectiveness is clearly distinctive from that of the classical antidepressant drugs, which take weeks to be effective. Additionally, ketamine seems to be effective in patients who are not helped by the usual antidepressants. The fact that it does not act on the monoamines provides new hope for developing a novel class of antidepressant drugs that act on the glutamate receptor. Because other NMDA receptor antagonists have antidepressant effects in a wide variety of animal models, the pressure to translate these findings to clinical use is significant. Unfortunately, as we have seen before, results from animal experiments do not necessarily transfer to human clinical benefits.

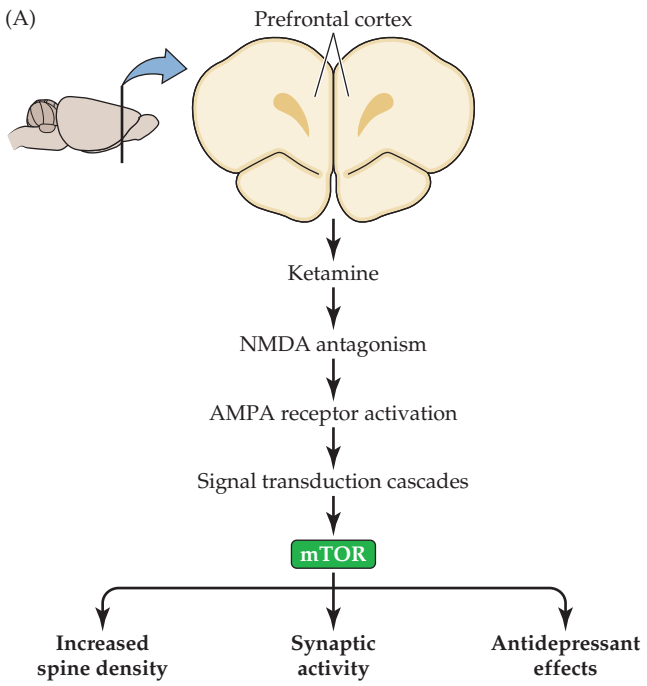


FIGURE 18.13 Ketamine's rapid activation of mTOR
(A) The cascade of events beginning with intravenous injection of ketamine inhibits NMDA receptor function but additionally enhances glutamate activation of AMPA receptors. Multiple signal transduction cascades mediate the activation of the protein kinase mTOR. The action of mTOR in turn increases dendritic spine density, enhances synaptic activity by modifying synaptic proteins, and produces rapid antidepressant effects. A reciprocal relationship between mTOR and BDNF forms a positive feedback loop (not shown). (B) Two-photon microscopy shows increased spine density on both distal and proximal segments of dendrites in medial prefrontal cortex (mPFC) pyramidal neurons 24 hours after ketamine administration, compared with control animals. There was an increase in large diameter, more mature (mushroom) spines. The increase was prevented by the mTOR antagonist rapamycin. (C) Whole-cell voltage clamp recordings show ketamine enhanced the 5-HT–induced excitatory postsynaptic currents in pyramidal cells of mPFC, 24 hours after ketamine injection. (A after Cryan and O'Leary, 2010; B,C after Li et al., 2010.)

For example, the NMDA antagonist memantine, which shows antidepressant effects in rodent tests, did not consistently show antidepressant effects in clinically depressed patients (Sanacora and Schatzberg, 2015). We have to ask what is different about ketamine than other NMDA antagonists.

The next question is how can we explain the prolonged effect of a single infusion of ketamine, which lasts far longer than the drug's short half-life. One potential explanation is that ketamine may initiate intracellular mechanisms that have an extended duration of effects. The concept of antidepressant enhancement of BDNF-stimulated neurogenesis and elaboration of dendritic spine growth and development has taken center stage because BDNF is the common factor involved with both conventional antidepressant effects and the rapid-acting ketamine. Additionally ketamine has no antidepressant effects in BDNF knockout mice or in mice without the BDNF receptor trkB (see Monteggia and Zarate, 2015). However, the question of how it can work so quickly remains, while conventional antidepressant drugs take weeks to enhance BDNF and its neurotrophic functions. Among the possible mechanisms is the rapid activation of the mTOR signaling cascade that is important in the synthesis of proteins that support the development, maturation, and function of new dendritic synapses promoting synaptic plasticity (Browne and Lucki, 2013). Through a series of events, blocking the NMDA receptor with ketamine subsequently enhances glutamate function at AMPA receptors and initiates multiple signal transduction cascades to ultimately activate the protein kinase mTOR that produces changes in synaptic plasticity (**FIGURE 18.13A**). Enhanced spine density was visualized on dendrites of pyramidal neurons in the medial prefrontal cortex (mPFC) 24 hours after ketamine infusion (**FIGURE 18.13B**). Coinciding with those changes, animals show antidepressant effects in the forced swim and learned helplessness tests. The inhibition of mTOR by rapamycin prevents the ketamine-induced synaptic

changes and the antidepressant effects. The ketamine-enhanced synaptic strengthening shown by increased electrophysiological responses to 5-HT was also reduced by rapamycin (Li et al., 2010; **FIGURE 18.13C**). mTOR and other downstream modulators, including BDNF, that are activated by ketamine provide other opportunities to identify drugs that have the rapid antidepressant action of ketamine without some if its liabilities, such as causing transient psychosis-like symptoms that last up to 2 hours, and dissociative symptoms. An additional incentive to find an alternative to ketamine is that although ketamine does not produce physical dependence, it is an abused substance (considered a "party drug"), which further limits its usefulness in treatment programs.

One of the newest explanations for the failure of other NMDA antagonists to be effective is that the antidepressant effects of ketamine may depend not so much on the molecule itself, but on one of its specific metabolites that rather than blocking NMDA receptors, activates AMPA receptors. Its importance was shown by administration of AMPA receptor antagonists, which prevented the antidepressant effects of ketamine. The metabolite, which was three times higher in the brains of female mice than in males, reversed depression-like behaviors more effectively in females than males. When the researchers prevented the formation of the metabolite, ketamine lost its effectiveness, so apparently the metabolite is necessary for the behavioral effect. Of special significance is that when the metabolite itself was administered, it did not cause the psychosis-like or anesthetic effects seen with ketamine. This approach provides one more avenue for research into a more effective, faster-acting drug to treat clinical depression (Zanos et al., 2016).

Because ketamine reduces NMDA receptor function but potentiates glutamate action at the AMPA receptor, it makes AMPA receptor *agonists* potentially useful targets. AMPA agonist action may explain the increased neural activity in the anterior cingulate cortex that predicts antidepressant response to ketamine as well as other antidepressant drugs. The synaptic resculpturing that is seen may again be related to the rapid activation of mTOR. It is especially interesting that the rapid (overnight) antidepressant effect of sleep deprivation (see **Web Box 18.4**) also enhances AMPA-induced synaptic plasticity.

Other potential explanations for these rapid synaptic changes exist. Ketamine has been shown to bind to μ-opioid receptors, initiate multiple second-messenger cascades, and increase BDNF, as well as altering the expression of several genes central to the operation of the molecular biological clock (Sanacora and Schatzberg, 2015). Its ability to alter circadian rhythms as well as increase BDNF may be due to the drug's ability to inhibit the signaling properties of the protein kinase GSK-3 (see the section on the mechanism of action of lithium below), but more preclinical research is needed and there are many potential pathways to investigate. Zunszain and colleagues (2013) provide an excellent summary of some of the possible mechanisms responsible for ketamine's unusual properties. Although much more research is needed before these approaches are adapted into clinical use, at this point ketamine has utility as a short-term solution in acute crisis situations, such as when emergency room physicians deal with suicidal depressed patients. For further discussion you may want to take the time to hear Professor Ronald Duman's lecture on the topic, available from Yale Psychiatry on YouTube (Duman, 2012).

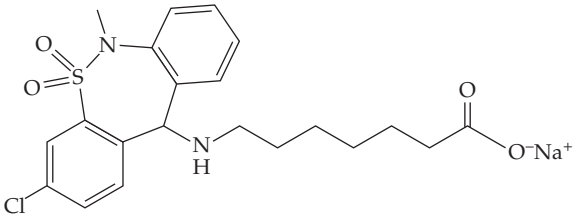


FIGURE 18.14 Chemical structure of tianeptine
Note the three-ring structure. While tianeptine is similar in structure to the tricyclic antidepressants, it has very different pharmacological properties.

Another effective antidepressant that modulates glutamate function is **tianeptine**, a TCA in structure but with different and complex pharmacological actions (**FIGURE 18.14**). Its unique neurobiological properties may explain its effectiveness in reducing symptoms of depression and comorbid anxiety with only mild side effects and little sedation or cognitive impairment. The drug increases phosphorylation of glutamate receptor subtypes in selective brain areas. Given the current interest in ketamine's potentiation of AMPA receptor function, it is interesting to note that the phosphorylation of glutamate receptor GluR1 by tianeptine potentiates AMPA receptor function. Its ability to enhance phosphorylation by intracellular kinases such as calcium/calmodulin-dependent protein kinase may further contribute to the synaptic plasticity characteristic of antidepressant drugs. Additionally, in animal studies, tianeptine prevents stress-induced changes in glutamate transmission (perhaps by adjusting the NMDA–AMPA balance) in hippocampus and amygdala, which contributes to its neuroprotective action. McEwen and colleagues (2010) provide a thorough description of the neurobiological effects of tianeptine.

GALANIN Galanin is a 30–amino acid neuropeptide implicated in mood disorders as well as in regulating feeding, cognitive performance, sleep, sexual activity, and stress responses. It is widely distributed in the brain and is co-localized with 5-HT in the nucleus of the raphe and with NE in the locus coeruleus. It acts as an inhibitory modulator of the two monoamines by hyperpolarizing the cells and reducing neurotransmitter release at their projection areas in the limbic system and cerebral cortex. Contradictory evidence regarding whether intracerebral

injections of galanin produce depressive or antidepressant effects may be explained by the existence of three distinct galanin G protein–coupled receptors that are differentially distributed in the brain and are coupled to distinct intracellular signaling mechanisms. Agonists at GalR1 and GalR3 cause depression-like behaviors in rodents, while agonists of GalR2 have antidepressant effects in the same rodent models (Kuteeva et al., 2008). Hence galanin may have a role in the pathophysiology of depression and represents a potential target for novel antidepressant medications. Of particular interest is the finding that 14 days of treatment with the SSRI fluoxetine up-regulated galanin mRNA expression by 100% and GalR2 (but not GalR1) expression by 50% in the dorsal raphe (Lu et al., 2005). Electroconvulsive shock also increased galanin mRNA in the nucleus of the raphe, but sleep deprivation increased it in the locus coeruleus. These researchers also found that intraventricular injection of a nonselective galanin antagonist could prevent the antidepressant effects of fluoxetine in the forced swim test, indicating that galanin may mediate the drug's clinical effectiveness. Furthermore, galnon, a nonspecific, nonpeptide galanin receptor agonist, produced a dose-dependent reduction in immobilization in the forced swim test. These results suggest that GalR2 agonists might augment standard antidepressant treatment. Additionally, on the basis of preclinical studies, antagonists at GalR1 or GalR3 would be expected to have clinically significant antidepressant effects, and several GalR3 antagonists have recently been developed for testing. One final fascinating aspect is that evidence suggests that galanin has neuroprotective effects. Ligands binding specifically to GalR1 or GalR1/2 significantly reduced the excitotoxic cell death in hippocampal neurons following intracerebroventricular injection of kainic acid (Webling et al., 2016). Furthermore galanin increases hippocampal neurogenesis, providing further support for the neurotrophic hypothesis described earlier.

NONDRUG THERAPY A less conventional but apparently effective antidepressant treatment is physical exercise. Yau and colleagues (2011) have shown in a series of studies that administration of corticosterone (the rodent equivalent of the human stress hormone cortisol) at low (30 mg/kg), moderate (40 mg/kg), or high (50 mg/kg) doses for 14 days produces a dose-dependent increase in depression-like behaviors seen in the forced swim and sucrose preference tests as well as impaired spatial learning in the Morris water maze. Voluntary running reversed these behavioral effects except at the highest corticosterone levels, and the positive behavioral effects were associated with increased hippocampal neurogenesis, dendritic length, and spine density. Preventing the increase in neurogenesis diminished the positive effect of running. A follow-up study examined the failure of exercise to reverse the high-stress-induced (50 mg/kg corticosterone) depressive behaviors and impairments in cognitive function. Their results provide motivation for all of us to exercise regularly because they found that exercise could reverse the damaging effects of high stress if the animals were allowed to exercise both before and during the corticosterone administration. Under these conditions, not only were the behaviors restored to normal but neurogenesis and levels of synaptic proteins were also normalized. Hence, it would appear that exercise makes us resilient to future stress (Yau et al., 2014).

Drugs for treating bipolar disorder stabilize the highs and the lows

For the majority of patients with bipolar disorder, **lithium carbonate** (Carbolith, Eskalith) is the most effective medication and is the usual drug of choice. Although lithium has no effect on mood or behavior in healthy individuals, J. Cade in 1949 discovered that it had powerful effects on patients with mania. After 1 to 2 weeks of lithium use, symptoms are eliminated or reduced in approximately 60% to 80% of manic episodes without causing depression or producing sedation. The drug is somewhat less effective in terminating episodes of depression, so it is often administered along with a TCA or other antidepressant drug. Most important is that it is useful for reducing the occurrence of future episodes of mania and depression. Additionally, it is particularly effective in reducing suicide in bipolar individuals. Patients who continue with lithium treatment have an average hospital stay of less than 2 weeks per year, but without lithium therapy patients spend an average of 8 to 13 weeks per year in the hospital. **FIGURE 18.15A** graphically demonstrates that without lithium maintenance, the typical patient with bipolar disorder has an episode of mania every 14 months and a period of depression every 17 months on average. Lithium maintenance reduces the recurrence of mania to once in 9 years and depressive episodes to about every 4 years (Lickey and Gordon, 1991).

Treatment of bipolar disorder with a mood stabilizer is a lifelong necessity for most patients. Either abrupt termination or gradual withdrawal of lithium results in recurring periods of mania and heightened suicide risk. Despite the risks, many patients stop taking the drug. In some cases, side effects are a significant problem for the patient, especially if they involve impaired memory and confusion. In other cases, patients stop taking the drug because they fail to experience normal mood changes, and this diminishes the richness of life. Finally, others object to the loss of the manic phase of bipolar disorder because this time is perceived as a period of heightened creativity and productiveness.

MECHANISM OF ACTION It is probably not surprising to find that lithium enhances 5-HT actions: it elevates brain tryptophan, 5-HT, and 5-HIAA (the principal

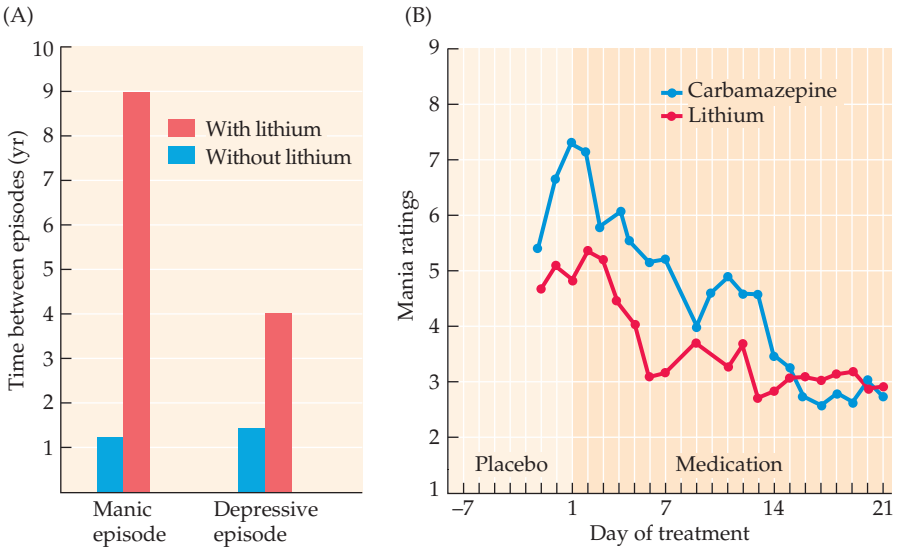


FIGURE 18.15 Effectiveness of lithium for bipolar disorder (A) Maintenance therapy with lithium significantly reduces the occurrence of manic episodes so that on average, the time between manic periods is 9 years, compared with 14 months without treatment. Depressive episodes also occur less often, averaging 4 years between episodes with lithium treatment and 17 months without. (B) The time course and extent of effectiveness of the newer drug carbamazepine are virtually identical to those of lithium in reducing manic symptoms in patients with bipolar disorder. (A after Lickey and Gordon, 1991; B after Post et al., 1984.)

5-HT metabolite) and increases 5-HT release, which ultimately alters receptor response in several brain areas. Lithium reduces catecholamine activity by enhancing reuptake and reducing release. Despite these neurochemical changes, it is unlikely that lithium acts on individual neurotransmitters to normalize mood swings of both mania and depression. Given that the drug flattens the extremes of emotion in both directions, it is more likely that it modifies synaptic transmission at points beyond the neurotransmitter receptors, for instance, in second-messenger function. Lithium has pronounced effects on adenylyl cyclase, phosphoinositide cycling, G protein coupling, brain neurotrophic factors, and multiple other intracellular cascades. One such signaling cascade involves the enzyme glycogen synthase kinase-3 (GSK-3). GSK-3 has numerous cell functions, but of particular importance for bipolar disorder is that the biological clock regulates GSK-3, but it in turn feeds back to modulate circadian rhythms. Since lithium inhibits GSK-3 in mouse brain and in blood cells of patients with bipolar disorder, it is possible that the mood-stabilizing effects of lithium are in part due to the inhibition of GSK-3 regulation of circadian rhythms. Lithium's ability to slow down the typical abnormally fast circadian rhythms in patients with bipolar disorder is associated with a stabilization of mood, while those patients with excessively slow circadian rhythms do not show symptom improvement (McClung, 2007). Further evidence for this hypothesis comes from rodent models of mania that can be attenuated by synthetic inhibitors of GSK-3, opening the inquiry into potentially new treatment strategies (see Logan and McClung, 2016). Additionally, polymorphisms of GSK-3 predict lithium's effects on circadian rhythms and are associated with several symptoms of the disorder. As you might expect, GSK-3 exerts other physiological effects, including the modulation of other intracellular signaling pathways. One effect of lithium-induced inhibition of GSK-3 in turn enhances the plasticity of neurons by altering dendritic spine stability and density, which is associated with reduced depression-like behavior and reduced amphetamine-induced hyperactivity and sensitization in rodents. Given the potential role of GSK-3 in lithium's effectiveness, continuing research is investigating the use of GSK-3–overexpressing mice as a rodent model (reviewed by Logan and McClung, 2016). Its ability to alter intracellular actions regardless of the triggering neurotransmitter may explain its effects in both mania and depression.

SIDE EFFECTS Lithium is not metabolized but is excreted by the kidney in its intact form. Sodium depletion due to extreme sweating, diarrhea, vomiting, dehydration, use of diuretic medication, or severely salt-restricted diets may lead to toxic levels of lithium because lithium is reabsorbed from kidney tubules instead of sodium. The effective therapeutic range of lithium concentration in the blood is 0.7 to 1.2 mM. Since toxic effects begin to occur at blood levels of 2.0 mM, the therapeutic index is very low, and a patient's blood level of lithium must be monitored on a regular basis. Side effects are generally quite mild at therapeutic doses but may include increased thirst and urination, impaired concentration and memory, fatigue, tremor, and weight gain. Toxic effects are more severe and include cramps, vomiting, diarrhea, kidney dysfunction, coarse tremor, confusion, and irritability. Levels of lithium above 3.0 mM may lead to seizures, coma, and death (Calabrese et al., 1995).

OTHER THERAPIES FOR BIPOLAR DISORDER Because only about 50% to 60% of patients show a good response to lithium, and because it has a significant potential for toxicity, alternative therapies have been developed. Of the alternatives, the anticonvulsant drugs carbamazepine

(Tegretol) and valproate (Depakene) are the most common and will be described briefly. However, several newer drugs such as topiramate (Topamax) and tiagabine (Gabitril) are similarly effective when compared with lithium and have a different toxicity profile. Further discussion of these drugs is beyond the scope of this text and is left to others (Calabrese et al., 1995; Guay, 1995).

Valproate (**Depakote**), a simple branched-chain fatty acid, was the first anticonvulsant approved by the U.S. Food and Drug Administration (FDA) for treatment of acute mania. Although valproate is readily absorbed after oral administration, it is low in potency, so high doses are administered. It is highly bound to plasma proteins, and because of the high dosage requirements, the depots may become saturated. Toxicity can occur with continued administration after saturation of the depots or after consumption of other free fatty acids that displace valproate from the depots, raising the level of free drug in the blood. Valproate is also capable of displacing other drugs bound to plasma protein, causing drug interactions. Valproate's metabolism creates a number of active metabolites that contribute to its action. Although its effectiveness is similar to that of lithium, one advantage of valproate is that it has a different side effect profile. Common side effects include drowsiness, lethargy, hand tremor, hair loss, weight gain, and gastrointestinal distress. Some evidence indicates that the drug can cause liver toxicity and pancreatitis, but the probability is low. However, overdose is potentially life threatening. Since valproate is teratogenic and is associated with neural tube defects and with increased risk of polycystic ovary syndrome, its use in women of childbearing age is limited. For a review of its use in psychiatry, see Haddad and coworkers (2009).

Valproate has a complex mechanism of action. Valproate increases GABA levels by stimulating glutamic acid decarboxylase, which enhances synthesis, and inhibiting GABA transaminase, which decreases GABA degradation. Additionally, it has multiple actions on DA and glutamate neurotransmission. However, it also may have a common mechanism of action with other mood stabilizers on intracellular signaling, including the inhibition of the circadian modulatory enzyme GSK-3 (see the section on lithium above) and regulation of several cell survival pathways involving neurotrophic factors.

Carbamazepine (**Tegretol**) is a structurally atypical anticonvulsant because it resembles tricyclic antidepressants, and this similarity allows it to inhibit NE reuptake. It also acutely blocks adenosine receptors and up-regulates them with chronic use. Its actions on intracellular signaling are similar to those of valproate and lithium. The time course and extent of effectiveness are similar to those of lithium (**FIGURE 18.15B**), but its side effects differ. The most common side effects, which usually diminish over time, include sedation, dizziness, somnolence, incoordination, nausea, and vomiting. More severe potential side effects include liver toxicity, severe skin rashes, and various blood conditions and diseases such as reduced white cell count, agranulocytosis, and aplastic anemia. The induction of several liver metabolizing enzymes in the cytochrome P450 family, including CYP3A4, CYP1A2, and CYP2C19, is also therapeutically significant because by increasing the amount of liver enzyme, carbamazepine accelerates its own rate of metabolism and that of many other drugs (enzyme induction is discussed in Chapter 1). The more effective metabolism leads to reduced blood levels and the need to monitor and increase drug dosages to optimize treatment response and prevent drug interactions.

Section Summary

- Antidepressants of all classes reduce symptoms in about two-thirds of individuals after 4 to 6 weeks of treatment. Total remission of symptoms occurs less often. Continued treatment prevents relapse.
- MAOIs elevate brain levels of monoamines by preventing their destruction in the presynaptic terminal by MAO and subsequently altering receptor number and intracellular signaling.
- The most common side effects of MAOIs include changes in blood pressure, sleep disturbances, and weight gain. More serious side effects are associated with enhanced response to sympathomimetics, hypertensive crisis following elevation of tyramine levels, and drug interactions due to liver enzyme inhibition.
- Tricyclic antidepressants block reuptake of NE or 5-HT or both, and this increases synaptic levels and produces subsequent compensatory changes in receptors and intracellular signaling.
- Side effects of TCAs include sedation, anticholinergic effects, and potentially dangerous cardiovascular effects.
- Second-generation antidepressants, including the SSRIs, are not more effective or more rapid in onset but are safer.
- Side effects of SSRIs are due to enhanced 5-HT function at multiple 5-HT receptors and include sexual dysfunction. Potentially fatal serotonin syndrome occurs when SSRIs are combined with other serotonergic drugs. Physical dependence occurs in 60% of cases.
- Mirtazapine enhances NE and 5-HT function by blocking α_2-autoreceptors and heteroreceptors on 5-HT cells. It also blocks selective 5-HT receptors to reduce side effects.
- Third-generation agents comprise CRF receptor antagonists, enhancers of the cAMP intracellular cascade, agomelatine, ketamine, galanin agonists,

tianeptine, and AMPA agonists. Multiple potential mechanisms may explain ketamine's rapid onset of antidepressant effects.

- The ability of physical exercise to relieve depression may depend on its ability to increase neurogenesis and dendritic resculpturing.
- Lithium carbonate reduces manic episodes without causing depression and reduces bipolar cycling. It is more effective than alternatives in reducing suicide rates.
- Side effects of lithium are relatively mild, but toxic effects at the highest doses lead to seizures, coma, and death. The therapeutic index is very small, so frequent monitoring of blood levels is needed.
- Lithium and other antimanic drugs modulate several intracellular signaling pathways, including the GSK-3 pathway, and neurotrophic factors.
- The anticonvulsant valproate is as effective as lithium and has different side effects but is teratogenic, so its use in women of childbearing age is limited.
- Carbamazepine has a time course and effectiveness similar to those of lithium with different side effects, some of which are liver toxicity and blood diseases. Induction of several cytochrome enzymes causes significant drug interactions.

STUDY QUESTIONS

1. Summarize the central characteristics of major depressive disorder and bipolar disorder.
2. Provide evidence for a genetic contribution to affective disorders.
3. Describe the HPA axis, including its negative feedback mechanism. What are the three most consistent neuroendocrine abnormalities found in clinically depressed patients?
4. Discuss the significance of an altered sleep–wake cycle in depression and bipolar disorder.
5. What are the three types of validity that ideally should be met by animal models of psychiatric disorders? Describe several models for bipolar disorder.
6. What is the monoamine hypothesis, and why has it been modified?
7. Provide evidence for the importance of 5-HT dysfunction in major depression.
8. What role does NE play in antidepressant action? What is the role of NE/5-HT interaction?
9. How do the structural and functional abnormalities in the brains of depressed individuals prompt the formulation of the glucocorticoid hypothesis? The neurotrophic hypothesis?
10. Describe the mechanism of action of MAOIs. How does their mechanism of action explain the occurrence of their major side effects?
11. What is the mechanism of action of the tricyclic antidepressants? Why have their side effects prompted the development of the SSRIs?
12. Describe the mechanism of action of the SSRIs. What are their significant side effects, and why are they different from the TCAs? Although considered safer than the older drugs, what potential serious outcomes are associated with the SSRIs?
13. Why is the mechanism of action of ketamine generating so much research attention? Describe several of the potential mechanisms of action.
14. How can galanin cause depression and also have antidepressant effects? What is its relationship to other antidepressant agents?
15. Provide evidence to suggest that exercise has antidepressant effects.
16. Describe the effectiveness of lithium for bipolar disorder, as well as its side effects. Describe one signaling cascade that may be responsible for the drug's ability to modify both the highs and the lows of mood.
17. Compare lithium, valproate, and carbamazepine in terms of efficacy and side effects. If you needed to be treated with one of these, which would you choose? Why?

Go to the *Psychopharmacology* Companion Website at oup-arc.com/access/meyer-3e for animations, web boxes, flashcards, and other study aids.

CHAPTER 19

The symptoms of schizophrenia frequently lead to personal isolation and failure to achieve a meaningful and productive lifestyle. (© iStock/PointImages.)

Schizophrenia: Antipsychotic Drugs

ARTHUR WAS 22 YEARS OLD WHEN HE WAS BROUGHT TO THE CLINIC because his parents were upset by his unusual behavior. He was an average student taking classes at a local junior college. He had taken a series of temporary jobs until he was laid off from the most recent one. It was then that he started talking about his blueprints to save all the starving children in the world. He said he had a secret plan that he would reveal only at the right time to the right person. His family became more distressed when he said he was going to the German embassy because they were the only ones who would listen to him. He said he would climb the fence at night and present his plan to the German ambassador. After several visits to the clinic the psychiatrist finally saw the plan, which consisted of random thoughts (e.g., "The poor, starving souls" and "The moon is the only place") and drawings of rocket ships that would go to the moon, where Arthur would create a community for the poor children. As time went on, Arthur began to show dramatic changes in emotion, often crying and acting apprehensive. He stopped wearing socks and underwear and, despite the extremely cold weather, would not wear a jacket outdoors. He had moved into his mother's apartment, but he wouldn't sleep much at night and kept the family up until early morning. For his mother, it was a living nightmare because she felt so unable to help her son (Barlow and Durand, 1995).

This chapter describes the characteristics of the devastating mental disorder known as schizophrenia and the drug therapies that are currently available to treat it. It also describes several models that attempt to explain the neuropathology that leads to its hallmark abnormal behavior and multiple symptoms. ■

Characteristics of Schizophrenia

Major mental disorders called psychoses are characterized by severe distortions of reality and disturbances in perception, intellectual functioning, affect (emotional expression), motivation, social relationships, and motor behavior. Schizophrenia is one relatively common form of psychosis. Other disorders that have psychosis as a component are schizophreniform disorder, schizoaffective disorder, delusional disorder, and bipolar disorder, which is classified as an affective disorder and was discussed in Chapter 18. Individuals with schizophrenia demonstrate many different symptoms, including hearing voices that are not there, holding unrealistic ideas and beliefs, and communicating in a way that is difficult to understand. They may be so incapacitated that voluntary or involuntary hospitalization is required at various times in their lives.

Although drug use or environmental toxins may cause brief episodes of psychosis, schizophrenia is generally a chronic condition. Although its symptoms can usually be controlled to some extent, schizophrenia cannot at this time be cured or prevented. Despite therapy, approximately 30% of people with schizophrenia spend a significant portion of their lives in psychiatric hospitals, accounting for a majority of the total hospital beds in these facilities. Approximately 1% to 1.5% of the world's population will suffer from schizophrenia during their lifetimes. Another 2% to 3% will suffer from less severe schizophrenic-like symptoms but will not meet the diagnostic criteria.

Symptoms of schizophrenia most often begin during the late teenage years and early 20s, although the disorder may first occur in childhood. The early onset of the disorder means that the episodes recurring throughout life disrupt the individual's most productive years. Although epidemiological studies have indicated that schizophrenia affects men and women equally, a clear gender difference in the age of onset and the course of this disorder exists. **FIGURE 19.1** shows that among 470 patients in one study the onset of schizophrenia was highest in early adulthood for both sexes. However, for men the chances of experiencing a first episode of schizophrenia decreased rapidly with age. The onset for women is lower than for men until age 36. At that time, more women than men demonstrate a first episode, and this difference continues into old age (Howard et al., 1993). Earlier age of onset is associated with more severe symptoms, particularly more frequent and intense negative symptoms and more severe loss of cognitive function, both of which are not well managed by the current antipsychotic drugs (see the section on diagnosis below). In addition, episodes of symptom relapse are more frequent in individuals with earlier age of onset, so males tend to experience an overall poorer prognosis. The implication of the gender difference for onset is not clear, but it may suggest the existence of two qualitatively distinct subtypes of the disorder. Recent studies suggest a gene × sex interaction, which supports the preclinical rodent findings of an impact of sex steroids on dopamine function, a neurotransmitter having a central role in the pathophysiology of psychosis. Female estrogen levels may explain their higher rate of symptom onset (compared with males) with increasing age (see Figure 19.1) as estrogen hormones gradually decline. Further, the age of onset in females is inversely related to puberty onset, indicating a possible neuroprotective effect of the female hormone on neurodevelopment leading up to the onset of symptoms (see Godar and Bartoloto, 2014). Schizophrenia can destroy lives and also cause a great deal of pain and suffering, not only for afflicted individuals but also for their families as they attempt to cope emotionally and financially with the disorder. On this basis, the direct (e.g., hospitalization and medication) and indirect (e.g., loss of productive employment, participation in society, and family stress) costs of schizophrenia have been estimated to be between $134 billion and $174 billion a year in the United States for 2013 (Cloutier et al., 2016).

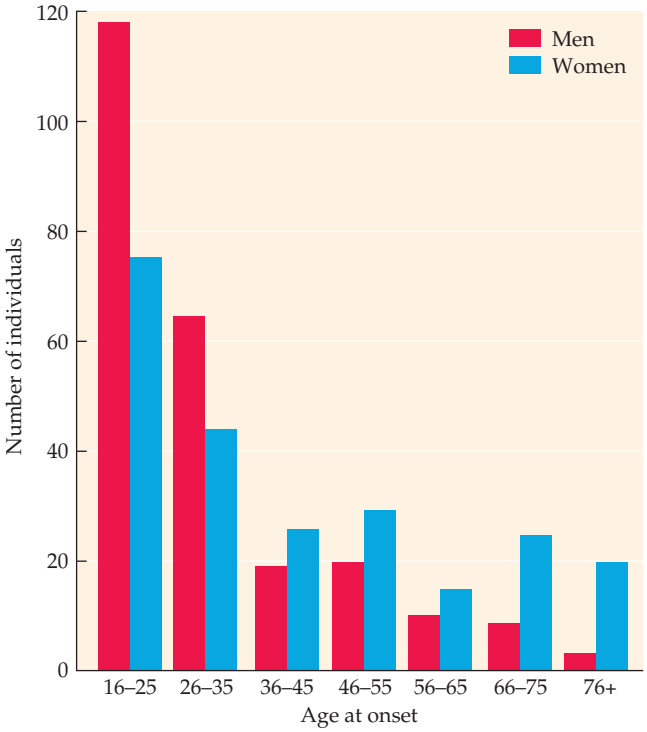


FIGURE 19.1 Gender differences in age at onset of schizophrenia Although both sexes show peak onset of symptoms between 16 and 25 years of age, this sample of 470 patients shows that more women than men experience their first episode after age 36, a difference that continues in every age bracket through old age. (After Howard et al., 1993.)

There is no defining cluster of schizophrenic symptoms

Schizophrenia is very clearly a thought disorder, characterized by illogical thinking, lack of reasoning, and

inability to recognize reality; however, the specific symptoms show a great deal of individual variation. Disturbances in perception (hallucinations) are a frequent occurrence in schizophrenia. These hallucinations are most often auditory and generally consist of voices that are insulting or commanding. For a closer look at auditory hallucinations, see **Web Box 19.1**. Tactile hallucinations are often electrical, tingling, or burning sensations. Bizarre delusions (beliefs not based on reality) are also common. Particularly prevalent are delusions of persecution involving the individual's belief that others are spying on or planning to harm him. Also quite common is the delusion that one's thoughts are broadcast from one's head to the world, or that thoughts and feelings are not one's own but are imposed by an outside source, such as from outer space. Because the form of thought is disturbed, communication is confused and illogical and often does not even follow the rules of semantics. Speech may be vague or repetitive or may shift from one subject to another, totally unrelated subject.

In many individuals with schizophrenia, emotions are either absent or totally inappropriate to the situation. Individuals who lack emotion show no expression, speak in a monotone, and report a lack of feeling. Inappropriate emotion is demonstrated, for example, by the individual who smiles or laughs while describing electrical tortures. Sudden and unpredictable changes of emotion are also common.

People with schizophrenia are frequently withdrawn, preoccupied with their own thoughts and delusions. Extreme apathy and an inability to initiate activities (avolition) frequently mean that the individual has no interest in performing everyday activities, including maintaining personal hygiene, which further isolates the individual from the mainstream. A variety of cognitive deficits impair the individual's ability to function at home, school, and the workplace. Motor activity is generally reduced and is characterized by inappropriate and bizarre postures, rigidity that resists efforts to be moved, or purposeless and stereotyped movements, for example, rocking or pacing. At times, people with schizophrenia, particularly the paranoid type, can be agitated and violent (Krakowski and Czobor, 1997).

DIAGNOSIS Although the symptoms described here seem to be easily recognizable, the diagnosis of schizophrenia is not so simple. One reason is that no two individuals show the identical pattern of symptoms, nor is there a single symptom that occurs in every patient with schizophrenia. Furthermore, symptoms increase and decrease over time, and the predominant symptoms or symptom clusters often change over the years in the same individual, which may lead to a change in diagnosis. The question of whether schizophrenia is a single disorder or a collection of disorders has never been fully resolved.

One useful classification scheme, stemming from the work of Crow (1980) and modified more recently by Andreasen (1990), is that of **positive**, **negative**, and **cognitive symptoms**. The positive symptoms of schizophrenia include the more dramatic symptoms of the disorder, such as delusions and hallucinations, disorganized speech, unusual ways of thinking, and bizarre behavior. Patients who demonstrate predominantly positive symptoms tend to be older when they experienced a sudden onset of symptoms and appeared relatively unremarkable in their younger years before the symptoms occurred. These patients respond well to conventional antipsychotic medications that block dopamine receptors (D_2), and their symptoms are made worse by drugs that enhance dopamine function. Current thinking suggests that neurochemical abnormalities are significant in this disorder (see the section on abnormal dopamine function below).

Negative symptoms are characterized by a decline in normal function and include reduced speech (alogia), deficits in emotional responsiveness (flattened affect), loss of initiative and motivation (avolition), social withdrawal, and loss of ability to derive pleasure from normally pleasurable activities (anhedonia). These symptoms are harder to recognize and may be mistaken for other conditions such as major depression. The cognitive symptoms characteristic of schizophrenia include impaired working memory, executive functioning, and attention. Cognitive deficits are responsible for poorer functioning in the community and greater isolation. Unfortunately, the negative and cognitive symptoms are among the most resistant to antipsychotic drugs and make it difficult for the individual to perform tasks of daily living or to lead a routine life, even when medication reduces the positive symptoms. Unlike patients with prominent positive symptoms, patients with dominant negative and cognitive symptoms tend to show early onset of some symptoms and a long course of progressive deterioration, perhaps reflecting long-term neurodegeneration or developmental errors. Although the film *A Beautiful Mind* does not reflect the medical realities of mental illness, it does provide an excellent insight into the experiences of a brilliant individual (John Nash as portrayed by Russell Crowe) as he copes with the onset of schizophrenia. A video interview with a patient with schizophrenia can be found on the Companion Website.

Section Summary

- Schizophrenia is a chronic psychosis that occurs in 1% to 1.5% of the population worldwide. Symptoms begin during late adolescence and early adulthood. Men have an earlier onset than women and a poorer prognosis.

- Schizophrenia is a thought disorder, characterized by illogical thinking, lack of reasoning, and failure to recognize reality. Specific symptoms show large variation among individuals, making diagnosis difficult.
- Positive symptoms are dramatic and lead to diagnosis. They include hallucinations, delusions, disorganized speech, unusual ways of thinking, and bizarre behavior. These symptoms respond to antipsychotic drug treatment.
- Negative symptoms are the absence of normal functions and include reduced speech, flat affect, loss of motivation, social withdrawal, apathy, and anhedonia.
- Cognitive deficits include impaired working memory, executive function, and attention. Negative symptoms and cognitive deficits are resistant to current treatments.

FIGURE 19.2 Brain images of twins not concordant for schizophrenia The arrows in the figure point to the ventricles filled with cerebrospinal fluid. The healthy twin has normal-sized ventricles, and his schizophrenic twin has ventricles that are much enlarged. (Courtesy of Drs. E. Fuller Torrey and Daniel Weinberger.)

Etiology of Schizophrenia

Scientists from several disciplines use a variety of strategies to uncover the causes of schizophrenia. The goal is to develop an integrated approach to psychopathology that considers anatomical, neurochemical, and functional factors. Schizophrenia is best understood as a disorder having a genetic component that makes the individual more vulnerable than the average person to particular environmental factors.

Abnormalities of brain structure and function occur in individuals with schizophrenia

Until recently, differences in the brains of individuals with schizophrenia compared with controls could not be detected. However, with the development of new techniques in neuroscience, differences of several kinds have been found, including structural differences, functional abnormalities, and abnormalities in immune function.

STRUCTURAL ABNORMALITIES Brain imaging techniques such as computerized tomography (CT) and magnetic resonance imaging (MRI) continue to produce evidence of structural abnormalities in the brains of people with schizophrenia. Many studies show cerebral atrophy (shrinking or wasting away) and enlargement of fluid-filled ventricles following cell shrinkage (**FIGURE 19.2**). Among the brain areas showing reduced volume are the basal ganglia, the temporal and prefrontal cortices, and several limbic regions such as the hippocampus, as well as white matter tracts connecting these regions. The temporal lobe and hippocampal differences in people with chronic schizophrenia compared with controls are some of the most consistent MRI findings (DeLisi et al., 1991). Structural differences in these areas also occur in monozygotic twins when only one has the disorder. Using postmortem brains, researchers found that the reduced volume of various brain regions was not due to a diminished number of cells, but rather to neurons having smaller somas, reduced dendritic trees and dendritic spine density, and increased cell packing. Numerous studies show that hippocampal cells of patients with schizophrenia are more disorganized (**FIGURE 19.3A**) than those of healthy people (**FIGURE 19.3B**) and that selected cortical layers in the brains of patients with schizophrenia are atrophied. Additionally, many brain areas showing shrinking of dendritic trees were associated with failures of connectivity among neurons and abnormal neuronal processing. With the use of new technologies such as diffusion tensor imaging (DTI) and resting-state fMRI (rs-fMRI; see Chapter 4 for a description of these techniques), the strength and integrity of myelinated white matter tracts can be more readily assessed. Studies using these techniques show altered patterns and inefficiency of networks of neurons that would impair the integration of signals among brain areas needed for sensory and cognitive processing as well as the organization of behavioral responses. In particular, a diminished amount of structural connectivity is especially apparent in white matter projections that link the frontal, temporal, and parietal regions. Both the extent of myelination and organization of the tracts are impaired in patients with schizophrenia, suggesting these fiber bundles play an important role in the pathophysiology of schizophrenia. Abnormalities in neural connections have been associated with lower IQ and performance on a variety of tests of cognitive function in healthy adults. In schizophrenia the severity of multiple connectivity deficits is correlated with the severity of positive and negative symptoms as well as cognitive function (details of connectivity assessment and function are provided by van

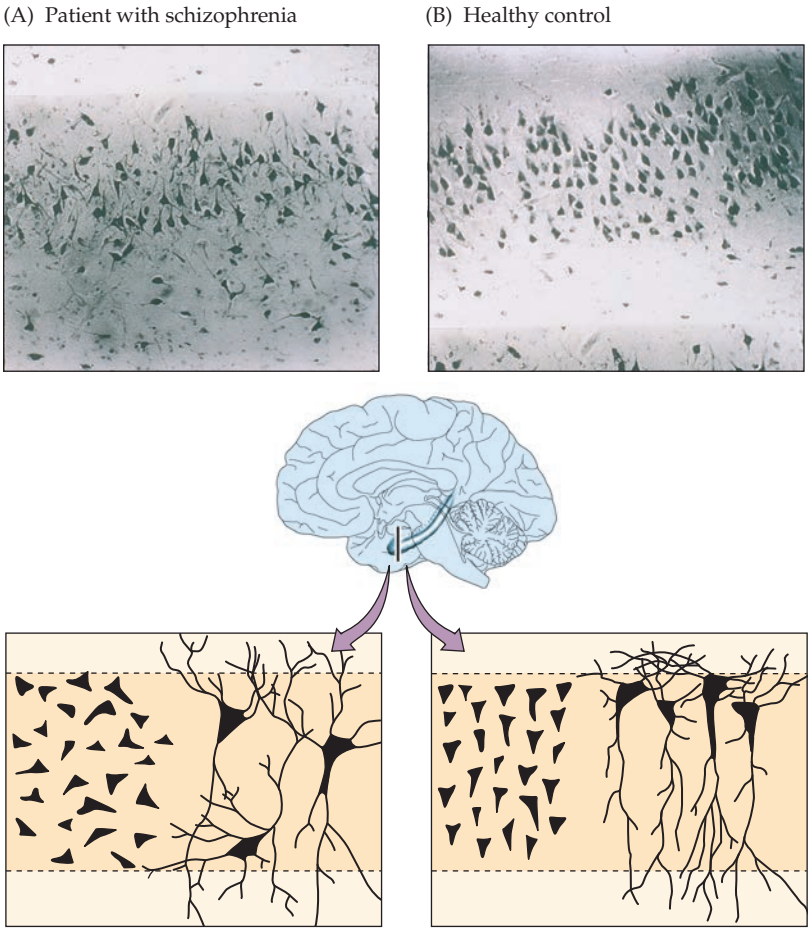


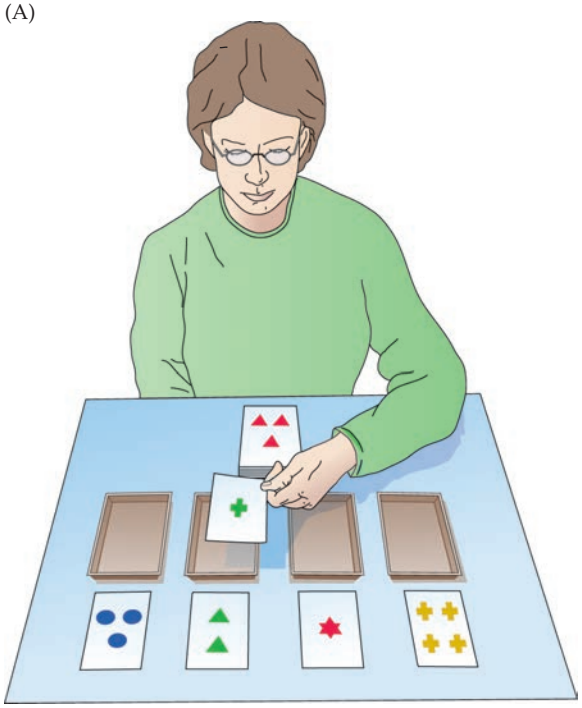
FIGURE 19.3 Disorganization of cells in the hippocampus
Histological cross sections of hippocampus showing the disorganized cells in the brain of a patient with schizophrenia (A) compared with the brain of a healthy control (B). Corresponding schematic diagrams show the haphazard arrangement of pyramidal cells in the hippocampus of patients with schizophrenia and the normal parallel organization in controls. (From Kovelman and Scheibel, 1984; histograms courtesy of Arnold Scheibel.)

den Heuvel and Fornito, 2014). Indeed, specific symptom clusters (e.g., positive symptoms in first-episode patients) have been linked to distinct abnormalities in functional connections between brain regions, such as between caudate and dorsolateral prefrontal cortex (DLPFC). Occurrence of some of the more subtle brain differences in healthy family members, in early-onset patients, and even neonatal offspring of patients argues for a genetic predisposition to developmentally abnormal connectivity, which increases vulnerability to psychosis (see Samartzis et al., 2014, and van den Heuvel and Fornito, 2014). Ideally these findings may ultimately provide a diagnostic tool to identify those individuals who are at most risk for developing schizophrenia so that prevention or treatment intervention may be utilized.

Investigators are always concerned that some brain changes may be due to progressive deterioration during the course of illness, rather than being the cause of the illness, or may be due to the effects of antipsychotic medication used chronically over many years. However, most brain changes, such as enlarged ventricles, are not correlated with either the duration of time since the onset of symptoms or the duration of time since the first hospitalization. In contrast, a significant correlation does exist between ventricle size and age of the individual when symptoms were first observed. On the basis of these results, researchers have concluded that ventricular enlargement is not due to progressive loss of brain cells but may represent abnormalities of brain growth and development preceding the onset of symptoms. Additional evidence for this idea is the discovery that the more subtle abnormalities in cell structure are rarely accompanied by gliosis (multiplication of astrocytes and microglia). Since gliosis is a response to neuronal damage that occurs in the mature brain, but not in the immature brain, it is likely that the cell abnormalities occurred during the developmental process (Weinberger, 1995). Prenatal exposure to inflammation represents one potential explanation for the abnormalities in brain structure (see the section Immune System Dysfunctions below).

FUNCTIONAL ABNORMALITIES In addition to structural abnormalities in the brains of individuals with schizophrenia, regional brain function in these individuals differs from that in controls. Measures of brain function include rate of cell metabolism, blood flow, electrical activity, and chemical changes. The most consistent difference is reduced function of the prefrontal cortex (PFC), called hypofrontality (Buchsbaum, 1990). Positron emission tomography (PET) and single-photon emission computerized tomography (SPECT) studies show less of an increase in cerebral blood flow in the frontal cortex of patients with schizophrenia than in healthy individuals while they perform cognitive tasks that depend on PFC function. These tasks include executive functioning, working memory, response inhibition, and problem solving that require planning, strategy, and attentional set shifting, such as the Wisconsin Card Sorting Test (WCST; **FIGURE 19.4A**). Reduced blood flow is associated with less glucose use, which in turn is a good indicator of how active the brain cells are. **FIGURE 19.4B** shows that the frontal cortex is less active in a patient with schizophrenia compared with

(A)



(B)

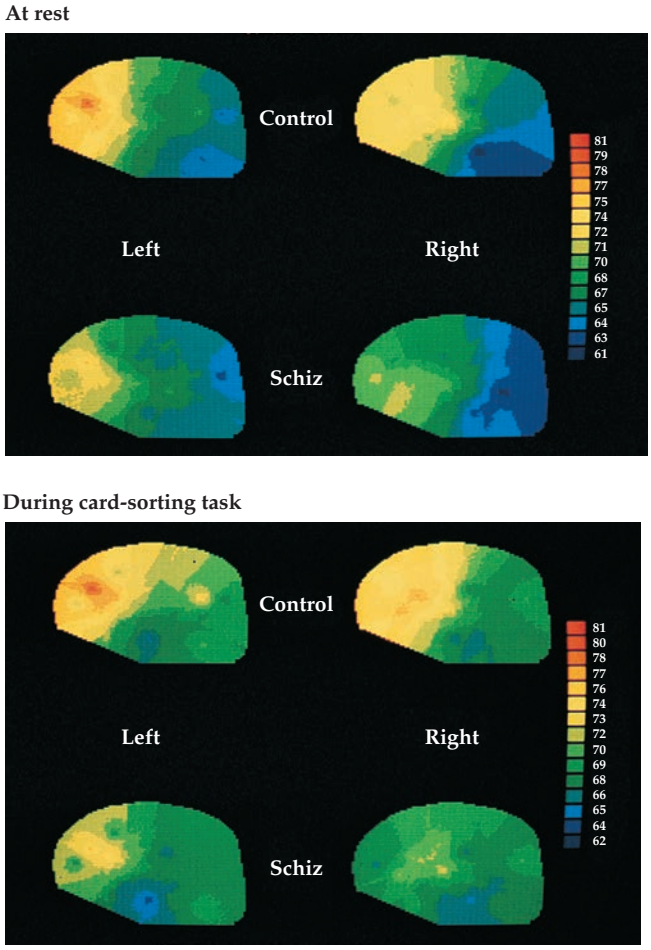


FIGURE 19.4 Hypofrontality in schizophrenia
(A) The Wisconsin Card Sorting Test is used to evaluate the ability of an individual to shift response strategies on the basis of feedback from the tester. The person is presented with stimulus cards having simple designs that differ in color, shape, and number of elements. The individual is asked to sort the remaining cards into piles. With each attempt, the person is told whether the choice is correct or incorrect. Over the test period, the sorting principle may first be color and then may shift to form or number. Patients with schizophrenia and those with frontal lobe lesions fail to shift strategies and may continue to sort on the basis of the original stimulus (e.g., color) despite being told that color sorting is no longer correct. (B) PET scans comparing frontal lobe activity of a patient with schizophrenia and a nonschizophrenic twin. The sibling with schizophrenia has less frontal lobe activity at rest (top) as well as during a frontal lobe challenge with the Wisconsin Card Sorting Test (bottom). (B courtesy of Karen Berman.)

a nonschizophrenic twin both at rest and during the WCST. Nevertheless, further research indicates that the picture is not quite so simple. When performing several different executive tasks, healthy individuals and those with schizophrenia activated the same brain regions, including the DLPFC, ventrolateral prefrontal cortex (VLPFC), anterior cingulate cortex (ACC), and thalamus. However, patients with schizophrenia showed much less activation in the left DLPFC, ACC, and left thalamus and in areas in the inferior and posterior cortex during selective tasks, which is consistent with their impairment in cognitive control. Somewhat surprising is the finding that individuals with schizophrenia showed *greater* activation than controls in several brain structures such as the VLPFC, amygdala, and insula. It is not clear whether the enhanced neural activity reflects a compensatory use of other brain areas to handle the cognitive tasks, or whether shifting of the network of activity represents a disease-specific pattern (Minzenberg et al., 2009). Hypofrontality in schizophrenia is especially interesting because the negative and cognitive symptoms of schizophrenia resemble the deficits seen following surgical disconnection of the frontal lobes (prefrontal lobotomy). Included in these deficits are poor social functioning, loss of motivation, defective attention, emotional blunting, and inability to shift strategies during problem solving (Gur, 1995).

IMMUNE SYSTEM DYSFUNCTIONS Almost 100 years ago inflammation was considered a potential etiological factor in the development of psychosis. It was observed that individuals with schizophrenia frequently have dysfunctional immune systems and that this dysfunction could be at least in part responsible for the loss of brain tissue and failure of connectivity among neurons. A confluence of evidence supports the association between immune dysfunction and schizophrenia.

According to multiple meta-analyses, blood levels of pro-inflammatory cytokines (a variety of small proteins released by immune cells) are elevated and anti-inflammatory cytokines are reduced in first-episode schizophrenic patients who have never taken antipsychotic drugs. Those cytokine levels return to normal after successful antipsychotic drug treatment. Further, blood levels of the pro-inflammatory cytokine IL-6 show a positive correlation with the severity of symptoms as well as duration of illness (reviewed by Khandaker et al., 2016). Whether the cytokines are causing the symptoms or are released in response to the disease is not clear, although animal studies suggest a causative role.

Epidemiological research has shown that maternal levels of cytokines, elevated due to exposure to any one of a variety of infectious diseases such as influenza, rubella, pneumonia, sinusitis, and others, predicts increased risk of schizophrenia in the offspring (see the section on developmental errors below). Additionally, individuals with immune disorders, such as type 1 diabetes, rheumatoid arthritis, and Crohn's disease, have an increased genetic risk of developing schizophrenia compared with the general population. It is of interest that individuals with schizophrenia experienced more infections as children, and the more infections these individuals experienced, the greater the likelihood of developing schizophrenia. Adults with schizophrenia also suffer from more infections, and it has been suggested that their sensitivity to environmental agents (e.g., viruses, food antigens) is due to early (perhaps prenatal) abnormal cytokine production. This early cytokine production sensitizes neural substrates that alter the set point of the immune system and subsequently more readily trigger neuroinflammation and progressive brain pathology (see Meyer, 2013). It has been suggested that their immune responses to these environmental agents may be related to the onset of symptom relapse. It is of further interest that genome-wide association studies (GWAS) show a strong relationship between a region on chromosome 6 that is responsible for proteins involved in multiple immune functions and schizophrenia (see Sperner-Unterweger and Fuchs, 2015 for further discussion of genetic links).

Maternal peripheral cytokines elevated by infection can bypass the placenta and fetal blood–brain barrier in several ways to influence brain function. They also activate microglia (glial cells with immune function in the brain; see Chapter 2), producing an inflammatory assault and potential structural brain changes such as modification of neuronal synapses. Microglia, with small somas and branching processes, normally detect infectious microorganisms, such as viruses and bacteria, and waste materials before becoming activated. Activation involves swelling up into an amoeboid shape and moving toward the pathogen to initiate processes for phagocytosis of the debris and production of cytokines, which initiates pro-inflammatory cascades. The extent of inflammation is normally under homeostatic control that ensures that the pathogens are destroyed and wound healing progresses, but the inflammation is then counteracted to protect uninfected, healthy tissue. Given that there must be a delicate balance between neuroprotection and neurotoxic functions for the brain to recover after injury, abnormal microglial action could have damaging outcomes regarding brain function in the developing individual.

Although accumulated evidence points to the significance of immune functioning in the etiology of schizophrenia, there has been limited research into the precise mechanism by which prenatal inflammation causes abnormal neurodevelopment that increases the risk for schizophrenia. Unfortunately, studies measuring microglia number or density in the brains of individuals with schizophrenia postmortem have not produced consistent results. Although in some studies microglia were increased, others showed no difference from control brains. Certainly there may be several technical reasons for the disparity; however, there is evidence that patients showing *active* psychosis represent a subgroup with enhanced microglia number, so variation in the study population selection may explain the differences among studies. When *activated* microglia and increased cytokine gene expression have been assessed in postmortem brains, much more consistent increases have been reported in selected brain regions such as frontal cortex, superior temporal gyrus, and subcortical regions, brain areas with gray and white matter abnormalities in schizophrenia. Once again, activation of microglia seems greatest during acute illness relapses (see Laskaris et al., 2016). However, Bloomfield and coworkers (2015) found elevated microglia activity *precedes* the onset of symptoms by using ultra-high-risk individuals, as assessed with the Comprehensive Assessment of At-Risk Mental States. These individuals did not have active psychosis and were medication-free. Using PET binding of the translocator protein TSPO, which is expressed on microglia, Bloomfield et al. found greater microglial activity in temporal lobe, frontal lobe, and total gray matter that was correlated to their risk assessment score.

Genetic, environmental, and developmental factors interact

Although schizophrenia is an ancient disorder described as early as 1000 BCE, its causes remain unknown. Schizophrenia is increasingly regarded as a neurodevelopmental disorder with a strong genetic component; however, psychological, biological, and

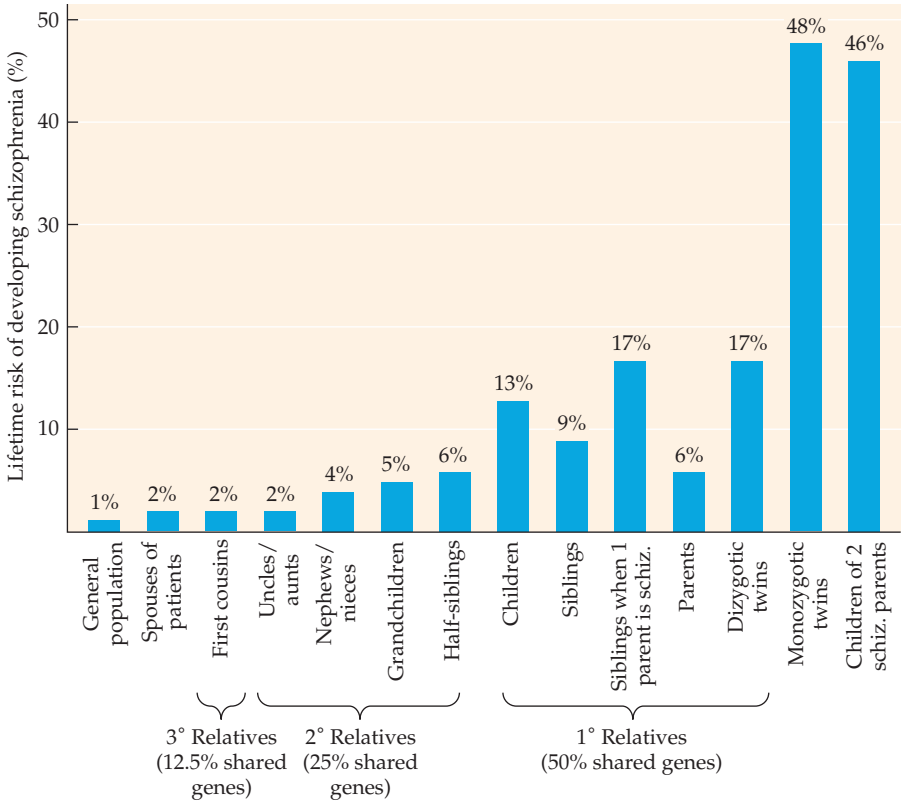


FIGURE 19.5 Lifetime risks of developing schizophrenia among relatives of an affected individual Data are summarized from about 40 family and twin studies conducted between 1920 and 1987. Compared with a 1% risk of developing schizophrenia in the general population, second-degree relatives have an average risk of 4%, first-degree relatives have a 6% to 17% risk, monozygotic twins have a 48% risk, and children having two schizophrenic parents have an average 47% risk. (After Gottesman, 1991.)

sociological factors combine in a unique manner to contribute to its psychopathology, course, and outcome.

HEREDITY The importance of heredity has been demonstrated by numerous family, twin, and adoption studies conducted by investigators who have taken advantage of the excellent record-keeping system of Denmark to show that relatives of individuals with schizophrenia are afflicted with the disorder much more frequently than members of the general population. In fact, the closer the genetic relationship, the greater is the probability of schizophrenia in the relative. In a classic study Gottesman (1991) summarized a large number of family and twin studies of individuals with schizophrenia that had been completed between 1920 and 1987 (**FIGURE 19.5**). These data demonstrate that the risk of having schizophrenia varies according to how many genes one shares with someone who has the disorder. Compared with the lifetime risk in the general population of about 1%, first-degree relatives such as parents, children, and siblings have an average lifetime risk 12 times greater (ranging from 6% to 17%), but more distant (second-degree) relatives, including uncles and aunts, nephews and nieces, grandchildren, and half-siblings, have an average risk of 4% (ranging from 2% to 6%). Dizygotic twins, who have the genetic similarity of siblings but who share the prenatal environment, show a concordance of 17%, which means that if one twin of the pair develops schizophrenia, the probability of the second twin developing the disorder is 17%. In comparison, monozygotic (identical) twins, who have identical genes, have a concordance of 48% or higher in many subsequent reports. This concordance exists even when the twins are reared apart in different environments, which further demonstrates the heritability of schizophrenia. However, although the concordance is striking, it is important to point out that other factors must be involved in the occurrence of the disorder, because if genetic abnormalities were totally responsible, concordance for identical twins would be 100%. Hence individuals having a gene that predisposes them to schizophrenia do not necessarily develop the disorder. Equally striking is the high risk for children born to two parents with schizophrenia.

Current molecular genetic research is trying to identify the specific genes that predict vulnerability to schizophrenia, which would allow early intervention to prevent onset of the disorder and to identify molecular pathways involved in its etiology (Muglia, 2011). The task is difficult because multiple genes located at different **loci** (sites on our chromosomes) are involved. Multiple gene abnormalities would explain why the risk of having schizophrenia increases with the number of affected relatives in the family. It also might explain why the symptom clusters vary in nature and intensity from individual to individual. Despite the difficulties, loci on a dozen chromosomes have been identified as likely sites for genes that increase the risk for developing schizophrenia, with the most promising being on chromosomes 13, 8, 22, and 6. Some have been identified by linkage studies, which look for similarities at the loci in families with affected members. A second approach considers **candidate genes**, genes that on prior physiological or theoretical grounds are

suspected to be involved in disease development, progression, or clinical manifestation. In the case of schizophrenia, identification of candidate genes falls into three possible areas. First, genetic correlates of neurophysiological characteristics typical of the schizophrenic individual are evaluated. These characteristics include the defective filtering of auditory stimuli, eye-tracking dysfunction, ventricular enlargement, and so forth. Second, neurochemical models or studies of pharmacological response may provide an additional focus in the search for candidate genes. A productive line of research evaluates differences in alleles associated with neurotransmitters and receptors, including dopamine, glutamate, and γ-aminobutyric acid (GABA), and with second-messenger systems, including G proteins, adenylyl cyclase, and protein kinases. Third, because schizophrenia is considered a neurodevelopmental disorder, gene mutations that affect proteins needed for key events during brain development, such as growth factors, are significant. Early gene-induced errors could produce the major permanent modifications of brain structure seen in schizophrenia.

Developments in technology such as **DNA microarray** and GWAS provide the means for rapid screening of large quantities of genomic data. These methods, described in Chapter 4, can identify complex gene expression patterns. For example, Maycox et al. (2009) reported multiple defects in the gene groups related to presynaptic function in the PFC of individuals with schizophrenia compared with healthy controls. In particular, they found the greatest and most consistent defects in proteins needed for synaptic vesicle recycling, neurotransmitter release, and cytoskeleton organization and function.

The new technology has produced a huge number of studies and identified hundreds of genetic risk variants in an attempt to predict vulnerability to schizophrenia. The work has generated an enormous amount of enthusiasm among researchers because it holds such tremendous promise for understanding the neural basis for the disorder, developing better animal models for drug testing, and improving treatment options. The excitement is in part due to refined statistical approaches capable of handling very large amounts of data, along with the establishment of the Psychiatric Genomics Consortium. The consortium provides the opportunity to pool data from multiple large research projects to enhance the statistical power of analysis (Devor at al., 2017). Nevertheless, others are more pessimistic because despite the great effort and cost, success has been quite modest. Certainly one reason is that there is little precision in diagnosis of schizophrenia (as is true for other psychiatric disorders) because there is no objective measure (as there is for diabetes or cancer), and researchers cannot know whether their sampling population is homogeneous or whether it contains multiple subsets of patients. Also, compared with other, more common diseases such as diabetes, the size of the population sampled has been much smaller for schizophrenia. It is also possible that the genetic variants identified are associated not with the complex disorder of schizophrenia, but with specific symptom clusters. Indeed, recent GWAS evidence suggests a large genetic overlap for schizophrenia, autism, and bipolar disorder, as well as cardiovascular risk factors and multiple sclerosis.

A very recent research paper reported that examination of a vast array of independent GWAS using a powerful statistical approach showed that many genes associated with schizophrenia involve the disruption of communication between large numbers of ionotropic and metabotropic families of receptors (Devor et al., 2017). The failure of information processing based on abnormal synaptic regulation supports our earlier discussion of schizophrenia as a disorder of connectivity, since the integration of synaptic signaling is critical for all neurotransmitter systems and intracellular functions. Devor et al. found genetic variations in a large array of ionotropic and metabotropic receptors, including those for dopamine (DA), glutamate, GABA, acetylcholine (ACh), opioids, and serotonin (5-HT), all of which impact symptoms of schizophrenia. Additionally, they all affect or are affected by a protein called DARPP-32 that has widespread effects in the brain and integrates various neurotransmitter systems. Since DARPP-32 acts as a converging point to integrate signaling, the authors hope that their findings may unite the other existing hypotheses of schizophrenic pathophysiology.

It is clear that a single gene that makes a large contribution to the susceptibility to such a complex disorder as schizophrenia will not be found. Instead it seems that large numbers of genetic variants will be found and that each will contribute only a small amount, as described by Devor and colleagues (2017). It has been estimated that combining all the identified polymorphisms together would explain less than 5% of the variability in disease risk, which means that predicting the disorder for a given individual is very limited (Rudan, 2010). Alternatively, it has been suggested that one way to explain the evidence for a high level of heritability from family, twin, and adoption studies is to consider epigenetic modifications as the source (Crow, 2008). It is possible that environmental factors causing epigenetic changes modify developmental mechanisms not by altering the gene itself, but by altering the expression of the gene (**BOX 19.1**). Another alternative is that some of the missing genetic component may be explained by rare chromosomal abnormalities in candidate genes such as *disrupted in schizophrenia 1* (*DISC1*).

Chubb and colleagues (2008) summarize the evidence suggesting that mutations of the ***DISC1* gene**

BOX 19.1 The Cutting Edge

Epigenetic Modifications and Risk for Schizophrenia

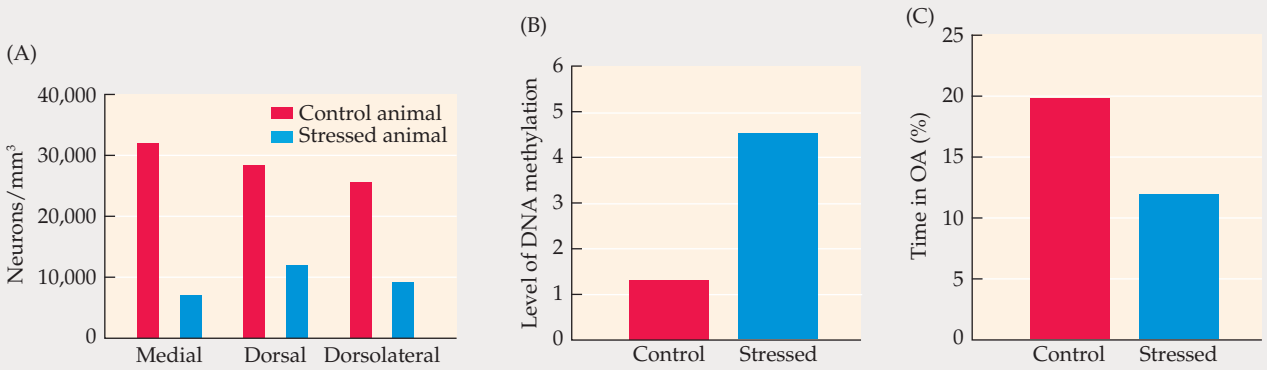
Schizophrenia is likely due to the interaction of a large number of susceptibility genes, each contributing a small amount to overall risk, in combination with environmentally induced epigenetic modifications. As discussed in the text, complications of labor and delivery, prenatal exposure to viral infection, malnutrition, and many other such events occur to a greater extent in people who develop schizophrenia. Although no single event will be predictive, all of these things as well as trauma later in life may be considered environmental stressors. Stress, especially early in life, produces epigenetic modifications that alter neurodevelopment. In earlier chapters (see Chapters 17 and 18), you learned that exposure to stress and neglect in the early years of life alters the development of the brain and endocrine stress circuits, leading to subsequent hyperarousal to stressors and an increased probability of psychopathology. Environmental stressors can also alter the expression of genes, such as the one for reelin (*RELN*).

Since abnormal neural connectivity characterizes schizophrenia, the discovery of epigenetic modification of the *RELN* gene has received a great deal of attention. Reelin is an extracellular glycoprotein secreted by neurons, and among its many functions is its role in guiding neuron positioning during fetal brain development. A reduction in reelin in the developing nervous system could explain the cell disorganization and morphological abnormalities typically seen in postmortem neuroanatomical studies of patients with schizophrenia. In adulthood, reelin seems to play a role in learning and memory by enhancing dendritic spine growth and synaptic plasticity. Consequently, low levels in the adult may reduce synaptic plasticity in the hippocampus and PFC, leading to cognitive deficits typical of schizophrenia. In fact, a number of studies have found up to 50% less reelin and its mRNA in the brains of patients with schizophrenia postmortem, compared with controls, particularly in the PFC, but also in the hippocampus, cerebellum, and basal ganglia.

At least some of the reduced reelin expression is due to epigenetic modulation, which has prompted some researchers to consider it a critical factor in psychosis (Guidotti and Grayson, 2011). Several postmortem studies have shown greater *RELN* hypermethylation (an epigenetic change described in Chapter 2) in several corticolimbic brain areas in patients with schizophrenia compared with controls. The added methyl groups would cause a more "closed" chromatin state and reduce transcription of the gene so that less reelin would be produced. The down-regulation of reelin may be due, at least in part, to an observed up-regulation of a methyltransferase enzyme responsible for the transfer of the methyl groups. Further evidence to support a role for epigenetic modification of *RELN* was noted when the amino acid methionine, which increases methylation, was given to patients with schizophrenia. It made the symptoms for more than 60% of the patients dramatically worse. Of special significance is the finding that there are differences in DNA methylation between monozygotic twins discordant for schizophrenia, which may explain how the differences in experientially induced epigenetic processes increase one individual's vulnerability despite the presence of identical genes.

These findings have several important ramifications. First, they suggest ways to create animal models that mimic the cognitive deficits of schizophrenia by prenatal administration of methionine, stress, or viral infection. In one such study, Palacios-Garcia and colleagues (2015) used 2 hours of restraint stress of pregnant dams during embryonic days 11 to 20, times corresponding to early and late cortical development in the fetus. Using immunohistochemistry on gestation day 20, they found a significant decrease in reelin-positive neurons that was not due to loss of cells but suggested a deficit in reelin gene expression in the stressed animals (**Figure A**). Of the cortical areas analyzed, the decrease in reelin immunoreactivity was greatest in PFC (79%) and dorsolateral parietal cortex (58%). Parallel changes in the reelin signaling pathway were found also. DNA methylation was significantly elevated in the cerebral cortex of stressed pups compared with controls, which would explain the reduced transcription of the reelin gene (**Figure B**). Behavioral measures were taken when the remaining pups reached young adulthood at 2 months. The researchers found the prenatally stressed animals showed increased locomotor activity and spontaneous rearing and spent significantly less time in the open arms of the elevated plus-maze, indicating increased anxiety (**Figure C**). In addition they showed deficits in emotional memory of an aversive situation, which may be related to deficits in synaptic plasticity in the amygdala–PFC circuit. This and other similar models that evaluate the association of early stress, reelin DNA methylation and subsequent decrease in gene expression, and behavioral changes in the adult provide a platform to evaluate the etiology of schizophrenia. Additionally, these models can then be used to identify drugs to reverse the cognitive symptoms that presently are not effectively treated.

BOX 19.1 The Cutting Edge (*continued*)



Reactions to prenatal stress (A) Prenatal stress reduces reelin-expressing neurons in the prenatal (E20) rat frontal cortex (medial, dorsal, and dorsolateral regions). (B) Level of DNA methylation at the reelin gene promoter region in samples of cerebral cortex from stressed and control rats. (C) Prenatally stressed animals spent less time in the open arms (OA) of the elevated plus-maze. (After Palacios-Garcia et al., 2015, CC-BY 4.0.)

A second ramification of the abovementioned findings is that unlike genetic impairments, epigenetic programming can be reversed, creating new treatment options. As you learned earlier (see Box 18.1), down-regulation of gene expression due to hypermethylation can be reversed by enhancing histone acetylation, either by activating histone acetylase or by inhibiting histone deacetylase. It is interesting that the mood stabilizer valproate, which is used to treat bipolar disorder (see Chapter 18), inhibits histone deacetylase and increases reelin expression in animals with suppressed reelin following methionine administration. It also enhances cognitive function and may be useful as an add-on treatment along with antipsychotic drugs in individuals with schizophrenia (see Guidotti et al., 2011). Several recent reviews provide more detail on the role of early stress in causing epigenetic modifications of reelin and potential neurodevelopmental outcomes (Guidotti et al., 2016; Negron-Oyarzo et al., 2016).

(as many as 15 variants of the gene exist) increase the probability of developing schizophrenia and other mental disorders. The initial finding showed that a chromosomal abnormality, specifically a translocation of pieces of DNA on chromosomes 1 and 11, was strongly linked to schizophrenia in a large Scottish family (St. Clair et al., 1990). One gene that was disrupted by the translocation is *DISC1*, which codes for proteins essential for neuronal functions such as embryonic neurogenesis and neuronal migration, intracellular transport functions, and axon elongation. Each of these could contribute to the morphological abnormalities seen in the schizophrenic brain. Since DISC1 protein is found in cell bodies, axon terminals, the postsynaptic density, and dendritic spines, it is likely to have multiple roles in synaptic function, in addition to its known role in regulating mitochondrial function. Subsequently, *DISC1* was implicated in several psychiatric disorders, including bipolar disorder, depression, and autism.

Individuals carrying the translocation show the reduced P300 event-related potential that is characteristic of those with schizophrenia and bipolar disorder. Various *DISC1* polymorphisms are also associated with impaired cognitive function in the areas of spatial working memory, verbal learning, sustained attention, performance on the WCST, and activation of the hippocampus during working memory tasks. Brain volume reductions in those with *DISC1* polymorphisms have been found in several studies, although the specific brain regions vary. Regional differences in the hippocampus, cingulate gyrus, and PFC have been found along with those in other brain areas. Using *DISC1* mutant mice, researchers have reported brain volume reductions similar to those found in schizophrenia and bipolar disorder. Some but not all have found schizophrenic-like behaviors such as deficits in prepulse inhibition of startle (PPI; see **Web Box 19.2**) and working memory in adult animals, which were normalized by antipsychotic drugs. These animal models provide further support for the importance of *DISC1* in increasing the vulnerability for schizophrenia. Perhaps most important to psychopharmacologists, the genetic model will potentially aid in screening drugs to alleviate the cognitive symptoms. Surprisingly, although we now know a lot about the gene and the protein it codes for, there seems to be no difference in *DISC1* expression in the brains of individuals with schizophrenia

postmortem. Furthermore, antipsychotic medication does not alter its expression in these individuals. Hence although *DISC1* is strongly implicated in mental illness, the mechanism by which it contributes to the pathogenesis is not understood.

DEVELOPMENTAL ERRORS Many investigators now believe that genetic vulnerability increases the probability that events during perinatal (including prenatal and postnatal) brain development will contribute to the occurrence of schizophrenia (Lewis and Levitt, 2002). The abnormal pattern of cortical connections and other brain structure irregularities that exist in the brains of individuals with schizophrenia are likely to be due to disruptions in the normal processes of cell multiplication and cell loss that continue into adolescence.

Evidence from several sources shows a higher occurrence of perinatal complications among individuals with schizophrenia than in the general population. Brain insult during pregnancy and delivery caused by oxygen deprivation, drug use, infection, endocrine disorders, or other factors occurs with higher frequency in individuals with schizophrenia. Severe malnutrition, as demonstrated in Holland during World War II, also represents an assault on the fetus that increases the probability of schizophrenia.

Exposure to viral infection (e.g., pneumonia, influenza, measles, or polio), especially during the second trimester of pregnancy, significantly increases the risk of schizophrenia in the child. The damaging effects of maternal infection are not likely due to the direct viral effects on the developing fetus, but rather to maternal and/or fetal inflammatory responses. Animal studies suggest that cytokines can have neurodevelopmental effects by affecting cell neurogenesis, proliferation, migration, and survival (see Meyer, 2013). Infectious pathogens of many types are known to increase microglial activation and cytokine release and have been suspected to cause neurodevelopmental brain abnormalities and increase the risk for schizophrenia (see previous discussion of immune system dysfunctions). However, multiple studies have shown that in addition to exposure to an infectious pathogen, many other prenatal assaults cause inflammation that predicts schizophrenia. Hence dysfunctions of the immune system may be the common denominator that explains increased risk of schizophrenia from a broad range of prenatal insults including various obstetric complications, starvation, exposure to war conditions, maternal gestational diabetes, maternal depression, and others (see Miller et al., 2013). Although none of these stresses or immune dysfunction alone explain the occurrence of the illness, the assault may increase the probability of schizophrenia in the individual who is genetically at risk. The finding that the elevated risk of schizophrenia associated with prenatal infection is much higher in offspring with a family history of psychotic disorders supports the idea of genetic predisposition. You have already seen in Chapter 17 and in Boxes 18.1 and 19.1 how environmental events that cause epigenetic modification of gene transcription may increase the risk for psychiatric disorders, which may include schizophrenia.

Further evidence for early developmental errors potentially predicting later schizophrenia is provided by the observation of several behavioral characteristics

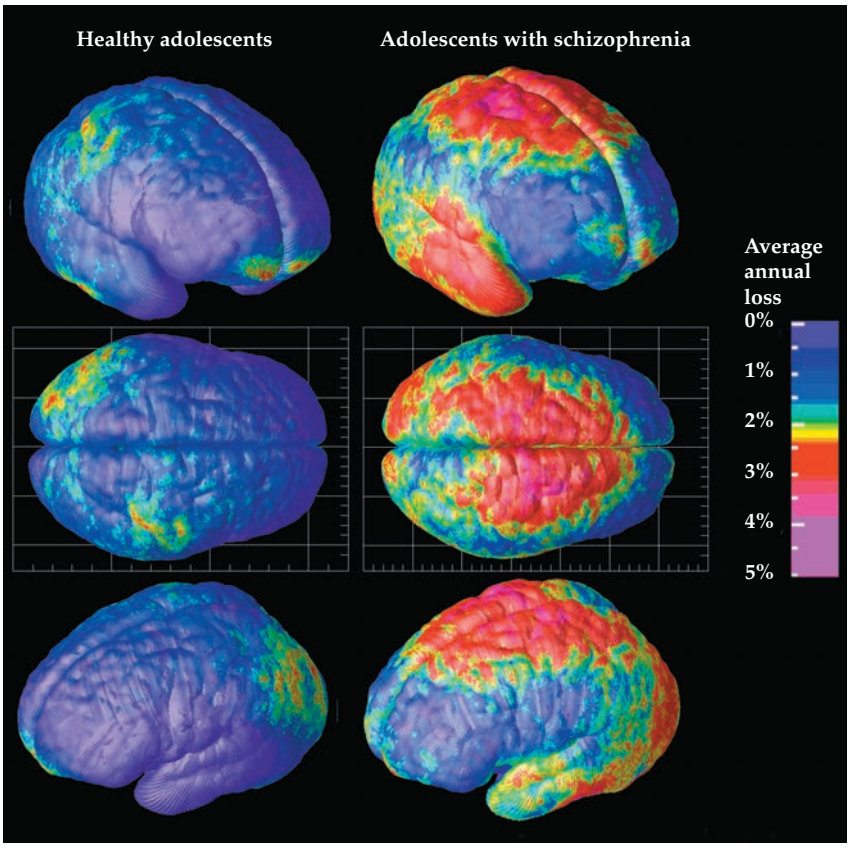


FIGURE 19.6 Cortical gray matter loss Three-dimensional maps of brain changes show the average annual rate of loss of cortical gray matter in healthy adolescents (left) and in adolescents with schizophrenia (right). (From Thompson et al., 2001; courtesy of Paul Thompson.)

of early infancy, particularly if the infant has other risk factors. The infant behaviors identified were passivity and apathy, reduced responsiveness to verbal commands, more difficult temperament, and poor sensorimotor performance. In later childhood, deficits in attentional and information-processing tasks, along with impairments in fine motor coordination, were the best predictors of psychiatric disorders.

Although evidence suggests that observers could identify subtle differences in the behavior of youngsters who later developed schizophrenia, such as more negative facial expression, increased social withdrawal, and unusual motor movements, the more flamboyant symptoms that lead to diagnosis do not appear until adolescence, which is also a period of significant brain development. Keshavan and colleagues (1994) found significant abnormalities in the elimination of synapses (pruning) that normally occurs during puberty. Excessive synaptic pruning in the PFC (associated with negative symptoms) and failure of pruning in certain subcortical structures (associated with positive symptoms) occur more often in the brains of individuals with schizophrenia than in healthy individuals. Using different technology, Thompson and coworkers (2001) imaged the brains of early-onset patients over several years with high-resolution MRI and compared them with age-matched controls. Although relatively rare, early-onset patients provide a unique opportunity to evaluate the timing and pattern of cortical gray matter changes to see how the disease emerges. What investigators found was that over the 5 years of the study, the patients lost twice as much cortical gray matter as the healthy controls. The excessive loss started in the parietal lobes and progressed anteriorly to the temporal lobes, to the DLPFC, and ultimately to the frontal eye fields (**FIGURE 19.6**). Of particular interest was that the extent of gray matter loss was correlated with the nature and severity of symptoms. Alterations in these normal developmental processes could be caused by genetic programming errors, early brain insults, and environmental factors. The nature and extent of interaction of these factors remain unclear.

BIOPSYCHOSOCIAL INTERACTION It is easy to imagine an interactive basis for schizophrenia that depends on genetic predisposition, structural brain-wiring errors, and subsequent biochemical abnormalities. Environmental or social factors that challenge the susceptible individual beyond her ability to deal with the stress further contribute to the development of the disorder. **Web Box 19.3** provides a fascinating case study demonstrating the interaction of genetic and environmental factors in a set of quadruplets with schizophrenia. **FIGURE 19.7** summarizes the stages in the development of schizophrenia on the basis of material presented in the text. This complex etiology and time course of the disorder is often referred to as the "two-hit" model, referring to the perinatal events in the genetically vulnerable individual that cause altered brain development as the first "hit." The second "hit" occurs at adolescence when neurodevelopmental errors in combination with environmental events produce the diagnosable symptoms of schizophrenia.

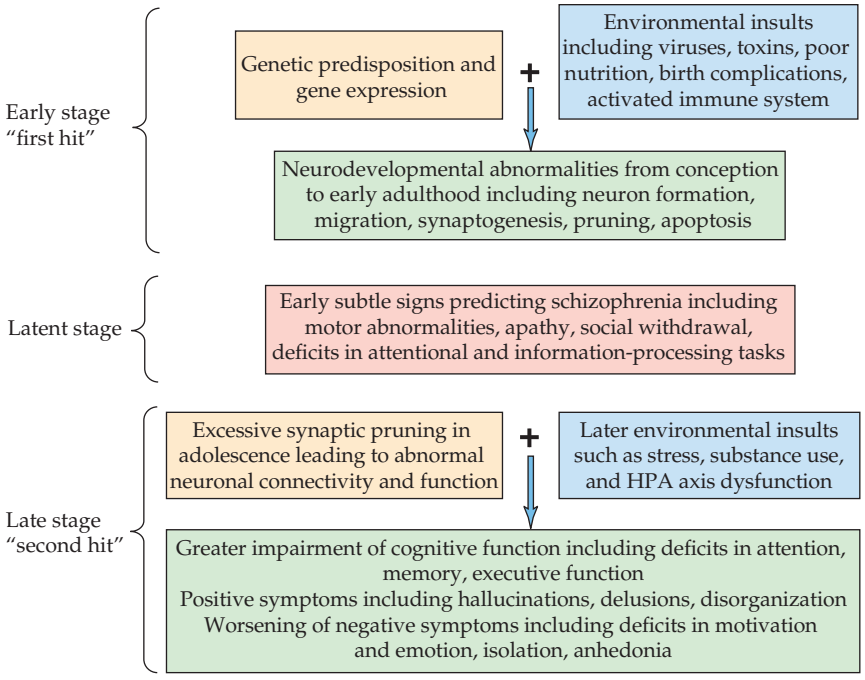


FIGURE 19.7 Etiology of schizophrenia This schematic diagram shows the importance of the interaction of genes and environmental factors in the development of schizophrenia, based on material presented in the text. Genes and environment interact to cause abnormal development of the nervous system. The neurodevelopmental abnormalities, such as errors in connectivity, make the individual susceptible to later environmental events at adolescence, which exacerbates the cognitive deficits and negative symptoms and elicits the positive symptoms leading to diagnosis.

Preclinical Models of Schizophrenia

Developing animal models for schizophrenia is especially difficult because the primary symptom is profound thought disorder, a cortical process not found in lower animals. No single animal model can mimic the complex symptomatology of schizophrenia, so each one tends to focus on one aspect of the disorder and experimentally induce homologous (similar) changes in animal behavior. It is assumed that subsequent attempts to manipulate the experimental response both neurochemically and neuroanatomically should provide evidence for the neurobiological and genetic bases of human behavior.

Animal models are also used to screen new therapeutic drugs for effectiveness. These models may not resemble the psychiatric condition in any way and may instead depend on neurochemically induced behaviors that are known to respond to currently useful drugs. The disadvantage, of course, is that such screening devices often fail to identify drugs with novel mechanisms of action, which may be of greatest importance to the researcher.

There are multiple approaches to creating these models, including pharmacological, developmental, and environmental manipulations as well as gene modification. In all cases, the models must be evaluated for face validity, construct validity, and predictive validity as discussed in Chapter 4. This section of the chapter will highlight just a few of the many techniques used, and recommended readings are provided on the Companion Website for further consideration.

The toxic reaction to high doses of central nervous system (CNS) stimulants is an early model that is still used because it is quick and easy to perform before further evaluation with other tests. It was found quite accidentally when clinicians realized that people who abuse CNS stimulants (amphetamine and cocaine) frequently show signs of thought disorder as well as well-formed paranoid delusions; various stereotyped, compulsive behaviors; and either visual or auditory hallucinations that are indistinguishable from those of paranoid schizophrenia. Also, when amphetamine is administered to patients with schizophrenia, the patients report that their existing symptoms get worse, not that new symptoms are produced. Finally, amphetamine-induced psychosis can be treated with the same drugs that are most effective in treating schizophrenia.

In animals, high doses of amphetamine produce a characteristic stereotyped sniffing, licking, and gnawing. Because stereotyped behavior also occurs in response to high doses of amphetamine in humans and is similar to the compulsive repetitions of meaningless behavior seen in schizophrenia, **amphetamine-induced stereotypy** is used in the laboratory as an animal model for schizophrenia. For many years, it has been a classic screening device to identify effective antipsychotic drugs that block D_2 receptors. Because high doses of amphetamine release dopamine, the abnormal behaviors produced by the drug support the dopamine hypothesis of schizophrenia (this hypothesis is described later in the chapter). The downside to this model is that it mimics only the positive symptoms, and since it depends on dopamine-regulated behavior, it is unlikely to identify drugs with novel mechanisms of action. A second pharmacological model, the **hypoglutamate model**, relies on the acute reduction of glutamate neurotransmission, which reflects the hypoglutamatergic activity seen in patients with schizophrenia (see the following section on neurochemical models). The administration of the *N*-methyl-D-aspartate (NMDA) receptor antagonists phencyclidine (PCP) and ketamine produce rodent behaviors reminiscent of positive (e.g., deficits in PPI and PCP-induced hyperactivity), negative (e.g., decreased social interaction and increased anxiety-like behaviors), and cognitive (e.g., deficits in working memory, novel object recognition, and attention) symptoms of schizophrenia. The drugs also produce positive, negative, and cognitive symptoms in healthy humans, which formed the basis for the hypoglutamate hypothesis of schizophrenia. The classic antipsychotics, such as haloperidol, are effective in reducing PCP- and ketamine-induced positive, but not negative or cognitive, symptoms. In contrast, the atypical antipsychotic clozapine can restore normal behavior. In the last section of this chapter you will see that these rodent results reflect human clinical experience. As was true for the high-dose amphetamine model, the hypoglutamate model is relatively easy and quick to use, but neither of them addresses the issue of neurodevelopmental abnormalities, believed to have a significant role in the etiology of schizophrenia. However, some evidence suggests that *chronic* NMDA antagonism causes inflammation and activation of microglia along with increased cytokines in specific brain regions. The fact that the cognitive deficits caused by chronic PCP administration can be reduced by anti-inflammatory drug treatment further supports the role of immune function in PCP-induced schizophrenia-like behavioral effects. Mattei and colleagues (2015) provide an excellent review of the pharmacological models as well as many others. The disruption of immune function with chronic PCP administration links this model to the neurodevelopmental model of prenatal inflammation described below.

A technique used to evaluate the motor side effects of antipsychotic drugs is the **vacuous chewing movements test**. In this test, mouth and facial dyskinesias (i.e., involuntary mouth opening not directed to physical material) that occur after chronic (between 4 and 36 weeks) antipsychotic medication are counted. Since these movements persist even after withdrawal of the drug, they are similar to the tardive dyskinesia

that occurs in some patients (see a later section on drug side effects). Clinical evidence and rodent testing both show that the movements are exacerbated by stress and that there is a higher incidence in older individuals.

Recently, cognitive symptoms have been an important focus for modeling because these symptoms are the core domain of schizophrenia and are highly disruptive to the individual and to his ability to function in the community. Also the presently available antipsychotics are relatively ineffective in reversing the deficits. Some commonly used tasks that reflect deficits in schizophrenia were described in Chapter 4 and include tests of working memory, attention, and sensory processing. Of special interest is the development of the **attentional set-shifting task**, the rodent version of the WCST (see Figure 19.4A). In the animal task, rodents are presented with pairs of bowls to dig into. Only one has a food reward. Unlike the human version, which requires sorting cards by color, shape, or number, the animals must choose the bowl on the basis of either odor or surface texture. More recently an automated visual discrimination analog of the WCST has been developed to be used with monkeys. Because of their similarity to the WCST, if these tasks are fully validated, their results are expected to have good translation to human behavior.

One very different type of model is based on evidence that schizophrenic individuals fail to "gate," or filter, most of the sensory stimuli they experience. Such a defect may lead to sensory overload and fragmented thinking because schizophrenic individuals are overwhelmed by sights and sounds and odors in the environment that they cannot filter out. The acoustic startle response, used in the technique called **prepulse inhibition of startle** (**PPI**), is one of the most reliable and generalizable models used to study sensory-filtering deficits. The procedure is almost identical when used with human participants and laboratory animals, so the translation of findings is excellent. Reversal of induced sensory-filtering deficits predicts antipsychotic effects. Furthermore, when deficits are induced by administration of glutamate antagonists like phencyclidine (PCP), it is a screening device that distinguishes between classical and atypical antipsychotics. The interested reader can find an update and review in Swerdlow et al. (2016). **Web Box 19.2** describes the technique and demonstrates the elegance of this model.

Since schizophrenia is a developmental disorder, models based on interfering with normal prenatal brain development have been developed. Many of the neurodevelopmental models are based on the epidemiological findings discussed earlier. That means that investigators subject pregnant rodents to inadequate diets, viral infections, elevated pro-inflammatory cytokines, or stressors that elevate glucocorticoids, or they create complications during delivery such as hypoxia. Each of these methods alters development of brain circuitry, produces neurochemical aberrations, and causes behavioral deficits that mimic some of the pathophysiology of schizophrenia. Since several methods produce a developmental delay in the onset of abnormalities, they may be particularly important for understanding the etiology of the disorder. One such model, the **neonatal ventral hippocampal lesion model** (**NVHL**), is described in **Web Box 19.4**.

There are many rodent models of **prenatal inflammation** based on the human epidemiological evidence and animal data suggesting that prenatal exposure to cytokine-induced inflammation and microglial activation increase the risk of neurodevelopmental abnormalities associated with schizophrenia. These are considered excellent examples of the neurodevelopmental hypothesis of schizophrenia because they do not require lesioning, genetic, or pharmacological manipulations. In each case the animals show a natural development of aberrations as they approach adolescence and adulthood. These models involve administration of pro-inflammatory agents, such as human influenza virus; polyiosinic–polytidylic acid (polyI:C), a synthetic agent used to simulate viral infections; one of the many cytokines (e.g., interleukin-6, or IL-6); or bacterial lipopolysaccharide, to pregnant rodents at various embryonic time points. The offspring are then evaluated for long-term brain and behavioral effects, compared with offspring of vehicle-treated mothers. The timing of the infection is important because the developmental outcome varies with gestational age of the fetus. **BOX 19.2** provides greater detail on the use of prenatal infections and their neurobiological and behavioral outcomes as they relate to schizophrenia.

As you probably suspect, there is a good deal of interest in creating **genetic models** with the hope that by modifying a schizophrenia susceptibility gene, abnormal behavior that is relevant to schizophrenia will be produced. This may entail creating knockout, knockin, or transgenic mice (see Chapter 4). For instance, when the gene for the dopamine reuptake transporter is deleted, the animals show hyperactivity in novel environments, deficits in PPI, stereotyped movements, and spatial learning impairments, behaviors analagous to schizophrenic behavior. Based on our earlier discussion, it should be clear that genetic factors can contribute only a portion of the vulnerability to schizophrenia. Hence newer animal models rely on integrating genetic and environmental factors. Thus, in addition to a genetic manipulation, environmental factors are manipulated. Some of these include enriched versus sensory-deprived housing, exposure to prenatal inflammation, postnatal stress, vitamin D deficiency, and exposure to drugs of abuse. For example, pre- or neonatal treatment with polyI:C in transgenetic mice expressing mutant *DISC1* (a candidate gene described earlier) may demonstrate synergistic gene–environment effects that would more closely resemble human

BOX 19.2 Pharmacology In Action

The Prenatal Inflammation Model of Schizophrenia

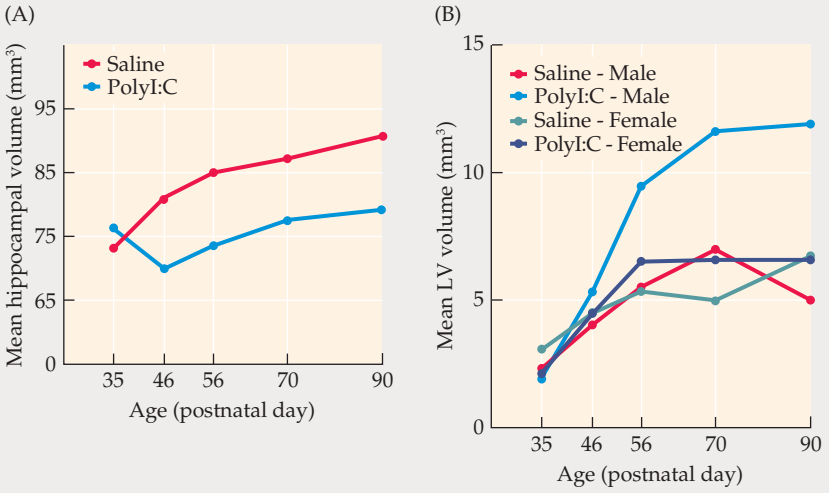
One early model of maternal immune activation in mice utilized infection with human influenza virus and was investigated in an extensive series of papers by Fatemi and coworkers (reviewed by Kneeland and Fatemi, 2012). When the researchers examined the offspring postnatally, they found significant differences (in some cases up-regulation, in others down-regulation) in gene expression in multiple brain areas, including frontal cortex, hippocampus, and cerebellum. For example, after viral exposure at embryonic day 16 (E16, considered the middle of the murine second trimester), 1463 genes were up-regulated and 797 genes were down-regulated. Many of these genes are involved in inflammation, cell structure, neuronal function, and myelination. As you might expect, protein levels also varied with brain region and timing of the infection. It is of importance that some of the significant proteins that had higher levels, such as nitric oxide synthase that manufactures nitric oxide, are responsible for fetal brain injury. In contrast other protein levels were reduced, as for reelin, which is important in migration and maturation of cells during brain development. Another protein with a reduced level was SNAP25, which is involved in axonal development, release of neurotransmitters, and apoptosis of infected cells. Structural brain and neurotransmitter abnormalities were reported in studies of both rodents and rhesus monkeys, along with behavioral differences, such as prepulse inhibition of startle (PPI) deficits, reduced spatial exploration, and social interaction, that were reversible with antipsychotic drug treatment. The testing with rhesus monkeys is particularly important because cortical development is more advanced in primates than rodents, so it verifies the relevance of this model and suggests that results translate to humans. Once again, all of the findings were highly dependent on the timing of the prenatal infection. Hence this model shows that exposure to viral infection during narrow windows of gestational development leads to elements of a schizophrenia-like phenotype in the maturing animal.

Two of the best established rodent models using maternal immune activation are those utilizing polyI:C as the infectious agent, mimicking the acute response to viral infection, or lipopolysaccharide, a bacterial endotoxin. Pro-inflammatory cytokines are elevated by these infectious agents, not only in the fetal brain, but also in the placenta and amniotic fluid, indicating widespread neuroinflammation during fetal development. As we saw for human influenza virus infections, these disease vectors also produce brain structural, neurochemical, cognitive, and behavioral outcomes that resemble components of schizophrenia in the adult offspring of infected mothers. An especially appealing characteristic of this model is that the adult offspring show behavioral correlates of positive, negative, and cognitive symptoms of schizophrenia. For psychopharmacologists, it is especially exciting to find that both classical and second-generation antipsychotics reverse some of the disease-like behavioral effects. More important is that the anti-inflammatory antibiotic and antioxidant minocycline also reverses some behavioral abnormalities (see the last section of this chapter on renewed efforts to treat the cognitive symptoms).

Many of the neuroanatomical effects of in utero exposure to inflammatory agents may begin early and persist postnatally. For example, maternal polyI:C challenge reduces hippocampal neurogenesis, and the condition persists into the offspring's adulthood. Also, the trajectory of postnatal brain development is altered by maternal exposure to polyI:C on gestational day 15. The volume of the hippocampus generally increases between adolescence and adulthood, but it is much reduced in polyI:C offspring (**Figure A**). During that developmental period the lateral ventricles increase in size, but they increased to a much greater extent in the animals experiencing embryonic inflammation. Interestingly the difference occurred only in male, but not female, offspring (**Figure B**; Piontkewitz et al., 2011). Other research suggests that the same maternal immune response increases DA neurons in the fetal midbrain and produces DA hyperactivity that can be seen in striatal slices from adult brains. This finding is appealing because of its link to the DA hypothesis of positive symptoms of schizophrenia and suggests that the abnormalities in DA mesolimbic function in the adolescent and adult may be due to prenatal developmental events (Meyer, 2013). Of course, other neurotransmitter systems are also altered by prenatal inflammation, including GABA, glutamate, and 5-HT levels as well as receptors and receptor subunit expression. Additionally, cell cytoarchitecture, such as reduced density and complexity of dendritic spines, a feature of psychopathology in schizophrenia, also occurs. Further polyI:C causes altered white matter connectivity especially in frontostriatal–limbic tracts, which may be responsible for the failure of coordinated neural communication between the prefrontal cortex and hippocampus, leading to deficits in PPI and cognitive dysfunction (Mattei et al., 2015). Once again, in each case the differences reported depend on the timing of the maternal infection (see Mattei et al., 2015).

Keep in mind that the events of pre- and perinatal development are progressive, and their effects may

BOX 19.2 Pharmacology In Action (*continued*)



Brain volume differences in animals treated prenatally with polyI:C
(A) Postnatal hippocampal volume differences in animals treated with polyI:C prenatally were compared to controls at five time points. (B) Mean lateral ventricle (LV) volume of male and female saline- or polyI:C-treated offspring at five postadolescent time points. (After Piontkewitz et al., 2011.)

not be apparent in behavior, brain morphology, and neurochemistry until early adulthood. So in effect, early events related to immune function and their persistence over time set the stage for later psychopathology when environmental stimuli (e.g., stress, drug use) in combination with abnormal adolescent neurodevelopment in myelination, synaptic pruning, and synaptic remodeling lead to the symptom-based diagnosis of schizophrenia. This is frequently referred to as the "two-hit" hypothesis, and it is summarized in Figure 19.7. One empirical test of the two-hit hypothesis found a *synergistic* interaction between prenatal exposure to inflammation, induced in the maternal mice with polyI:C on gestational day 9, and exposure to variable and unpredictable stress of the offspring at peripuberty. That means the effect of significant increase in activated microglia and elevated pro-inflammatory cytokines in the hippocampus and prefrontal cortex, indicative of potentially damaging neuroinflammatory processes in utero, plus the neurobiological effect of adolescent stress was greater than the sum of their individual effects. These synergistic effects were seen for PPI disruptions, psychomimetic behavioral hypersensitivity to amphetamine and an NMDA antagonist, and activation of microglia in the hippocampus and prefrontal cortex. Interestingly, just as the time of exposure to inflammation in utero determines the outcome, these researchers found that the timing of adolescent stress is critical. These synergistic effects occurred only when stress was initiated at peripubertal age but not later in adolescence. Hence, the latent neuropathological consequences of prenatal immune activation ("first hit") were unmasked by stress during puberty ("second hit") (Giovanoli et al., 2013).

psychosis than either manipulation alone. A discussion of the development of such gene–environment models is provided by Kannan and coworkers (2013).

Section Summary

- Imaging shows cerebral atrophy, enlargement of ventricles, and smaller basal ganglia, temporal lobe, and hippocampus in patients with schizophrenia. Hippocampal cells are disorganized. Lack of gliosis indicates developmental error rather than degeneration.
- Abnormal myelination and organization of white matter tracts is responsible for reduced connectivity between brain regions, which prevents integration of signals for cognitive processing.
- Brain function deficits include hypofrontality during tasks of working memory, executive function, response inhibition, and planning and strategy.
- Elevated cytokines and microglia activation are associated with higher risk for schizophrenia. The immune responses are localized to brain regions with gray and white matter abnormalities. Microglia activation is greatest during acute illness relapses.
- Family studies show that relatives of individuals with schizophrenia have increased risk for the disorder. The closer the genetic relationship, the greater is the risk.
- Concordance for schizophrenia is much higher in monozygotic than dizygotic twins, indicating significant genetic contribution.
- Linkage studies and genome-wide association studies have identified numerous gene variants that may each contribute a small amount to the increased risk for schizophrenia.
- Mutations of the *DISC1* gene increase the probability of developing schizophrenia. This gene codes for proteins necessary for embryonic brain development.

- Gene variants are associated with EEG abnormalities, impaired cognitive function, and brain volume reductions.
- Perinatal events such as stress during pregnancy and delivery, exposure to viral infection, and malnutrition may alter the trajectory of brain development and predispose the individual to schizophrenia. Activation of the immune response by the perinatal events may be the common factor explaining altered brain development.
- Behavioral abnormalities, apparent in young children who later develop schizophrenia, suggest early developmental errors. Other developmental errors during adolescence cause twice the cell loss in cortical areas compared with that seen in healthy teens.
- Animal models of schizophrenia created by pharmacological or environmental manipulations generally focus on one aspect of the disorder and induce similar changes in animal behavior. Neurodevelopmental models and genetic mutations are also used.

Neurochemical Models of Schizophrenia

To identify the neurochemical basis for any mental disorder, three general approaches can be taken. First, neurochemical correlates of animal and human models of the disorder can be studied. Second, the neuronal mechanisms of effective drug treatment are considered, keeping in mind that it is dangerous to assume that because the neurochemical effects of a drug reverse the symptoms, the drug is acting at the specific site of the disorder. Third, the functioning of neurotransmitter systems in patient populations is assessed by measuring neurochemicals in blood, cerebrospinal fluid (CSF), or urine or in postmortem brain tissue. Brain imaging techniques provide the newest way of evaluating CNS function in patient populations. Based on these three approaches, evidence strongly suggests that although several neurotransmitters probably play a role in schizophrenic symptoms, malfunction of dopaminergic transmission is almost certainly involved.

Abnormal dopamine function contributes to schizophrenic symptoms

The finding that amphetamine can produce a psychotic reaction in healthy individuals that can be reversed by DA antagonists initially suggested the **dopamine hypothesis of schizophrenia**. Also, patients with schizophrenia who have been given amphetamine and cocaine say that the drugs make their symptoms worse but do not produce different symptoms. In addition, stereotyped behavior in rats can be elicited by intracerebral injection of amphetamine into forebrain DA areas and can be blocked by administration of DA receptor blockers, such as haloperidol.

As you will see in the following section, there is a strong correlation between D_2 receptor blockade and effectiveness in reducing schizophrenic symptoms. Finally, the finding that antipsychotic treatment induces changes in DA turnover, as determined by plasma levels of the dopamine metabolite homovanillic acid (HVA), further supports the DA hypothesis of schizophrenia, which suggests that excess DA function is related to the manifestation of the positive symptoms.

Of the approaches used to understand the neurochemistry of schizophrenia, evaluation of DA functioning in patient populations has been the least consistent. To substantiate the DA hypothesis, we would expect to see an increase in DA activity either through increased turnover (synthesis, release, metabolism) or by altered receptor number. Significant increases in DA synthesis have been found not only in patients with schizophrenia, but also in very early prodromal stages of the disorder, that is, a time when initial symptoms occur, preceding full development of the disorder. Early abnormal DA synthesis predicts progression to psychosis. With the onset of psychosis, synthesis capacity increases further. Although the change in the dopamine metabolite HVA in response to antipsychotic treatment (i.e., an initial increase followed by a significant decrease over several weeks) predicts treatment outcome, under baseline (nondrug) conditions neither plasma nor CSF levels of HVA are consistently different in patients with schizophrenia compared with controls. Nor are HVA levels correlated with symptom type or severity (Friedhoff and Silva, 1995). However, high-risk individuals and those with schizophrenia show significantly greater HVA response to stress, indicating a more robust impact of stress on DA synaptic function.

In addition, Laruelle and colleagues (1999) and subsequent replicated studies found that in patients with schizophrenia, a challenge dose of amphetamine elicited a significantly greater release of DA than was seen in controls. Baseline levels of DA are also elevated, indicating higher resting levels of DA. This effect was found at the onset of illness and in patients who had never taken antipsychotic drugs. Hence the hyperdopaminergic state is not due to prolonged illness, hospitalization, or chronic drug treatment. Further, a correlation was found between the exaggerated DA response and worsening of the positive symptoms. Other evidence for DA involvement comes from several studies that found increased D_2 receptors in the basal ganglia, nucleus accumbens, and substantia nigra of postmortem schizophrenic brains. PET scan quantification of DA receptors also suggests increased D_2 receptors in drug-free patients with schizophrenia, particularly in patients with positive symptoms as well as in those who are more acutely ill (Kahn and Davis, 1995). Recent genetic research suggests

genes encoding the D_2 receptor and regulation of DA synthesis are associated with increased risk for schizophrenia. Evidence summarized by Howes and colleagues (2017) indicates that increased presynaptic synthesis and release represent the major DA dysfunction in schizophrenia, appear early in the prodromal stage, and cause the occurrence of psychosis. Acute stress releases DA and triggers psychotic symptoms. In addition, it is of particular interest that many stress-inducing environmental factors such as neurodevelopmental assaults, inflammation, childhood adversity, cannabis use, urban living, and others increase risk for developing schizophrenia. Therefore early stress-inducing release of DA may make those individuals less tolerant to later stress and hence more vulnerable to psychosis.

Much of the evidence has been synthesized into a **DA imbalance hypothesis**, as described by Davis and colleagues (1991). They suggest that schizophrenic symptoms are due to reduced DA function in mesocortical neurons along with excess DA function in mesolimbic dopaminergic neurons. The negative symptoms and impaired thinking may be explained by impaired PFC function (low mesocortical activity). In contrast, positive symptoms seem to be improved by reducing DA function in mesolimbic neurons.

The neurodevelopmental model integrates anatomical and neurochemical evidence

Weinberger (1995) has developed a **neurodevelopmental model** that combines evidence of altered dopaminergic function with the loss of specific nerve cells (as described earlier in the section on etiology) and symptom clusters. The first part of the model is supported by several pieces of evidence that associate negative symptoms (flat affect, social withdrawal, lack of motivation, poor insight, and intellectual impairment) and cognitive symptoms (poor executive function, lack of attention, hypofrontality, and so forth) with reduced frontal lobe function. First, the negative symptoms of schizophrenia resemble the characteristics of patients with lesions of the frontal lobe (e.g., following frontal lobotomy). Also, the severity of the negative symptoms is correlated with reduced prefrontal cell metabolism, as evaluated by PET scan. In addition, neuropsychological testing in humans shows a relationship between poor performance on tasks requiring frontal lobe function, reduced cerebral blood flow in the PFC, and decreased DA function, as determined by lowered HVA levels in CSF. Further, in animal experiments, prefrontal lesions produce deficits in behaviors that require insight and strategy. Intracerebral injection of D_1 receptor antagonists impairs delayed-response performance and produces impulsivity and deficits in responding for delayed reward. Conversely, D_1 agonists improve cognitive deficits caused by injection of a DA neurotoxin into the PFC. These experiments indicate an important role for DA acting at the D_1 receptor in PFC in cognitive processes.

Animal studies also implicate mesocortical cells in normal response to stress. Mesocortical cells are cells originating in the ventral tegmental area (VTA) that innervate the frontal cortex and other cortical areas. These cells respond with increased DA turnover not only to acute stress but also to learned stress, for instance, when an animal is returned to a previously stressful environment. In summary, these results suggest that the onset of negative symptoms of schizophrenia is due to the occurrence of early mesocortical failure. However, although the cell loss occurs relatively early in life, the abnormal behavior may not appear until the system would normally reach functional maturity (i.e., after puberty, when development, myelination, and synaptic pruning are complete). Thus complex cognitive functions, including insightful behavior and the ability to respond to the social stresses commonly occurring at adolescence, would be expected to be compromised.

The second part of the model attempts to explain positive symptoms of schizophrenia with evidence of hyperactive subcortical cells. In animal studies, lesioning of prefrontal dopaminergic neurons produces chronic subcortical DA hyperactivity, manifested by increased DA turnover (Kahn and Davis, 1995). In addition, when DA agonists such as apomorphine are injected into the PFC, DA metabolites are reduced in the striatum. Thus when the inhibitory cortical feedback is lost, mesolimbic cells increase their activity. Furthermore, studies of epileptic patients suggest that psychotic experiences, hallucinations, perceptual distortions, and irrational fears are associated with electrical discharge in limbic regions. Thus Weinberger (1995) suggests that excessive mesolimbic DA activity following mesocortical cell loss could explain the more dramatic positive symptoms of schizophrenia. Those are the same symptoms that are most readily reversed by antipsychotic-induced DA receptor blockade.

The neurodevelopmental model makes no attempt to identify the cause of the proposed early mesocortical cell loss. The defect could be due to one of many factors, including genetically programmed errors, inadequate maternal nutrition, obstetrical complications, viral infection, and other possibilities discussed earlier in the chapter. Weinberger argues that such a lesion produces few symptoms early in life but reveals itself later, at a time when social stresses demand maximum prefrontal cognitive function. Loss of the DA input prevents the individual from making appropriate responses and instead leads to confused thinking, perseveration of inappropriate behavior, and social withdrawal. The loss of inhibitory cortical feedback onto subcortical neurons plus the stress-induced increase in mesolimbic cell function leads to agitation, fearfulness, and hallucinations. The appeal of this

model of schizophrenia is in its ability to incorporate many distinct pieces of the puzzle (neurochemical, anatomical, and developmental pieces, and social stress). It also provides several testable hypotheses on which future research can be designed.

Glutamate and other neurotransmitters contribute to symptoms

Since glutamate is known to have an important role in learning and memory as well as synaptic plasticity (see Chapter 8), glutamate hypofunction may explain the negative symptoms, abnormal cognitive function, and impaired neural connectivity seen in schizophrenia. Since there is a great deal of similarity in the cognitive deficits caused by either D_1 or NMDA receptor blockade, it is clear that both receptors contribute to working memory processes. Inadequate glutamate function at the NMDA receptor may be a precursor to the DA dysfunction and may explain the apparent *increase* in mesolimbic DA and *decrease* in PFC. **FIGURE 19.8A** shows the relationship of the brain structures involved and how descending glutamatergic neurons influence both DA pathways. **FIGURE 19.8B** is a schematic diagram that shows the details of the glutamate–dopamine interaction. Descending excitatory glutamatergic cells (green) projecting from the PFC activate NMDA receptors on mesocortical DA cells (black) in the VTA. These mesocortical DA cells reciprocally project back to the cortex. If glutamate levels are insufficient to activate mesocortical cells, low cortical DA neurotransmission and subsequent low D_1 receptor activation will lead to deficits in working memory, hypofrontality, and negative symptoms. In contrast, other PFC glutamatergic neurons acting on NMDA receptors have indirect *inhibitory* control of midbrain mesolimbic DA neurons (blue) that project to limbic regions. This happens because the glutamate neurons excite midbrain GABA cells (red; not shown in part A) that have an inhibitory effect on mesolimbic DA neurons. In this case, low levels of glutamate signaling would fail to inhibit mesolimbic neurons and would produce excessive DA release and associated positive symptoms, which can be relieved with DA receptor–blocking antipsychotic drugs. Evidence in support of this relationship was demonstrated in rats by blocking NMDA receptors in VTA, which resulted in an increase in DA release in the nucleus accumbens and reduced release of DA in the PFC. Hence the PFC provides tonic inhibition of mesolimbic neurons and tonic excitatory regulation of mesocortical cells. A more detailed discussion of glutamate–dopamine interaction is provided by Winterer and Weinberger (2004).

Evidence for the importance of NMDA receptors in schizophrenia comes from multiple sources. Challenge studies consistently show that blocking the

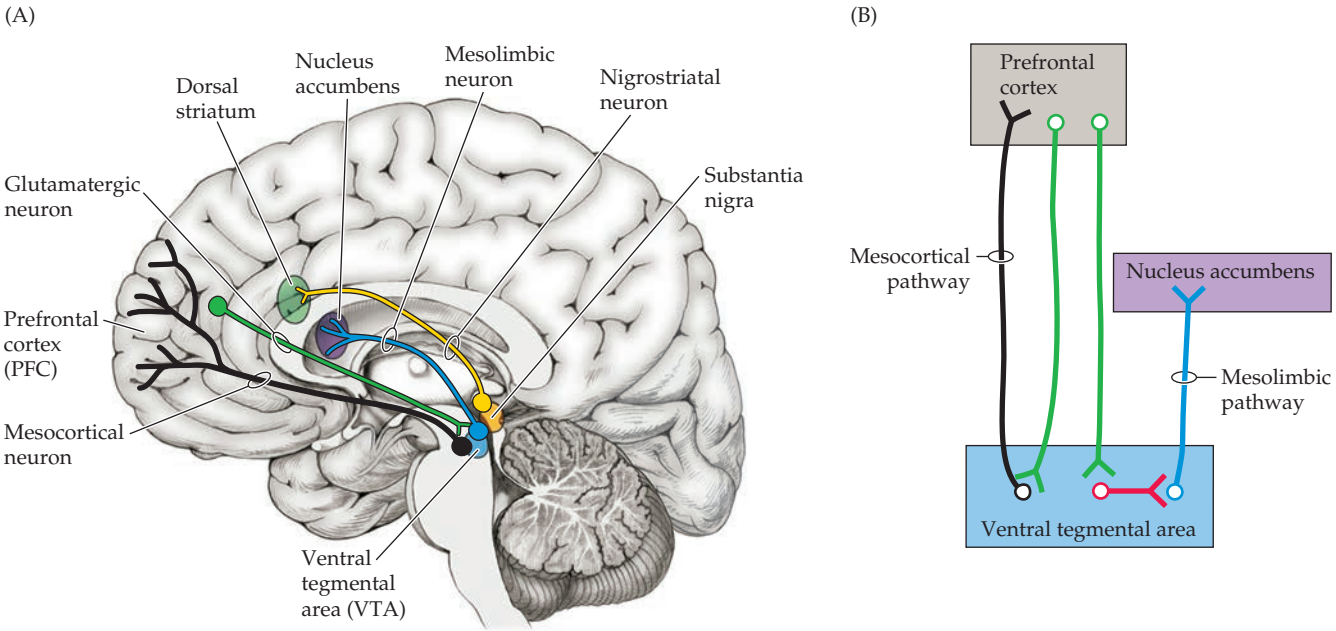


FIGURE 19.8 Hypoglutamate hypothesis of schizophrenia (A) Midsagittal view showing nigrostriatal (yellow), mesolimbic (blue), and mesocortical (black) dopaminergic neurons as well as a descending glutamatergic neuron (green) originating in the prefrontal cortex (PFC). (B) Since descending glutamate neurons (green) excite mesocortical DA neurons (black), poor glutamate signaling would produce low DA release in PFC, exacerbating negative and cognitive symptoms. Other glutamate neurons (green) excite inhibitory GABA neurons (red) that subsequently inhibit the dopaminergic mesolimbic pathway. (These tiny neurons within the VTA could not be depicted in part A.) In this case, low glutamate signaling would fail to inhibit mesolimbic firing, leading to excess DA release in the nucleus accumbens and positive symptoms. (B after Winterer and Weinberger, 2004.)

glutamatergic NMDA receptor with PCP ("angel dust") or ketamine produces a psychotic syndrome that closely resembles schizophrenia in healthy individuals and exacerbates symptoms in patients with schizophrenia. Of special significance is the fact that PCP and ketamine produce the positive, negative, and disorganized symptoms of schizophrenia. NMDA blockade also produces cognitive deficits indicative of schizophrenia, including poor performance on the WCST, impairments in verbal memory, and reduced spatial and verbal learning. Loss of such functions indicates hippocampal and frontal lobe dysfunctions (see Lin et al., 2012). Rodent studies showed that even brief treatment with an NMDA channel blocker impaired cognitive flexibility and working memory in young animals. Of special interest was the study of individuals with a rare autoimmune disorder that causes them to form antibodies against their own NMDA receptor subunit NR1a. That disruption of the subunits causes a loss of synaptic NMDA receptors. These patients present with a variety of neurological symptoms, but the most common first symptoms are behavioral in nature. These schizophrenia-like symptoms include agitation, paranoia, psychosis, and violence. Finally, changes in glutamate receptor subunit composition in several brain areas of individuals with schizophrenia have been described, and these may affect the quality of glutamate signaling (Ulas and Cotman, 1993). For instance, reduced mRNA and protein levels of the NR1 and NR2 subunits of the NMDA receptor were found in the DLPFC of patients with schizophrenia. Additional genetic evidence also implicates the NMDA receptor. A very large genome-wide association study (GWAS) identified 128 significant associations, many of which involved glutamatergic function. (See Balu and Coyle, 2015, for more details.)

There is accumulating evidence that the glutamatergic signaling deficit associated with schizophrenia is due to hypofunction of the NMDA receptors rather than levels of the amino acid. Nevertheless, some direct evidence for low CSF glutamate levels in patients with schizophrenia compared with controls has been found, although the differences are not consistent. More consistently, in postmortem studies, decreased levels are reported for both glutamate and aspartate in PFC and hippocampus, compared with controls, as well as reduced NMDA function. Additionally, some studies have found that lower levels of the amino acid are correlated with greater brain atrophy and cognitive impairment. Also, in synaptosomes (i.e., preparation of isolated nerve terminals) prepared from the postmortem brains of patients with schizophrenia, depolarization-induced release of glutamate is reduced. However, there have been multiple reports of *increased* glutamate levels in many brain areas, as determined by magnetic resonance spectroscopy. Coyle and Konopaske (2016) and Poels and collegues (2014) provide some potential explanations for the difficulties in interpreting the data in an effort to resolve the discrepancies.

Of particular interest is that the antipsychotic drug clozapine interacts with the glutamate receptor and increases glutamate levels in the PFC of rats, which may explain the drug's unique ability to reduce negative and cognitive symptoms in patients (see the section on atypical antipsychotics below). This characteristic of clozapine suggests that glutamate may be a new target for the development of antipsychotic drugs (see the final section of this chapter). For a more detailed discussion of the interaction of glutamate and DA in the production of schizophrenic symptoms and its potential therapeutic benefits, see Yang and Chen (2005).

Because the circuitry of the limbic structures and of the frontal cortex is complex, it is not surprising that many other neurotransmitters modulate or interact with DA transmission. Acetylcholine, GABA, norepinephrine, serotonin, and endorphins each may play a part in the presentation of individual symptoms of the disorder.

Section Summary

- To understand the neurobiology of any psychiatric disorder, researchers create animal models, evaluate mechanisms of effective drug treatment, and measure biological substances in patients or image their brains.
- The dopamine hypothesis suggests that positive symptoms are caused by excessive mesolimbic DA activity. Evidence includes the following: (1) amphetamine produces positive symptoms in healthy individuals and makes symptoms worse in patients with schizophrenia; (2) intracerebral DA into the forebrain of rodents produces stereotyped behavior reversed by antipsychotics; (3) a strong correlation exits between D_2 receptor blockade and antipsychotic efficacy; (4) schizophrenic individuals show exaggerated DA release after amphetamine challenge as well as in basal conditions; and (5) there is some evidence for increased D_2 receptors in schizophrenia.
- Evidence that the negative and cognitive symptoms are due to reduced frontal lobe function includes the following: (1) the negative/cognitive symptoms resemble characteristics after frontal lobotomy; (2) the severity of negative symptoms is negatively correlated with prefrontal brain activity and decreased DA function; and (3) prefrontal brain lesions or D_1 receptor antagonists injected into PFC impair cognitive performance.
- The neurodevelopmental model suggests that early mesocortical deficits due to genetics or

environmental events that alter brain development are followed by loss of inhibitory control of mesolimbic cells and the onset of positive symptoms.

- Poor glutamate signaling due to hypofunction of NMDA receptors may be responsible for positive, negative, and cognitive symptoms and corresponding changes in DA activity.
- Descending glutamate neurons from PFC activate mesocortical neurons. Low glutamate signaling at NMDA receptors (or NMDA receptor antagonist administration) reduces mesocortical function, causing negative/cognitive symptoms.
- Descending glutamate neurons from PFC activate midbrain inhibitory GABA cells that reduce mesolimbic DA cell firing. Low glutamate signaling at NMDA receptors (or NMDA receptor antagonist administration) prevents the inhibition, leading to excessive mesolimbic firing and positive symptoms.
- Accumulating evidence from challenge studies, rodent studies, receptor subunit studies, and genetics suggests that NMDA hypofunction is central to schizophrenia.
- Low levels of glutamate are found in hippocampus and PFC of patients with schizophrenia postmortem, and low levels are correlated with greater brain atrophy. Glutamate release is lower in patients with schizophrenia than in controls, and glutamate receptor subunits are different.

Classic Neuroleptics and Atypical Antipsychotics

Drugs useful in treating schizophrenia are called antipsychotic drugs or **neuroleptics**, an older term that refers to their ability to selectively reduce emotionality and psychomotor activity. A large number of drugs are included in this category, and they are commonly divided into two classes: classic neuroleptics and the newer second-generation (often called "atypical") antipsychotics. Although none of the drugs is consistently more effective than the others, a particular individual may respond better to one drug than to another. Therefore, treatment may require testing several antipsychotic drugs to find the one that is most effective for a given patient. The classic antipsychotic drugs are the phenothiazines, such as chlorpromazine (Thorazine), and the butyrophenones, such as haloperidol (Haldol). The second-generation antipsychotics, such as clozapine (Clozaril), risperidone (Risperdal), and aripiprazole (Abilify), are noteworthy because they appear to produce fewer side effects involving abnormal movements (e.g., tremors, rigidity). A review of classic and atypical antipsychotics is provided by Li and colleagues (2016).

Phenothiazines and butyrophenones are classic neuroleptics

Chlorpromazine was the first phenothiazine used in psychiatry, but many small changes in the shape of the drug molecule produced a family of related compounds that differ in potency, clinical effectiveness, and side effects. **FIGURE 19.9** shows the three-ring phenothiazine nucleus and the structural relationships of several other drugs in this class. By changing the chemical groups at the R_1 and R_2 positions, many new compounds can be created that vary in their effects. For example, chlorpromazine (which has a chlorine at R_2) is much more potent than promazine (which has hydrogen at the R_2 position). By substituting at R_1, an antipsychotic (thioridazine [Mellaril]) with fewer motor side effects is created. Further changes at R_1 and R_2 produce drugs (trifluoperazine [Stelazine], fluphenazine [Prolixene]) that further vary in potency and side effects. This structure–activity relationship provides clear evidence that molecular modifications alter the ability of the drugs to bind to specific receptor recognition sites in the cell membranes.

EFFECTIVENESS The introduction of antipsychotic drugs during the 1950s dramatically improved the treatment of patients with schizophrenia. The effectiveness of these drugs has been demonstrated hundreds of times in double-blind, placebo-controlled trials. For a significant number of patients, the antipsychotics are more effective than placebo in reducing symptoms and decreasing the average length of hospitalization. After only a few doses, the hyperactive and manic symptoms usually disappear, whereas the positive symptoms of schizophrenia may gradually improve over several weeks. Delusions, hallucinations, and disordered thinking are reduced, and improvements in insight, judgment, self-care, and seclusiveness are seen. More resistant to treatment are the negative and cognitive symptoms of schizophrenia.

Although estimates of effectiveness vary, psychiatrists often refer to the law of thirds. One-third of the patients treated with antipsychotics show excellent symptom reduction in response to the drugs and may not experience subsequent hospitalizations even when they discontinue medication. These individuals show few residual signs of the disorder. They are employed outside the institution, may marry, and maintain a relatively normal social life. The second third show significant improvement in symptoms but may experience relapses that require hospitalization from time to time. These individuals may be employed, although usually at a reduced occupational level, and they may remain socially isolated. Some require significant help in day-to-day living, for example, in maintaining personal hygiene, preparing meals, or keeping scheduled appointments. The final third show a lesser degree of recovery and may spend a significant amount of time

Phenothiazine nucleus

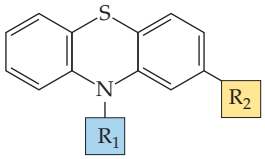


FIGURE 19.9 Phenothiazine nucleus and related compounds Minor molecular modifications determine the three major subgroups of phenothiazines and change drug potency, pharmacological activity, and side effects.

	R_1	R_2
	Aliphatic group	
Promazine (Prazine)	$-CH_2-CH_2-CH_2-N(CH_3)_2$	$-H$
Chlorpromazine (Thorazine)	$-CH_2-CH_2-CH_2-N(CH_3)_2$	$-Cl$
Trifluopromazine (Psyquil)	$-CH_2-CH_2-CH_2-N(CH_3)_2$	$-CF_3$
	Piperidine group	
Thioridazine (Melleril)	$-CH_2-CH_2-$ (N, CH_3)	$-SCH_3$
Mesoridazine (Serentil)	$-CH_2-CH_2-$ (N, CH_3)	$-S(=O)-CH_3$
	Piperazine group	
Trifluoperazine (Stelazine)	$-CH_2-CH_2-CH_2-$ (N, N) $-CH_3$	$-CF_3$
Perphenazine (Trilafon)	$-CH_2-CH_2-CH_2-$ (N, N) $-CH_2-CH_2-OH$	$-Cl$
Fluphenazine (Prolixene)	$-CH_2-CH_2-CH_2-$ (N, N) $-CH_2-CH_2-OH$	$-CF_3$

each year in a psychiatric institution. These patients need much more help in dealing with the stresses of everyday living. Since many of the behavioral abnormalities remain, these individuals are often unemployed, have few social relationships, and exist on the margins of society. Some portion of this final third fail to respond to any drug treatment and remain institutionalized. Estimates suggest that more than 30% of the adult homeless population in the United States may suffer from unmedicated or inadequately medicated psychosis.

Following a patient's initial recovery, antipsychotic drugs are prescribed as maintenance therapy to prevent relapse. Recovered patients maintained on antipsychotics have about a 55% chance of remaining in the community for 2 years after leaving the hospital, compared with a 20% chance for those on placebo. Thus drug maintenance more than doubles an individual's chances of avoiding significant relapse. Unfortunately, because the side effects of these drugs (discussed later in this chapter) are often debilitating and extremely unpleasant, many patients fail to continue treatment, which leads to a high relapse rate.

Although psychotherapy and group therapy are not considered substitutes for pharmacotherapy, social skills training and family therapy are important additions to drug treatment. Psychoeducation involves enhancing social competence and family problem solving, teaching vocational skills, minimizing stress, and enhancing cooperation with medication schedules (Goldstein, 1995; see the description of a multimodal approach called NAVIGATE at the end of this section).

Dopamine receptor antagonism is responsible for antipsychotic action

Antipsychotic drugs modify several neurotransmitter systems; however, their clinical effectiveness is best correlated with their ability to antagonize DA transmission by competitively blocking DA receptors or by inhibiting DA release. Evidence comes from several sources, including receptor binding studies, changes in DA turnover, second-messenger function, and neuroendocrine effects.

RECEPTOR BINDING Both first- and second-generation antipsychotics block D_2 receptors. Drugs that readily bind to the DA receptor at low concentration because of their high affinity also reduce symptoms at low doses (**FIGURE 19.10A**). Likewise, antipsychotics that require higher concentrations to bind to DA receptors require higher doses to be clinically effective. Although antipsychotic drugs bind to other neurotransmitter receptors in addition to DA, there is no clear relationship between clinical effectiveness and binding to serotonin (**FIGURE 19.10B**), α-adrenergic, or histamine receptors. Nor is there a correlation for D_1 receptor binding. Therefore, the correlation with D_2 receptor binding establishes quite clearly the mechanism of antipsychotic drug action.

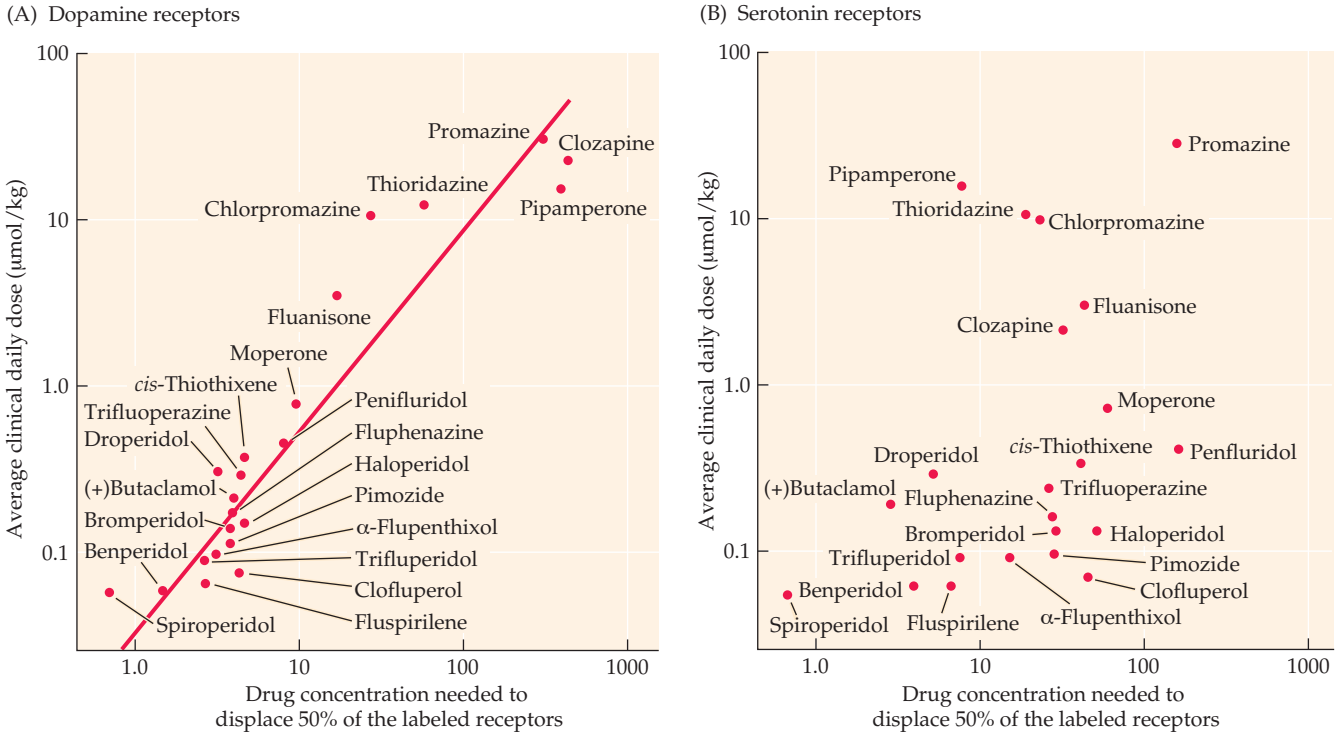


FIGURE 19.10 Correlation between antipsychotic drug binding to neurotransmitter receptors and clinical effectiveness The receptor binding studies were accomplished by first labeling the receptors with an appropriate radioactive ligand for each neurotransmitter. The antipsychotic drug was added in increasing concentrations until it competed successfully for half of the labeled sites. That value (K_i) is plotted along the *x*-axis, and the corresponding average clinical daily dose for that drug is plotted on the *y*-axis. A clear positive correlation is found for dopamine receptor binding (A), but serotonin receptor binding shows no apparent correlation with effectiveness (B). Further experiments found no correlation between clinical effects and binding to either α-adrenergic or histamine receptors. (After Snyder, 1996.)

Antipsychotics have a particularly high affinity for D_2 receptors, which serve as both normal postsynaptic receptors and autoreceptors and are located in the basal ganglia, nucleus accumbens, amygdala, hippocampus, and cerebral cortex. **FIGURE 19.11** shows a series of PET images in which D_2 receptors in the basal ganglia were labeled with [^{11}C]raclopride. The bright areas show the binding of the labeled drug to D_2 receptors. The control is a scan of a healthy man injected only with the [^{11}C] raclopride to show maximum binding. The remaining scans are from patients with schizophrenia given [^{11}C] raclopride in addition to one of the antipsychotic drugs. Reduction of radioactive ligand binding indicates competition for the sites. Striatal D_2 receptors were almost completely blocked by haloperidol and risperidone, but clozapine had less affinity. Although a drug's ability to bind to the D_2 receptor is closely correlated with its effectiveness in reducing psychotic symptoms, some of the atypical antipsychotics, such as clozapine, may produce their unique effects by acting on a combination of receptor types.

In addition to reducing dopaminergic transmission by blocking postsynaptic D_2 receptors, the antipsychotics also readily block D_2 autoreceptors. The inhibitory autoreceptors are responsible for controlling the rate of firing of the cell as well as the rate of synthesis and release of neurotransmitter. Applying a DA agonist, such as apomorphine, to the DA cell bodies in the substantia nigra (origin of nigrostriatal cells) or ventral tegmentum (origin of mesolimbic and mesocortical neurons) stimulates the autoreceptors and decreases the rate of firing of the dopaminergic neurons. This inhibition is antagonized by administration of an effective antipsychotic drug such as chlorpromazine, but not by an inactive phenothiazine. The increase in firing rate after antipsychotic administration is accompanied by increased turnover (synthesis, release, and metabolism) of DA.

DA TURNOVER Clinical response to antipsychotic treatment is associated with an initial increase in dopamine metabolism, which is determined by measuring the concentration of the principal DA metabolite, HVA. An increase in metabolism is assumed to reflect an increase in neurotransmitter release. The increase in DA utilization follows the acute blockade of autoreceptors on dopaminergic cells. If the dopamine hypothesis is correct, this enhanced synaptic DA should make the symptoms worse. However, recall that postsynaptic

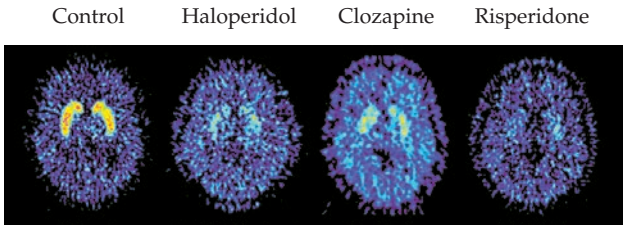


FIGURE 19.11 D_2 receptor occupancy by antipsychotic drugs PET scans of a healthy, untreated male (control) and three patients with schizophrenia treated with the classic neuroleptic haloperidol or with an atypical antipsychotic drug—clozapine or risperidone. In all individuals, striatal D_2 receptors were labeled with [^{11}C]raclopride. The scans show that the radioactive label of striatal D_2 receptors was displaced almost completely by haloperidol and risperidone binding but less effectively by clozapine. These and other differences in receptor antagonism are thought to be responsible for the ability of the clozapine-like neuroleptics to reduce symptoms without producing serious motor side effects. (Courtesy of Svante Nyberg and Anna-Lena Nordström, Karolinska Institute.)

DA receptors are also blocked, so there is no worsening of symptoms. The initial increase in HVA is followed by a gradual decrease with chronic treatment, because the chronic blockade with antipsychotics leads to supersensitivity (up-regulation) of the autoreceptors. The up-regulation once again allows them to respond appropriately to DA by reducing DA synthesis, release, and metabolism. An alternative explanation for the gradual decrease in turnover, originally posed by Grace (1992), suggests that after the initial neuroleptic-induced increase in DA turnover, dopaminergic cells have the ability to temporarily inactivate themselves. This temporary inactivation, called depolarization block, would reduce the release of DA and its subsequent metabolism. The time-dependent change in receptors and the depolarization block help to explain the gradual onset of effectiveness of antipsychotic drugs.

PROLACTIN RELEASE Further evidence for DA receptor blockade comes from neuroendocrine measures of prolactin. Under normal conditions, DA inhibits prolactin release. By blocking D_2 receptors in the pituitary gland, antipsychotics stimulate the secretion of prolactin, which leads to lactation and breast enlargement, even in males, disturbing side effects of antipsychotic drug use. Measuring serum prolactin in patients provides an easy measure of D_2 receptor function in the CNS.

SLOW HOMEOSTATIC CHANGES Although the clinical effectiveness of antipsychotics is closely correlated with dopamine receptor blockade, the time courses of the two events are significantly different. Since the receptor blockade is almost immediate, it is surprising that several weeks of treatment are necessary before symptoms begin to subside. This disparity in time course suggests that the drugs are not directly targeting the locus of the disorder but are gradually inducing the nervous system to make adaptive changes that lead to clinical improvement. Some of the slow homeostatic changes that occur over time are depolarization block, change in receptor number, and altered dopamine turnover.

Side effects are directly related to neurochemical action

Unfortunately, both traditional and atypical antipsychotic drugs frequently produce a large number of side effects, some of which are so disturbing that nonhospitalized patients stop taking the drug and suffer a relapse of psychiatric symptoms. Each drug may have different potential side effects based on the neurotransmitter receptors it binds to. Because patient compliance (cooperation in following the treatment schedule) is a large problem, clinicians most often choose the antipsychotic to prescribe on the basis of minimizing the side effects for a given patient. **TABLE 19.1** summarizes some of the benefits and side effects associated with the blockade of various receptors.

Antipsychotic-induced DA receptor antagonism occurs in each of the DA pathways described in Chapter 5 and is responsible not only for the clinical effectiveness of antipsychotics but also for many of their side effects. There are four dopamine pathways in the brain that are important for understanding antipsychotic drug action (three are illustrated in Figure 19.8A):

1. The mesolimbic pathway projects from the ventral tegmental area to the nucleus accumbens and other limbic areas. It is involved in many behaviors, as well as in the pleasure derived from drugs of abuse and the delusions and hallucinations of schizophrenia. It is reasonable to consider the mesolimbic pathway as the site for the drug-induced reduction of positive symptoms.
2. The mesocortical pathway also projects from the ventral tegmental area but sends axons to the prefrontal and limbic cortex, where it may have a role in the cognitive effects and negative symptoms of schizophrenia.
3. The nigrostriatal pathway begins in the substantia nigra and projects to the striatum, where it contributes to the modulation of movement. Parkinsonian symptoms are caused by insufficient DA binding to receptors in the striatum. Therefore, neuroleptic effects on nigrostriatal DA are likely to be responsible for Parkinsonian tremors and other motor side effects.
4. Projecting from the hypothalamus to the pituitary gland are the short neurons that constitute the

TABLE 19.1 Clinical Implications of the Blockade of Various Receptors by Antipsychotics

Receptor	Possible benefits	Possible side effects
Dopamine D_2	Reduced positive symptoms	Extrapyramidal side effects (EPS) including Parkinsonism, akathisia, tardive dyskinesia; endocrine effects such as prolactin secretion, menstrual changes, sexual dysfunction
Serotonin 5-HT_{2A}	Reduced EPS?	Sexual dysfunction
Serotonin 5-HT_{2C}	Unknown	Weight gain
Histamine H_1	Sedation	Sedation, increased appetite, weight gain, hypotension
Muscarinic cholinergic	Reduced EPS	Autonomic side effects such as blurred vision, dry mouth, constipation, urinary retention, tachycardia; memory dysfunction
α_1-adrenergic	Unknown	Orthostatic hypotension, dizziness, reflex tachycardia
α_2-adrenergic	Unknown	Drug interactions

tuberohypophyseal pathway, which regulates pituitary hormone secretion. Blockade of DA receptors in this pathway is the likely source of the neuroendocrine side effects.

PARKINSONISM The most serious and troublesome side effects of classic antipsychotics are the movement disorders that resemble the symptoms of Parkinson's disease (see Chapter 20) and involve the extrapyramidal motor system, collectively called extrapyramidal side effects (EPS). **Parkinsonian symptoms** include tremors, akinesia (slowing or loss of voluntary movement), muscle rigidity, akathisia (a strong feeling of discomfort in the legs and an inability to sit still, which compels the patient to get up and walk around), and loss of facial expression. We know that Parkinson's disease is caused by a loss of cell bodies in the substantia nigra, which gives rise to the nigrostriatal pathway (see Figure 19.8A). The lack of inhibitory dopamine function in the striatum (a subcortical brain area that modulates movement) causes excess cholinergic neural activity (**FIGURE 19.12**). Knowing that the classic antipsychotic drugs block dopamine receptors, we assume that drug-induced Parkinsonism is due to dopamine blockade in that area of the brain. To verify this hypothesis, experiments using PET showed that neuroleptic-treated patients with Parkinsonian symptoms had *more* dopamine receptors of the D_2 type in the striatum than did those without those side effects (Farde et al., 1992). Such compensatory receptor up-regulation is likely to occur after reduced dopamine transmission. Therefore, it is assumed that the antipsychotic-induced tremors are due to the blockade of dopamine receptors in the striatum, the projection region of the nigrostriatal dopaminergic neurons. Since one way to treat the symptoms of Parkinson's disease is to reduce excess acetylcholine activity, antipsychotic drugs that have anticholinergic action were developed to

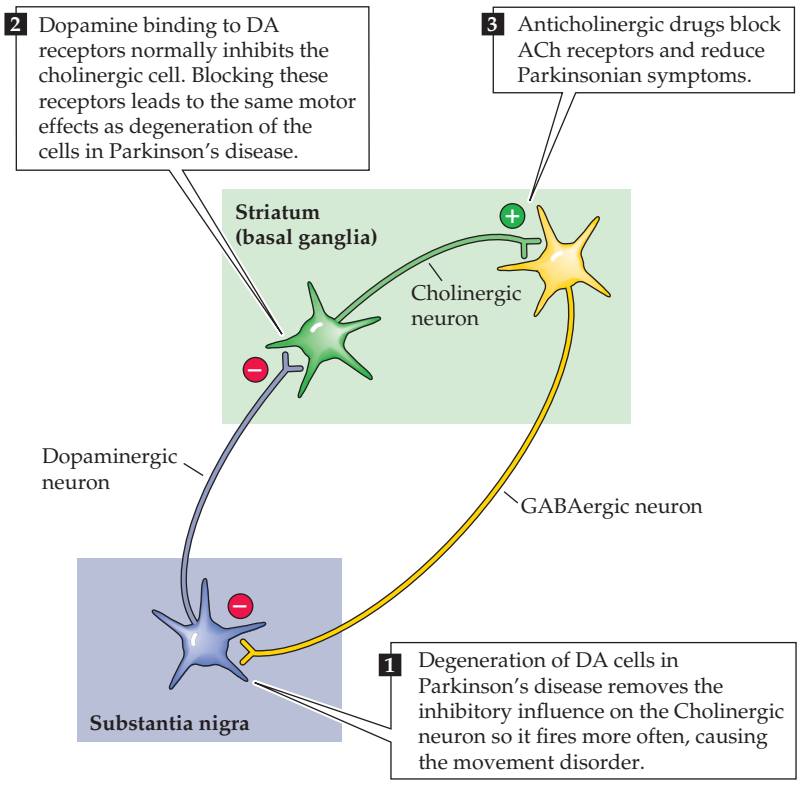


FIGURE 19.12 Schematic diagram showing the neurotransmitters involved in Parkinsonian symptoms Parkinson's disease is caused by degeneration of the nigrostriatal dopaminergic neurons, which begin in the substantia nigra. The reduced dopaminergic cell function causes a loss of inhibitory control of the cholinergic cells in the striatum, so the cholinergic cells fire at higher rates. Drug-induced Parkinsonian symptoms follow DA receptor blockade in the striatum and subsequent excess acetylcholine activity, which is functionally similar to the loss of dopaminergic cells in Parkinson's disease. Anticholinergic drugs reduce the symptoms of Parkinson's disease and the side effects of antipsychotic drug treatment.

minimize the Parkinsonian side effects. One such example is thioridazine. Alternatively, combining antipsychotic drug treatment with an anticholinergic drug such as benztropine (Cogentin) is also a common treatment approach. In addition, several of the atypical antipsychotics, such as clozapine and risperidone, produce a lower than normal incidence of extrapyramidal side effects (see the section on atypical antipsychotics below).

TARDIVE DYSKINESIA A second type of motor side effect associated with prolonged use of antipsychotic drugs is **tardive dyskinesia** (**TD**). TD is characterized by stereotyped involuntary movements, particularly of the face and jaw, such as sucking and lip smacking, lateral jaw movements, and "fly-catching" movements of the tongue. There may also be purposeless, quick, and uncontrolled movements of the arms and legs or slow squirming movements of the trunk, limbs, and neck. Estimates suggest that TD appears in about 10% to 20% of patients treated with neuroleptics overall. Although TD may appear in any age group, the incidence increases to 50% in patients older than 60 years and may exceed 70% in geriatric patients. It is generally assumed that the dose of antipsychotic and the duration of treatment are related to the occurrence of TD. To demonstrate the importance of treatment duration, **FIGURE 19.13** shows the cumulative incidence of TD in a group of 362 chronic psychiatric patients who were maintained on antipsychotic drugs. The conclusion that two out of three patients maintained on antipsychotics for a period of 25 years will develop TD is a sobering one that should encourage further research into treatment strategies that minimize such side effects. Although the symptoms are considered to be irreversible in some patients, for many individuals improvement does gradually occur. However, in many cases, the symptoms are much worse when the drug is first terminated and persist for long periods after the withdrawal of antipsychotics. Reversal of TD occurs most readily in younger patients. Despite a good deal of research with both animal and human models, the underlying neuropathology responsible for TD is not known, although Casey (2004) provides some possibilities.

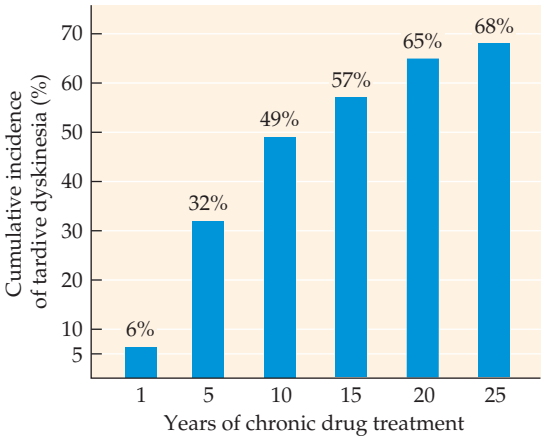


FIGURE 19.13 Cumulative incidence of tardive dyskinesia Evaluation of a group of psychiatric patients maintained on antipsychotic medication showed an average incidence of approximately 6% for each of the first 5 years, with a cumulative incidence of 32% at the end of 5 years of chronic medication. By combining data, investigators estimated the 10-year and 25-year risks. Clearly, long-term treatment increases the probability of developing tardive dyskinesia. (After Glazer et al.,1993.)

NEUROENDOCRINE EFFECTS Blockade of receptors in the dopamine pathway that regulates pituitary function produces a variety of neuroendocrine effects. These effects include breast enlargement and tenderness, decreased sex drive, lack of menstruation, increased release of prolactin (frequently producing lactation), and inhibition of growth hormone release. Reduced growth hormone release represents a significant therapeutic issue when children and adolescents are medicated. In addition, significant weight gain and the inability to regulate body temperature can be disturbing side effects, particularly for young people who are concerned with body image.

NEUROLEPTIC MALIGNANT SYNDROME Of the possible side effects, **neuroleptic malignant syndrome** (**NMS**) is the most serious and life threatening. NMS is characterized by fever, rigidity, altered consciousness, and autonomic nervous system instability (including rapid heart rate and fluctuations in blood pressure). NMS is potentially lethal, but rapid diagnosis and immediate action have significantly reduced the mortality risk.

ADDITIONAL SIDE EFFECTS Many of the older and newer antipsychotic drugs have not only dopamine-blocking effects but also anticholinergic and antiadrenergic actions. These complex interactions produce widespread effects on the autonomic nervous system. For example, blocking cholinergic synapses produces effects such as dry mouth, blurred vision, constipation, difficulty in urination, and decreased gastric secretion and motility. You may notice that although anticholinergic effects in the autonomic nervous system are problematic, anticholinergic effects in the striatum reduce Parkinsonian side effects. Orthostatic hypotension (low blood pressure when an individual stands upright) from the antiadrenergic action of antipsychotics leads to dizziness, faintness, or blacking out. Many of these drugs produce significant sedation, which may be very troublesome for some patients but useful for those who suffer from agitation and restlessness.

In general, the particular drug chosen for a patient depends on its side effects. For example,

TABLE **19.2** Relative Benefits and Risks of Atypical and Conventional Antipsychotic Drugs[a]

	Atypical antipsychotic agents						Conventional antipsychotic agents by potency[b]		
Property	Aripip-razole	Cloza-pine	Olan-zapine	Que-tiapine	Risper-idone	Zipra-sidone	High	Moderate	Low
Efficacy in terms of:									
Positive symptoms	++	++++	+++	++	+++	+++	+++	+++	+++
Negative symptoms	+	++	+	+	+	+	+	+	+
Relapse[c]	++	++++	+++	?	+++	+	++	++	++
Adverse effects:									
Anticholinergic	0	+++	+	0	0	0	0	++	+++
Hypotension	+	+++	++	++	+++	+	+	++	+++
Hyperprolactinemia	0	0	+	0	++	+	++	++	++
Diabetes mellitus	+	++	++	+	+	+	+	+	+
Sexual dysfunction	+	++	++	+	++	+	++	++	+++
Weight gain	0	+++	+++	++	+	0	0	+	++
Extrapyramidal symptoms	+	0	+	0	++	+	++++	+++	++
Neuroleptic malignant syndrome	?	+	+	+	+	+	+++	++	+

Source: From Gardner et al., 2005.

[a]Benefit or risk: ++++, very high; +++, high; ++, moderate; +, low; 0, negligible; ?, not defined.

[b]High-potency agents are flupenthixol, fluphenazine, haloperidol, and trifluoperazine; moderate-potency agents are lozapine and zuclopenthixol; low-potency are chlorpromazine, methotrimeprazine, and thioridazine.

[c]Relapse was compared with placebo after 1 year.

chlorpromazine or thioridazine may be used because they tend to minimize the extrapyramidal side effects, although their sedative effects may be undesirable and the probability of autonomic side effects is relatively high. Haloperidol, in contrast, tends to produce less sedation and fewer autonomic side effects but is associated with a greater probability of movement disorders. Many of the newer antipsychotic drugs have been developed to provide professionals with more options for matching a particular patient and the side effects that she can tolerate. **TABLE 19.2** compares a number of traditional and atypical antipsychotic drugs and rates the incidence of specific side effects for each drug.

TOLERANCE AND DEPENDENCE Clinically, the antipsychotic drugs cause little or no tolerance, physical dependence, or abuse potential (psychological dependence). Patients can take the same dose of one of these drugs for years without seeing a reduction in the effectiveness in reducing psychotic symptoms. However, some tolerance to the sedative, hypotensive, and anticholinergic effects develops gradually over a period of weeks. The lack of physical dependence is demonstrated by the absence of withdrawal symptoms following abrupt cessation of these drugs even after years of administration. The lack of abstinence syndrome may be due to the long half-life of the drugs and the prolonged presence of the drugs and their active metabolites in the body before excretion. However, abrupt termination of the drugs may unmask signs of TD.

Since the neuroleptics do not produce euphoria and have subjectively unpleasant effects, these drugs are rarely abused. Animal studies also demonstrate a low incidence of self-administration and a tendency to avoid these drugs. Despite the drugs' unpleasant nature and disagreeable side effects, the antipsychotics are not lethal and have a high therapeutic index, which makes them unlikely candidates for drug overdose.

Atypical antipsychotics are distinctive in several ways

Presently, the designation of "atypical" or "second generation" is reserved for antipsychotics that reduce positive symptoms of schizophrenia as well as the classical drugs do, but without causing significant extrapyramidal side effects. In addition, some of the newer agents fail to increase serum prolactin and have a low incidence of tardive dyskinesia. Three general approaches have been taken to develop these second-generation drugs: selective D_2 receptor antagonists, DA system stabilizers, and broad-spectrum antipsychotics.

TABLE **19.3** Relative Neurotransmitter Receptor Affinities for Selected Antipsychotics at Therapeutic Dose[a]

	Broad spectrum						Selective D_2		Stabilizer	Classical
Receptor[b]	Clozapine	Risperidone	Olanzapine	Quetiapine	Ziprasidone	Sertindole	Sulpiride	Amisulpride	Aripiprazole	Haloperidol
D_1	+	+	++	–	+	++	–	–	–	+
D_2	+	+++	++	+	+++	+++	++++	++++	++++	++++
D_3	+	++	+	–	++	++	++	++	++	+++
D_4	++	–	++	–	++	+	–	–	+	+++
5-HT_{1A}	–	–	–	–	+++	ND	ND	ND	++	–
5-HT_{1D}	–	+	–	–	+++	ND	ND	ND	+	–
5-HT_{2A}	+++	++++	+++	++	++++	++++	–	–	+++	+
5-HT_{2C}	++	++	++	–	++++	++	–	–	+	–
5-HT_6	++	–	++	–	+	ND	ND	ND	+	–
5-HT_7	++	+++	–	–	++	ND	ND	ND	++	–
α_1	+++	+++	++	+++	++	++	–	–	+	+++
α_2	+	++	+	–	–	+	–	–	+	–
H_1	+++	–	+++	++	–	+	–	–	+	–
m_1	++++	–	+++	++	–	–	–	–	–	–

Source: From Miyamoto et al., 2005.
[a]–, minimal to none; +, low; ++, moderate; +++, high; ++++, very high; ND, no data.
[b]D, dopamine; 5-HT, serotonin; α, adrenergic; H, histamine; m, muscarinic cholinergic.

SELECTIVE D_2 RECEPTOR ANTAGONISTS Since effective antipsychotic drugs block D_2 receptors, the first attempts to develop new drugs with fewer side effects evaluated **selective D_2 receptor antagonists**. Examples of such drugs include sulpiride and amisulpride. These drugs bind specifically to D_2 receptors and have a slight affinity for D_3 receptors (**TABLE 19.3**), which may explain why their behavioral effects differ to some extent from those of traditional neuroleptics. Their selectivity for DA receptors also means that effects on the autonomic nervous system and the cardiovascular system are minimal and sedation is mild. However, hormonal side effects tend to be common, and the risk of fatal blood disorders reduces the utility of the drugs.

DOPAMINE SYSTEM STABILIZERS Among the newer of the atypical antipsychotics are the **dopamine system stabilizers**. The prototypical drug aripiprazole (Abilify) is a DA partial agonist, which means that the drug readily binds to DA receptors but produces less of an effect than DA itself. Hence aripiprazole competes with DA for DA receptors in overactive synapses, reducing the effect of DA for as long as the drug is bound. When excessive DA activity is reduced, the positive symptoms are reduced. In contrast, the same drug stimulates DA receptors (but to a lesser extent than DA) in brain areas where there may be too little DA, thus in principle reducing negative symptoms. In clinical trials, the drug had a relatively low incidence of side effects (see Table 19.2). There was little evidence of cardiotoxicity, weight gain, or motor side effects. Reported adverse effects such as headache, agitation, insomnia, and nervousness were minor. Aripiprazole may represent a new class of antipsychotics that is more readily accepted because of fewer unpleasant side effects.

BROAD-SPECTRUM ANTIPSYCHOTICS A second trend in neuropharmacology is to evaluate **broad-spectrum antipsychotics** that block other receptor types in addition to D_2 receptors. The rationale for this work is the clinical effectiveness of clozapine, a drug that has relatively weak affinities for D_1 and D_2 and substantial serotonergic, muscarinic, and histaminergic affinities, as well a high affinity for the D_4 receptor (see Table 19.3). Clozapine is the best-known atypical antipsychotic. Although clozapine is no more effective than standard neuroleptics in treating the positive symptoms of schizophrenia, it is often effective in patients who are treatment resistant. Clozapine produces significant improvement in 60% of patients who do not respond to typical neuroleptics. Clozapine is also the first antipsychotic that can reduce some of the negative and cognitive symptoms as well as reduce anxiety and tension. Unfortunately, the drug has a wide variety of side effects because of its action on multiple receptors. Clozapine reduces the seizure threshold, making seizures more likely in the vulnerable individual. It can also produce hypersalivation, weight gain, and

cardiovascular problems. A more dangerous side effect is the occurrence of agranulocytosis, a serious blood abnormality that can be detected only with frequent (i.e., weekly or biweekly) blood screening tests. The increased expense of testing and the seriousness of side effects restrict the use of clozapine to selected patients.

The initial discovery of the effectiveness of clozapine led to a great deal of excitement and efforts to design a new class of antipsychotics with similar efficacy but without dangerous side effects. These atypical drugs, which include risperidone, olanzapine, quetiapine, ziprasidone, and sertindole, bind with varying affinities for multiple neurotransmitter receptor subtypes. Table 19.3 allows you to compare binding affinities of these agents with those of the selective D_2 antagonists, the DA system stabilizer aripiprazole, and the classic drug haloperidol. Keep in mind that these atypical drugs are a heterogeneous group neurochemically and in clinical profiles, making it difficult to allow broad generalizations about the class, but you can predict side effects on the basis of receptor affinity (refer to Table 19.1). You can compare clinical benefits and risks of these drugs by examining Table 19.2. Although they all reduce positive symptoms better than placebo, there is little evidence to suggest *superiority* of the atypical drugs over conventional drugs. Keep in mind that clinical studies are difficult to perform and that variability in results may be explained by differences in sampling of patient populations, durations of treatment, high dropout rates, inappropriate dose comparisons, and small outcome measures that may be statistically significant but not significant to the patient (Gardner et al., 2005). Superiority of the newer agents in treating negative symptoms is even more difficult to demonstrate, because evaluating behavior such as social and emotional withdrawal and lack of motivation is problematic with the usual measuring devices. Although a number of clinical studies and meta-analyses suggest that cognitive deficits such as verbal memory, attention, psychomotor processing, and verbal fluency frequently show improvement with some atypical drugs such as risperidone compared with haloperidol, other studies and meta-analyses report no difference.

Most significant for atypical status is the low incidence of motor side effects (called extrapyramidal side effects or EPS) described earlier. Clozapine does not appear to cause EPS even at high doses, and reports of TD are uncommon. Although all of the newer drugs are generally considered less likely to cause EPS, keep in mind that the occurrence of motor side effects varies significantly among the atypical agents (see Table 19.2). Furthermore, the occurrence of EPS is frequently dose-dependent, which means that a drug may act as an atypical antipsychotic at low doses but become more conventional at higher doses. A prominent endocrine side effect with the classical antipsychotics is hyperprolactinemia and associated sexual dysfunction. Several of the newer agents, including quetiapine and the dopamine system stabilizer aripiprazole, lack this effect entirely, although some of the newer drugs such as risperidone elevate prolactin to the same moderate level as the older agents. Finally, some cases of neuroleptic malignant syndrome have been reported for the atypical drugs.

Metabolic side effects including weight gain, hyperglycemia, and elevated plasma cholesterol are troublesome characteristics for at least some of the atypical antipsychotics, although these side effects also occur with some of the older drugs. Significant weight gain is common with atypical antipsychotics and with some classical agents and is most robustly correlated with the extent of histamine H_1 receptor binding, although 5-HT_{2C} receptor antagonism may also contribute to this effect. Adolescents tend to gain even more weight than adults. Clozapine and olanzapine seem to cause the most weight gain, probably through suppression of the satiety response, and the most prolonged weight gain over time. Clozapine-induced weight gain was also most persistent despite efforts to behaviorally reverse the gain with diet and exercise. Some of the atypical antipsychotics cause sometimes severe elevations in blood sugar and insulin resistance, leading to type 2 diabetes. The risk of developing diabetes is 9% to 14% greater in patients taking atypical antipsychotics than in those taking the first-generation drugs. The elevated plasma lipids caused by some of the drugs increases risk for high blood pressure and heart disease. Metabolic syndrome side effects are reviewed by Yogaratnam and colleagues (2013).

Several traditional and second-generation drugs cause alterations in the electrical activity of the heart in some individuals. If severe enough, these arrhythmias can cause sudden death. Several antipsychotics have been removed from the market because of this side effect. Because of these cardiac changes, off-label use (i.e., for a condition not approved by the Food and Drug Administration [FDA]) for the treatment of behavioral disorders in the elderly with dementia has been evaluated. The FDA reported a twofold increase in sudden death in this population caused by cerebral ischemia, stroke, cerebrovascular events, or infection. Subsequent evaluation suggests as much as a tenfold increased risk for sudden death in elderly patients taking the drug for 1 week, which gradually decreases to control levels after 3 months. Nevertheless, the FDA (2008) has recommended a boxed warning on drug labels describing the potential risks. As is always the case, the potential risks and benefits along with the costs must be weighed for each patient.

Although there is general agreement that D_2 receptors are important in antipsychotic effects for all antipsychotics, we have to wonder what is different about clozapine that explains its efficacy for reducing negative symptoms and its low potential for EPS. Some

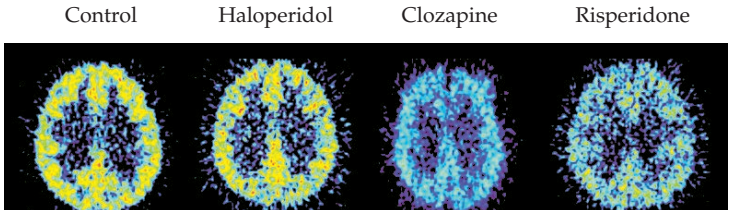


FIGURE 19.14 5-HT$_2$ receptor binding by atypical antipsychotics PET scans of a healthy, untreated male (control) and three patients with schizophrenia treated with the traditional neuroleptic haloperidol or with an atypical antipsychotic drug—clozapine or risperidone. In all individuals, neocortical 5-HT$_2$ receptors were labeled with [^{11}C]*N*-methylspiperone ([^{11}C]NMSP). The scans show that both atypical antipsychotics but not haloperidol reduced 5-HT$_2$ binding. These and other differences in receptor antagonism are thought to be responsible for the ability of clozapine to reduce schizophrenic symptoms (negative as well as positive) with a minimum of motor side effects. (Courtesy of Svante Nyberg and Anna-Lena Nordström, Karolinska Institute.)

laboratories hypothesize that typical and atypical neuroleptics can be differentiated by their ratio of antagonism for various receptors. For instance, a high degree of binding to D_4 receptors, found in high concentration in the mesolimbic system and the frontal cortex, may enhance the therapeutic effect but minimize the nigrostriatal motor symptoms associated with D_2 occupation. Other evidence suggests the importance of antagonism of the 5-HT$_2$ receptor in combination with D_2 blockade. **FIGURE 19.14** shows that the atypical antipsychotics clozapine and risperidone readily bind to 5-HT$_2$ receptors, but haloperidol does not. In contrast, haloperidol almost completely blocks D_2 receptors (see Figure 19.11), but the clozapine shows only a partial effect on D_2. Because 5-HT$_2$ receptors modulate DA release, it has been suggested that this difference in receptor antagonism is responsible for increasing DA efflux in cortex and hippocampus, potentially improving cognitive symptoms while producing less DA release in striatum, which would be expected to reduce EPS. For this reason, extensive research is being conducted into the potential enhancement of cognitive function by modulating 5-HT at the 5-HT$_{2A}$ and several other 5-HT receptor subtypes (Meltzer and Massey, 2011). In contrast, other researchers believe that atypicality can be explained solely by low D_2 receptor binding affinity, regardless of any other receptor type function (Seeman, 2002). Receptor affinity (K_d) is the ratio of how readily the drug moves off the receptor compared with how readily it binds (see Chapter 4 for radioligand binding). Hence, a drug with low affinity could be one that binds slowly or that after binding moves off the receptor rapidly. Most antipsychotic drugs bind at similar rates, but there are large differences in how quickly they unbind. For example, clozapine binds to and unbinds from the D_2 receptor 100 times faster than haloperidol. Low affinity means that the drug is not constantly bound to the receptor, and when it is not bound, normal DA transmission can occur. This characteristic might prevent EPS, maintain prolactin levels, and protect cognitive function.

Practical clinical trials help clinicians make decisions about drugs

Since their inception, the second-generation antipsychotics have been the first line of treatment and have been prescribed in about 90% of cases, despite no conclusive evidence that they are superior to the first-generation drugs. In many instances, the clinical effects seen in the "real-world" applications have been less robust than the results from randomized, placebo-controlled trials performed as part of drug development. There can be several reasons for this disparity, such as differences in the duration of drug treatment. Others have suggested that the drug development trials are biased toward the atypical agents because they use as the comparator a high-potency classical drug like haloperidol, which is known to have significant EPS. Bias would also occur when first-generation drugs are used at higher than necessary doses, which increases the potential for side effects. Often an older drug is used to compare outcomes without the usual coadministration of an anticholinergic agent that would reduce the appearance of EPS. At other times, the outcome measures, although statistically significant, are insufficient to make the patient himself feel better. Finally, well-controlled trials, by necessity, exclude patients with comorbid medical or psychiatric conditions, although these excluded individuals are more typical of the usual schizophrenic patient in the real world. Furthermore, drug development trials generally select a carefully defined and limited subset of patients who do not reflect the heterogeneous schizophrenic population. For these reasons, drug trial results comparing antipsychotic drugs may not be generalizable to the larger population. Because the newer drugs generally cost about 10 times what older generic agents cost, cost–benefit considerations are important in clinical decisions.

For all these reasons, the National Institute of Mental Health (NIMH) between January 2001 and December 2004 initiated the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE), a blinded and controlled study, to compare effectiveness, tolerability, and cost-effectiveness of treatments. These variables were determined by measuring the duration of use before discontinuation of treatment by a patient (Lieberman et al., 2005). Discontinuation of treatment (i.e., switching to another drug) was chosen as a critical measure because it combined several factors, including *both* the patients' and the clinicians' judgments of effectiveness, safety, and side effects, into an overall measure of effectiveness that balances benefits and undesirable effects. Secondary measures of outcome included the specific reasons for discontinuation of treatment and scores on

two symptom scales. Additional secondary measures of safety and tolerability were made every 3 months for 18 months. These measures included the incidence of serious adverse effects, the incidence of serious neurological effects, and changes in body weight, electrocardiographic findings, and lab blood analyses. At 57 sites in a variety of treatment settings in the United States, almost 1500 patients were recruited and randomly assigned to receive the classic antipsychotic perphenazine or one of the atypical agents, olanzapine, quetiapine, risperidone, or ziprasidone. The goal of the study was to replicate the "real-world" prescription of these drugs to patients who are more representative of the typical outpatient population, without the normally extensive exclusion criteria required in clinical studies. Hence the results of this practical clinical trial are intended to serve as a guide for clinical practice and to allow patients to make more informed decisions.

The biggest surprise of the study results, counter to the hypothesis, was that the newer drugs were no more effective than the first-generation drug perphenazine. Overall rates of discontinuation (74%) were high, which means that the majority of patients had to switch to another drug either because of lack of effectiveness or because they could not tolerate the side effects. Among all the drugs, olanzapine had the longest duration before discontinuation and produced the greatest improvement initially, although this advantage decreased over the 18 months. Also, olanzapine showed the highest discontinuation rate as the result of adverse side effects, particularly weight gain and metabolic effects. To the researchers' surprise, there were no differences among the drugs in terms of effectiveness for reducing negative symptoms or cognitive deficits. Further, the incidence of EPS was the same for all agents, and this may be attributed to the low but effective dose of perphenazine used. This study was important because it showed that drug effectiveness is quite different under conditions similar to routine clinical practice compared with tightly controlled trials. The database has been used in subsequent studies looking at other factors, including cost-effectiveness, quality of life, and patient characteristics that predict response (see Lieberman and Stroup, 2011, for a list of references). Subsequent naturalistic research, such as the Cost Utility of the Latest Antipsychotic Drugs in Schizophrenia Study (CUtLASS) performed in the United Kingdom, supported the findings from CATIE and found no difference among the first- and second-generation drugs, except that clozapine was superior (see Lewis and Lieberman, 2008, for a brief review). It would seem that only clozapine is truly "atypical." These findings should encourage the use of older first-generation drugs, which are effective at a significantly lower cost. For the patient, this means more treatment options are available to optimize benefits and side effects for the individual.

There are renewed efforts to treat the cognitive symptoms

It is quite clear that the cognitive symptoms along with the negative symptoms represent aspects of the disorder that are the most damaging to the quality of life of the schizophrenic individual. These symptoms prevent integration into the community and reduce the probability of functioning in a productive manner. Since neither the first- nor second-generation antipsychotics improve the cognitive impairments of schizophrenia, several new pharmacological approaches are being considered (Buchanan et al., 2007).

ACETYLCHOLINE Because acetylcholine (ACh) has a role in attention, sensory processing, and several aspects of memory, enhancing ACh is one reasonable approach to treating cognitive symptoms. In addition, the fact that the rate of cigarette smoking is extremely high among individuals with schizophrenia (58% to 88% compared with about 17% in the general population) has led some to suggest that nicotine use may be a form of self-medication (Boggs et al., 2014). Nicotine binds to and activates nicotinic cholinergic receptors and has been found to enhance performance in cognitive tests (see Chapter 13). Furthermore, ACh is an appropriate target because postmortem and imaging studies have shown cholinergic deficits in patients with schizophrenia. For example, reduced numbers of ACh receptors have been reported in the DLPFC and hippocampus, brain areas critical to cognitive function. It is interesting that clozapine, the only antipsychotic drug currently available that enhances cognitive deficits, increases the release of ACh in the hippocampus and DA in the PFC. Acute administration of nicotine or subtype-selective nicotinic agonists for the α7 nicotinic acetylcholine receptors (α7 nAChRs; see Chapter 7 for a description of ACh receptors and their subtypes) produces some limited cognitive improvement, particularly enhancing selective attention. Several subtype-specific nicotinic partial agonists were found to normalize auditory gating deficits in rodents, as well as in early trials with patients with schizophrenia. At least part of the problem in developing drugs for enhancing cholinergic function is that the receptors undergo desensitization (see Chapter 7). One way around that problem is to develop drugs that bind to sites on the receptor other than the ACh binding site and enhance agonist function in that way. These types of drugs are called positive allosteric modulators (PAMs). One recent preclinical study of interest tested several PAMs of the α7 nAChRs on ketamine-induced cognitive deficits (Nikiforuk et al., 2016). The study found that the drugs reversed ketamine-induced cognitive inflexibility in the attentional set-shifting task, and in fact, the animals performed somewhat better than controls. The PAMs also enhanced performance in the novel object recognition task, in which the animals demonstrated that they could remember, after treatment with either of

two PAMs, which object was familiar and which was novel (**FIGURE 19.15**). Interestingly, earlier these same researchers had shown that the two PAMs could also improve *natural* (not drug-induced) forgetting, an effect that was dependent on the α7 nAChR. Finally, they also found that the social withdrawal induced by ketamine was reversed by the PAMs. Hence these PAMs improved cognitive and social behaviors in the ketamine model of schizophrenia and suggest further study as a potential therapeutic approach.

Since enhancing ACh functioning by inhibiting its synaptic breakdown by acetylcholinesterase has been used to enhance cognitive function in patients with Alzheimer's disease, it has potential for treating that dysfunction in schizophrenia. Several studies found some improvement in attention, verbal memory, and executive function, but the effects were so small that they are unlikely to have clinical utility. See Boggs et al. (2014) for a review of efforts to use cholinergic agents to enhance cognitive function in schizophrenia.

DOPAMINE In an earlier section, you saw that negative symptoms and cognitive deficits including hypofrontality are associated with reduced DA function in PFC, particularly at D_1 receptors, which are the most common receptor subtype in that region. It is well known that increasing synaptic DA with amphetamine improves cognitive deficits in patients with schizophrenia; however, since the positive symptoms are due to excessive DA release from mesolimbic neurons, those symptoms worsen. The goal then is to selectively enhance D_1 receptor signaling in PFC with D_1 agonists. The first agent, dihydrexidine, was found to reverse cognitive performance deficits in aged primates and in other animals after neurotoxin-induced lesions of DA cells. In a small number of patients with schizophrenia, the drug increased blood flow in prefrontal regions but unfortunately failed to improve task performance in delayed recall or working memory. Recent research showed that very high plasma concentrations are needed to reach detectable D_1 receptor occupancy of the brain. Because of poor bioavailability of the drug, insufficient dosage may in part explain the failure to improve cognitive function (see Arnsten et al., 2017).

A second approach to enhancing PFC DA function is to inhibit the enzyme catechol-*O*-methyltransferase (COMT), which degrades DA in the synapse, ending its signaling. Inhibition of the enzyme with a drug such as tolcapone causes a relatively selective increase of DA in PFC because there are few reuptake transporters in that region; hence, metabolism is the principal way in which synaptic DA in the PFC is inactivated. In COMT knockout mice, DA levels are increased in PFC but not in striatum, and performance on memory tasks is improved. In patients with advanced Parkinson's disease, tolcapone improved performance on attentional tasks, verbal short-term memory, and visuospatial recall. Others found tolcapone-induced improvement in executive function and verbal episodic memory in healthy volunteers that was accompanied by improvement in information processing in PFC, as visualized with functional MRI (fMRI). Given that COMT polymorphisms influence frontal lobe function and performance on tasks of working memory, the genetic variation may represent a predictive marker for effective pharmacotherapy. Unfortunately, tolcapone can produce serious liver dysfunction, which requires frequent liver enzyme testing; it has already been withdrawn from the market in Canada and Europe. Gupta and colleagues (2011) provide a review of COMT function and its significance as a therapeutic target for cognitive disorders.

GLUTAMATE In a previous section, you learned that glutamate antagonists such as PCP and ketamine produce behaviors that resemble the positive, negative, and cognitive symptoms of schizophrenia. Figure 19.8

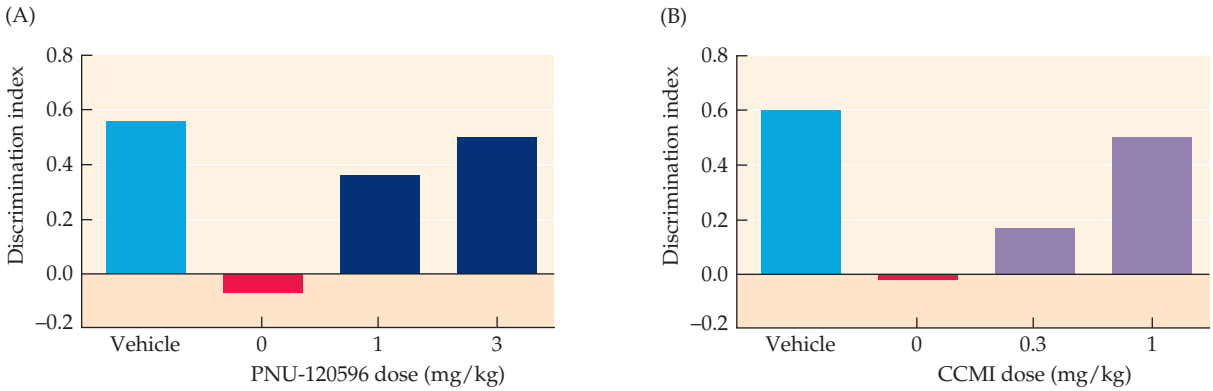


FIGURE 19.15 Effects of PAMs on novel object recognition The discrimination index represents the animals' ability to remember an object that was presented on day 1 and to spend more time investigating a novel object when both the old and new object are presented on day 2. Both bar graphs show that 20 mg/kg of ketamine (red) dramatically reduced the cognitive function in the absence of either PAM. As PAM (PNU-120596 and CCMI) dosages increased, the ability to discriminate increased to approximately control levels. (After Nikiforuk et al., 2016.)

showed that low glutamate signaling could explain the decrease in mesocortical activity that is believed to cause negative and cognitive symptoms. On the basis of this notion, enhancing glutamate activity at NMDA receptors might reverse these symptoms. Although administering an NMDA receptor agonist seems reasonable, it is not possible because these drugs produce neuronal hyperexcitability and seizures. Instead, glycine site agonists can be used to enhance NMDA signaling because glycine is an obligatory co-agonist at this receptor and is as necessary as glutamate for receptor activation (see Chapter 8). The glycine receptor agonists glycine, D-cycloserine, and D-serine, when combined with antipsychotics, reduced negative and cognitive symptoms in some but not all clinical trials. Despite the frustration of inconsistent results, there are many potential methodological explanations to explore. For instance, studies vary in drug dosage, duration of trials, outcome measures, differences in which classic or second-generation antipsychotics are used adjunctively, and even whether those studied were outpatient or inpatients (the latter have assured compliance with the drug regimen). Despite the difficulties, a meta-analysis of double-blind, placebo-controlled studies looked at the efficacy of the NMDA enhancers on multiple symptom domains, dose–response relationships, the effects of concomitant antipsychotics (both conventional and second-generation), and side effects in patients with schizophrenia. Using 800 patients from 26 studies, the meta-analysis found that these glycine modulatory site agonists were effective in most symptom domains, including depressive, negative, cognitive, positive, and general psychopathology. Glycine and D-serine were overall more effective than D-cycloserine, which may be explained because glycine and D-serine are full agonists at the glycine modulatory site while D-cycloserine is a partial agonist that would not maximally activate the receptor. Since no trials reported significant side effects, these agents appear to be safe and effective.

An alternative way to increase synaptic glycine is to administer an inhibitor of the glycine transporter that moves synaptic glycine from the synapse to neurons and glial cells. A recent clinical trial administering the glycine transporter inhibitor sarcosine, along with atypical antipsychotics, shows that sarcosine has greater efficacy than the glycine agonist D-serine in overall symptom reduction, reduction of negative symptoms, and improvement in quality-of-life ratings of social activity, sense of purpose, motivation, anhedonia, capacity for empathy, aimless inactivity, and emotional interaction (Lane et al., 2010). Both were superior to placebo. Meanwhile other approaches for enhancing NMDA function are being studied. Since D-serine appears to be effective in treatment, enhancing the function of its synthesizing enzyme, serine racemase, could elevate endogenous levels of D-serine. Using the same logic, inhibiting the metabolism of D-serine by D-amino acid oxidase could increase endogenous D-serine and have potential antipsychotic effects. Only with time will we know whether NMDA enhancement lives up to its promise.

ANTI-INFLAMMATORY DRUGS Earlier we discussed the potential role that fetal inflammation may play in the neurodevelopmental pathogenesis of schizophrenia. If brain inflammation leads to schizophrenic symptoms, then it is possible that anti-inflammatory drugs may be a useful therapeutic approach. A wide variety of anti-inflammatory drugs have been tested to varying extents, including aspirin, celecoxib, polyunsaturated fatty acids, minocycline, and others. One of the more interesting is minocycline, an antibiotic that reduces inflammation and also reduces microglia activation, so fewer pro-inflammatory cytokines are released. It also has neurotrophic, antioxidant, and antiapoptotic properties. Animal studies have shown minocycline provides neuroprotection following ischemic stroke or glutamate-induced excitotoxicity and in animal models of neurodegenerative diseases such as Huntington's disease and Parkinson's disease, as well as in animal models of schizophrenia. In one study Monte and colleagues (2013) found that minocycline both *prevented* and reversed ketamine-induced impairment of PPI and hyperlocomotion (models of positive symptoms) as effectively as the antipsychotic risperidone. The anti-inflammatory also prevented and reversed ketamine-induced social withdrawal (negative symptom) and impaired Y-maze performance (cognitive symptom), neither of which were improved by risperidone. Their results encouraged the further study of minocycline as a novel approach to schizophrenia therapy. As is frequently the case, clinical trials with patients with schizophrenia have produced mixed results. Multiple reports suggest that minocycline, along with antipsychotic drugs, improves negative symptoms and general outcome along with executive functioning and working memory. Others found it to be ineffective. There is some indication that anti-inflammatory drugs may be helpful primarily for early-phase schizophrenia or for patients with high levels of CRP, a protein that increases in the blood during inflammation. Perhaps more subgroups of patients who respond to particular anti-inflammatories will be identified. As is always the case, risk–benefit ratios must be considered, since many of the anti-inflammatory drugs have the potential for serious consequences. In the case of minocycline, because of potential damaging effects it is not likely to be the first choice for adjunctive use (Fond et al., 2014). For a review of minocycline and its potential in the treatment of schizophrenia, see Zhang and Zhao (2014).

Another potential therapeutic approach, based on the animal studies described earlier, is to use

immunotherapy that targets specific cytokines such as IL-6 with antibodies that reduce the IL-6 induction of inflammatory cascades. Although there is an FDA-approved anti–IL-6 receptor antibody (tocilizumab) used to treat rheumatoid arthritis, it has not yet been tested in patients with schizophrenia. Preliminary testing involves measuring cytokine levels before and after treatment to determine the relationship to treatment outcomes. Additionally it may be ascertained whether this immunotargeting is overall effective or limited to only a subset of patients, for example, those with high baseline cytokine levels. If subsets are identified based on individual physiological function, we are one step closer to personalized medicine. Another key issue to be investigated is the interaction with antipsychotic drugs, the effects of which might be augmented or diminished. An additional question posed is about what time in the course of the disease process immunotherapy is effective. Immunotherapy and other treatments based on the cytokine findings in schizophrenia provide one more potential approach to therapy and are described by Girgis and colleagues (2014).

NAVIGATE COMBINED TREATMENT APPROACH Thus far our emphasis has been on pharmacotherapies for schizophrenia, but a new initiative based on earlier European designs has refocused care for first-episode patients on a comprehensive multimodal, multidisciplinary, team-based approach. The program called NAVIGATE involves several components: education of the family to increase their understanding of the disease and relapse prevention; supported employment and education to help patients decide on appropriate classes or job opportunities that are most suitable for them based on their symptoms; individual resiliency training provided by one-on-one talk therapy intended to help the patients reduce substance use, build social relationships, and cope with symptoms such as suicidal thoughts as well as positive symptoms; and individualized low-dose medication treatment to minimize side effects and enhance compliance. Every effort is made to include the family and the patient in the decision-making processes and help them navigate through the complexities of the mental health care system. Details are beyond the scope of this chapter, but Mueser and colleagues (2015) summarize the program, team staffing, and conceptual foundations. Large controlled studies have compared outcomes of patients getting standard drug-focused community care with those in NAVIGATE. After 2 years patients in the comprehensive program showed enhanced quality-of-life scores, motivation, social interactions, sense of purpose, and engagement in regular activities. Improvements were greatest for those treated earliest after the onset of psychosis. In most cases drug dosages were 20% to 50% lower than standard, leading to fewer side effects. Because of lower drug costs with generic drugs and, in some cases, reduced relapse and hospitalization and increased productivity and quality of life, NAVIGATE can be considered a cost-effective treatment approach. In 2014, Congress awarded $25 million in block grants to states to fund NAVIGATE combined treatment services. In the near future, the team approach may be supported by a mobile phone health app designed by University of Washington professor Dror Ben-Zeev. The app provides a list of options, for example, "medication," "voices," or "social." The individual selects the category she wants help with and provides some input before getting some helpful advice or encouragement to deal with the current situation. This therapeutic approach gives individuals with schizophrenia more control and a greater role in their own treatment.

Section Summary

- All antipsychotics are significantly better than placebo in reducing positive symptoms and decreasing length of hospitalization.
- The law of thirds says one-third of patients treated with antipsychotics improve dramatically and return to normal lives. A second third show some improvement but experience relapses and need help with day-to-day living. The final third show little improvement and have significant periods of hospitalization.
- Prolonged maintenance therapy doubles the odds of avoiding relapse.
- There is a strong positive correlation between antipsychotic binding to D_2 receptors and clinical effectiveness.
- Antipsychotic blocking of D_2 autoreceptors causes an initial increase in DA neuron firing and increased turnover of DA, followed by a gradual decrease as the autoreceptors up-regulate. Depolarization block may contribute to the decrease in turnover. These adaptive changes may explain the gradual onset of effectiveness.
- Parkinsonian symptoms are the most troubling side effects with traditional antipsychotic treatment. Combining the drugs with anticholinergic agents reduces risk. Second-generation drugs have a lower incidence of motor side effects.
- Tardive dyskinesia involves involuntary movement of face, jaw, tongue, neck, or extremities, which may be irreversible in some patients.
- Neuroendocrine side effects are caused by DA receptor blockade of tuberohypophyseal neurons that project to the pituitary.
- Neuroleptic malignant syndrome is a potentially life-threatening effect of antipsychotics.

- Anticholinergic effects of antipsychotics produce widespread effects on autonomic nervous system function.
- Antipsychotics cause little tolerance or physical dependence and have no addiction potential.
- Atypical antipsychotics may be selective D_2 antagonists, DA partial agonists (DA system stabilizers), or broad-spectrum antipsychotics.
- The atypical antipsychotics are heterogeneous neurochemically and clinically but overall cause fewer motor side effects than the older drugs.
- Several of the atypical antipsychotics are especially problematic in causing weight gain, hyperglycemia, cardiotoxicity, elevated cholesterol, and increased risk for diabetes.
- Only clozapine improves negative and cognitive symptoms without motor side effects and is often effective for treatment-resistant patients. Serious side effects limit its use.
- The neurochemical property that makes clozapine unique is not known. It may be high affinity for D_4 receptors, antagonism of 5-HT_2 receptors, or low D_2 affinity.
- A practical clinical trial (CATIE) using 1500 patients under "real-world" conditions showed that the atypical antipsychotics were no more effective than the traditional agents in reducing positive, negative, or cognitive symptoms. Occurrence of EPS was the same for both. Only clozapine is superior.
- Nicotinic partial agonists, D_1 agonists, COMT inhibitors, glycine agonists, glycine transporter inhibitors, anti-inflammatories, and combined drug with behavioral intervention programs are being investigated as methods to improve cognitive processing and negative symptoms.

STUDY QUESTIONS

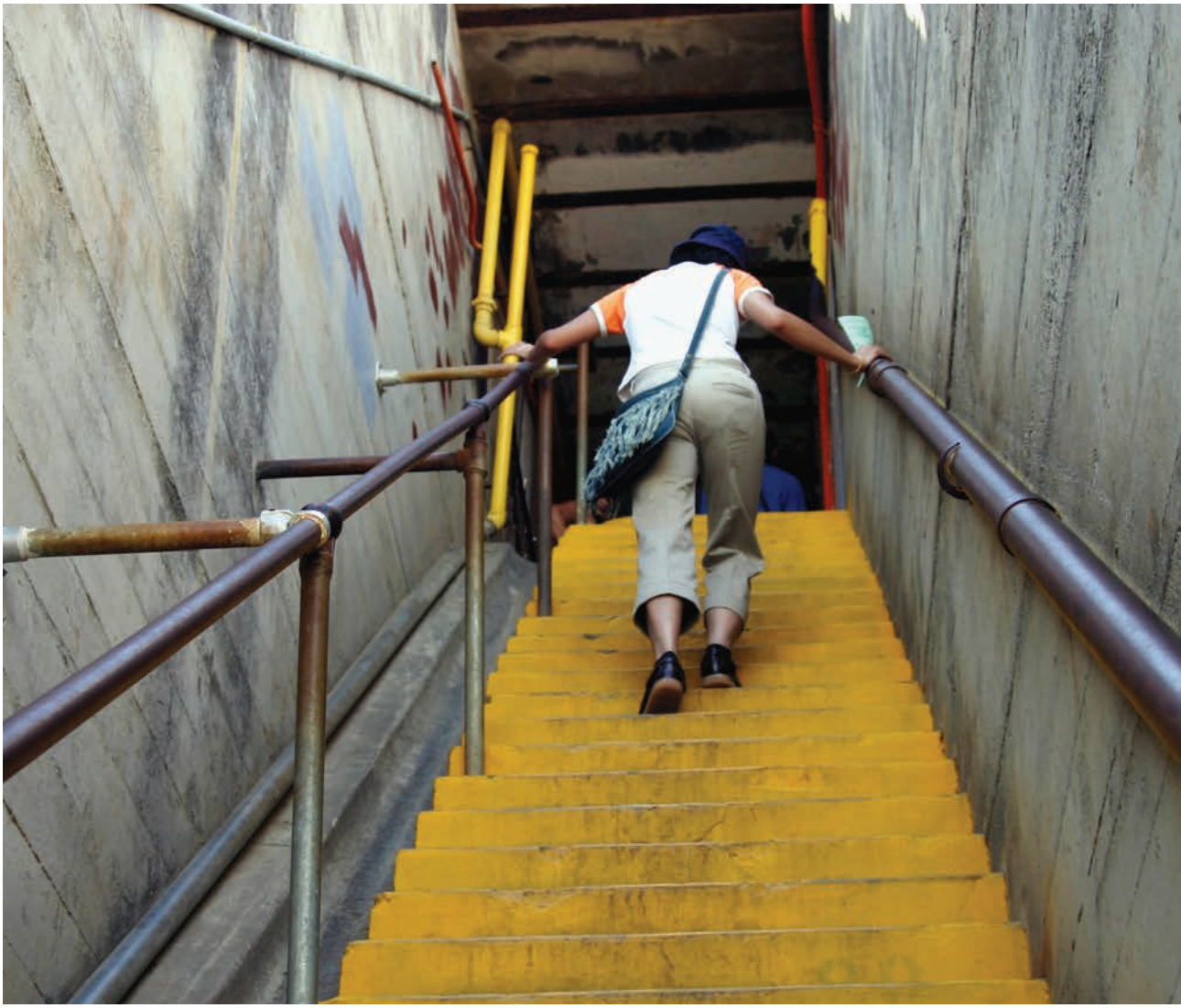
1. Describe the symptoms of schizophrenia. What makes it difficult to diagnose? What are the positive, negative, and cognitive symptoms?
2. What are some of the brain structure abnormalities found in patients with schizophrenia? Functional abnormalities? Immune system dysfunctions?
3. Provide evidence for the hereditary nature of schizophrenia. Why do researchers believe the candidate gene *DISC1* contributes to the risk for developing schizophrenia?
4. What evidence exists to suggest there are developmental errors early in the life of a patient? At adolescence? How is that evidence incorporated into the "two-hit" model?
5. Describe each of the following animal models: amphetamine-induced stereotypy, hypoglutamate model, vacuous chewing movements test, attentional set shifting, prepulse inhibition of startle, prenatal inflammation, and genetic models.
6. Summarize the dopamine hypothesis of schizophrenia, and provide several pieces of evidence.
7. How does the neurodevelopmental model attempt to explain the positive and negative symptoms?
8. How does glutamate regulate mesocortical and mesolimbic neurons? What seems to be responsible for the hypoglutamate function?
9. Discuss the importance of D_2 receptor antagonism in antipsychotic drug action.
10. Describe the four DA pathways, and tell how each is responsible for the effectiveness and side effects of antipsychotics.
11. What are Parkinsonian side effects? How are they treated?
12. What is tardive dyskinesia? Why is this side effect especially troubling?
13. List side effects of antipsychotics and their neurochemical basis.
14. All the antipsychotics block D_2 receptors except aripiprazole. Describe its mechanism of action.
15. What is the principal benefit of the "atypical" antipsychotics? Describe the three general approaches used to develop those drugs.
16. What are the benefits and risks of the broad-spectrum antipsychotic clozapine?
17. Discuss the metabolic side effects and cardiac arrhythmias caused by some of the broad-spectrum antipsychotics.
18. What are the possible neurochemical explanations for the atypical effects of clozapine?

STUDY QUESTIONS *(continued)*

19. Why are "real-world" clinical trials important, and how do they differ from the usual well-controlled drug development trials? What did the CATIE study demonstrate?
20. In what three ways have researchers attempted to enhance cholinergic function to treat cognitive symptoms of schizophrenia?
21. Describe the two approaches for enhancing PFC DA function.
22. Why are glycine modulatory site agonists, glycine transporter inhibitors, and serine racemase enhancers considered potential therapeutic adjuncts to antipsychotic drugs? How do they work?
23. Describe the therapeutic potential of anti-inflammatory drugs.
24. What is the treatment approach called NAVIGATE, and what are its benefits?

Go to the *Psychopharmacology* Companion Website at oup-arc.com/access/meyer-3e for animations, web boxes, flashcards, and other study aids.

CHAPTER 20



Exercise can help alleviate some of the symptoms of Parkinson's disease. (© iStock.com/juliardi.)

Neurodegenerative Diseases

WHILE RETURNING FROM A VISIT WITH FRIENDS, 54-year-old Ms. S. noticed a familiar sensation, a creeping heaviness throughout her body. As she parked her car at her apartment complex, she found herself "frozen" in the driver's seat. She called a friend for help getting out of her car and then pondered how to get her unmoving muscles to carry her to her third-floor apartment. Her solution? To sing her way upstairs. Singing her favorite song allowed her muscles to move with the beat of the music. With her friend helping by opening her apartment door, Ms. S. was able to get into her apartment and collapse on her couch before freezing again.

Ms. S. is suffering from a deficiency of dopamine signaling in her brain. She has Parkinson's disease. The creeping heaviness that she experiences signals the beginning of an "off period," that is, the wearing off of her dopamine-enhancing medication. As the medication leaves her system, she has difficulty with limb movement (as for many with Parkinson's, this is an asymmetrical effect; for Ms. S., it happens more on the right side), her muscles become stiff, and she has difficulty lifting her feet (this results in a shuffling gait). She has a tendency to lean forward and has a corresponding anterior lean of her head. With medication, her body feels lighter, and movement feels normal and natural; her motivation to move is automatically translated to the movement. There is a noticeable change in her thinking when the medication takes effect. Limitations in movement as her Parkinson's disease progressed caused Ms. S. to give up her career as a chiropractor, but she is still able to swim for 30 minutes three times a week.

Parkinson's disease is one of several disorders in which the primary pathological mechanism is loss of cells in the nervous system. Other disorders that are classified this way and discussed in this chapter are Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis, and multiple sclerosis. ■

Parkinson's Disease and Alzheimer's Disease

Parkinson's Disease

Parkinson's disease (**PD**), the disorder suffered by Ms. S. in the chapter opener, is a chronic, progressive, neurodegenerative disorder (Davie, 2008). The symptoms of the disorder are not reversible and get worse as the degeneration of neurons progresses. Although there are genetic and environmental risk factors, a definitive cause of the disorder has not yet been discovered. Treatments are symptomatic in nature; no treatments are known to affect disease progression. Generally, age is the most significant risk factor for development of PD, and the incidence of the disorder increases with age. But there is an early-onset variant of the disease in which symptom onset occurs before the age of 40. Perhaps the most famous case of early-onset PD is that of Michael J. Fox (**FIGURE 20.1**), whose disclosure of his diagnosis came the day before it was to be reported in a tabloid newspaper. He has since become a vocal and dedicated advocate for research on PD and care for those with the disorder.

The clinical features of PD are primarily motor related

Often the most visible outward sign of PD is a 4- to 6-hertz **resting tremor**, which occurs in about 70% of patients with PD. It generally starts in the hand (where it is called a "pill-rolling" tremor for its characteristic motion) or foot on one side of the body, although it can also happen in the jaw or face. The tremor is present when the limb is relaxed and generally disappears with intentional movement. The tremor can be exacerbated by stress or excitement and can spread to the other side of the body with disease progression (although the initially affected limb generally shows a more severe tremor throughout the disease process).

FIGURE 20.1 Among the most public faces of Parkinson's disease are Muhammad Ali and Michael J. Fox In addition to educating the public and federal leaders, Fox (shown here testifying before the U.S. Senate health committee) created and advocates for the Michael J. Fox Foundation for Parkinson's Research. (MediaPunch Inc./Alamy Stock Photo.)

Parkinson's disease is characterized by both difficulty in initiating movement (**akinesia**) and slowing of movement in general. This slowing, called **bradykinesia** (from the Greek *brady*s, "slow," and *kinesis*, "motion"), leads to several seemingly unrelated symptoms. Many patients with PD are described as "stone-faced" because their facial muscles do not move as much and therefore don't allow the range of facial expression that was previously achievable. Perhaps less obvious as consequences of slowed/reduced movement are the micrographia (smaller handwriting), hypophonia (decreased volume of speaking), and monotonous speech (decreased prosody in the voice) that accompany PD.

Rigidity, or stiffness and inflexibility in the joints, is another common symptom of PD. This difficulty in joint movement often manifests (together with bradykinesia) as lack of arm swing when walking. This contributes to the "Parkinson's shuffle" gait often seen in patients. The two particular types of rigidity that may be seen with PD are described as "lead-pipe" rigidity, a state where the inflexibility of the joint is maintained consistently through a range of passive movement, and "cog-wheel" rigidity, which is characterized by ratchet-like interruptions in muscle tone. Cog-wheel rigidity is likely the result of a superimposition of a tremor over the rigidity.

Postural instability—impaired balance and coordination—causes patients to exhibit a forward or backward lean in their upright posture. This leads to **retropulsion** in some patients: when bumped from the front or when starting to walk, patients with a backward lean have a tendency to step backward. Postural instability can cause patients to have **anteropulsion**, a stooped posture in which the head is bowed and the shoulders are drooped. Both anteropulsion and retropulsion can manifest as counterintuitive symptoms of **festination**, which is defined as an uncontrollable acceleration of gait. This is likely what Ms. S. was experiencing as she entered her apartment in our chapter opener. Unwanted acceleration of movement can also happen in a patient's speech, a condition called tachyphemia.

Another early sign of motor dysfunction is evident in eye movement, primarily as difficulty in tracking moving objects. This occurs in both medicated and nonmedicated patients with PD and may be useful in the differential diagnosis of PD. Other, less consistently seen, motor symptoms include **dystonia** (persistent involuntary muscle contractions), impaired gross and fine motor control, **akathisia** (a constant urge to move certain body parts), speech problems,

difficulty swallowing, sexual dysfunction, cramping, and drooling.

Although the symptoms of PD are primarily motor related, other difficulties accompany the disorder. Early signs include loss of the sense of smell, constipation, REM (rapid-eye-movement) sleep behavior disorder (lack of normal loss of muscle tone during REM sleep), mood disorders, and orthostatic hypotension (also known as postural hypotension—the sudden drop in a person's blood pressure upon standing, resulting in dizziness or fainting). As the disease progresses, patients may also experience sleep disturbances, bladder problems, weight loss or gain, vision problems, dental issues, fatigue or loss of energy, skin problems, and medication side effects.

Several other disorders can mimic the effects of PD. Symptoms of Parkinson's disease in the absence of the hallmark pathology of PD are referred to as Parkinsonism. Some disorders that may bring about Parkinsonian symptoms are strokes, encephalitis, and repeated brain trauma. Additionally, medications, including many antipsychotic drugs (e.g., haloperidol), some drugs used to treat high blood pressure (e.g., reserpine), and some mood stabilizers (e.g., valproate and lithium), can cause Parkinsonian symptoms as side effects.

Patients with Parkinson's may also develop dementia

Parkinson's disease dementia (**PDD**) is diagnosed when one or more cognitive functions are impaired to the point of interfering with the ability of the patient to navigate everyday life. Prevalence estimates vary, but it is likely between 15% and 40% and increases to near 70% after 15 years of disease progression. The tricky part of the diagnosis of PDD is differentiating it from comorbid Alzheimer's disease (AD) or **Lewy body dementia** (**LBD**). Generally, AD and PDD share symptoms, but they tend to occur in a different order. Early PDD may be characterized by **bradyphrenia** (slowed answers), although patients may still be capable of giving correct answers if allowed adequate time. They may also show mental inflexibility and changes in visuospatial function. Hallucinations are fairly common in PDD early on in the dementia process, but they don't show up in AD until very late in the disease progression. Patients may show improvement on cognitive tests of dementia severity, such as the **Mini-Mental State Examination** (**MMSE**), if they take cholinesterase inhibitors, particularly rivastigmine (Exelon) (Emre et al., 2004), a medication that is also used to treat symptoms of AD.

The primary pathology of PD is a loss of dopaminergic neurons in the substantia nigra

The **substantia nigra** is generally considered part of the **basal ganglia**, a group of structures that are instrumental in translating motivation into action (see Chapter 2). A common model uses the concepts of accelerator and brake for voluntary movements. But these neurons are not the start of the pathology in PD. In 2003, Braak et al. described stages (now called Braak stages) of pathological changes that happen in PD. The degeneration starts in the dorsal motor nucleus of the vagus and the anterior olfactory structures. This loss of olfactory processing accounts for the **anosmia** experienced by many of those with PD. The degeneration then moves to two sets of brainstem nuclei: the raphe and the locus coeruleus (**FIGURE 20.2**). Only in the third stage do we start to see degeneration of the substantia nigra (**FIGURE 20.3**), along with the amygdala and the nucleus basalis of Meynert. It is in this third stage that clinical diagnosis generally occurs with the onset of motor symptoms. Degeneration of the temporal lobe mesocortex (an area of cortex where layers 3, 4, and 5 are fused into one; e.g., the parahippocampal cortex) follows in stage 4, followed by degeneration of the neocortex in the temporal lobe along with neocortex sensory association and premotor areas in stage 5. Finally, the neocortex areas of primary sensory function and motor areas show degeneration in the final stage, 6.

Motor symptoms, other than the resting tremor, are generally explained by the loss of dopaminergic substantia nigra cells. Loss of these cells results in less dopaminergic input to the striatum (the putamen and the caudate nucleus) of the basal ganglia. This loss increases excitation to the subthalamic nucleus and the internal globus pallidus through direct and indirect pathways of the basal ganglia. Excitation of inhibitory neurons leads to inhibition of the thalamus and subsequent inhibition of motor structures in the cortex (**FIGURE 20.4**). This results in the akinesia and bradykinesia of PD.

In most cases, the onset of PD is a sporadic event. Estimates of the percentage of cases resulting from familial inheritance generally are around 10%. Age is the most significant risk factor for PD; the incidence increases as the population ages. Environmental contributors are also suspected in the development of PD (a result of the observation of heroin addicts who had mistakenly taken MPTP [a neurotoxin that destroys DA neurons], as discussed in Chapter 5). Although research has not identified any one specific cause, it has supported the idea that pesticides and other toxins may contribute to PD onset (Tanner et al., 2011).

Regardless of the cause of PD, several processes contribute to the degeneration of neurons, including mitochondrial dysfunction, oxidative stress, inflammation, excitotoxicity, protein misfolding, and proteosomal dysfunction (Davie, 2008). These mechanisms then lead to **Lewy body** formation (see later in this chapter), other protein accumulation, and ultimately apoptosis of the cells (see Chapter 8 for a discussion of apoptosis).

Supporting evidence for these mechanisms comes from many different places. Mitochondrial dysfunction

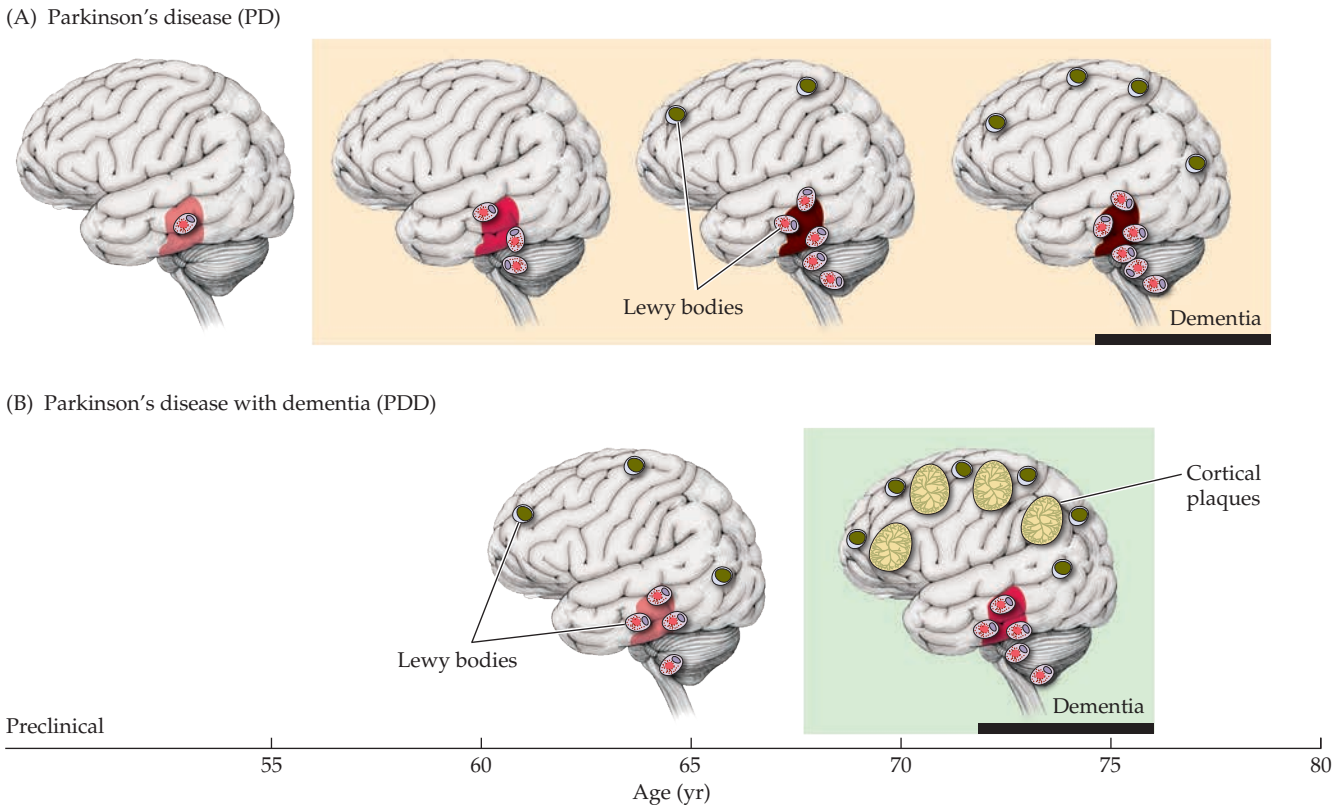


FIGURE 20.2 Disease progression in early- and late-onset Parkinson's disease Cellular degeneration (represented by progressively darker red shading in the midbrain) and the accumulation of Lewy bodies spread at different rates through the brainstem nuclei and midbrain depending on the age of onset. Compared with those with earlier-onset PD (A), more pronounced pathology earlier in disease progression is seen in those with later-onset PD (B). Latency to dementia onset from disease onset is also faster in later-onset cases. (After Obeso et al., 2010.)

is indicated from the process by which **MPTP** (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) destroys cells. Actually, it is not the MPTP itself that damages the cells, but the oxidation product MPP^+. MPP^+ is formed by the activity of monoamine oxidase B (MAO-B) in astrocytes and serotonergic neurons and is transported by the dopamine (DA) transporter into substantia nigra cells. Once inside the cells, MPP^+ accumulates in mitochondria, blocking mitochondrial respiration (i.e., oxygen-dependent energy production). Mutations in genes supporting mitochondrial function have been identified in familial forms of PD. The "MitoPark" mouse model was designed to test the hypothesis that mitochondrial dysfunction is particularly important in the pathogenesis of PD. In this mouse, mitochondrial transcription factor A (TFAM) was inactivated in substantia nigra dopaminergic neurons. The results of this inactivation are depletion of mitochondrial DNA (mtDNA), loss of gene transcripts, and deficiency in the respiratory chain, the end result of which is cell death (Sorensen et al., 2001). The behavioral outcomes in these mice were similar in manner and scope to PD and were reversed by L-DOPA treatment, one of the most common therapies for PD (discussed in detail later in this chapter).

Oxidative stress is indicated as a contributor to the pathology of PD by postmortem analysis of patient brain tissue, which shows oxidative damage, accumulation of iron, glutathione (GSH) depletion (Martin and Teismann, 2009), and a decrease in

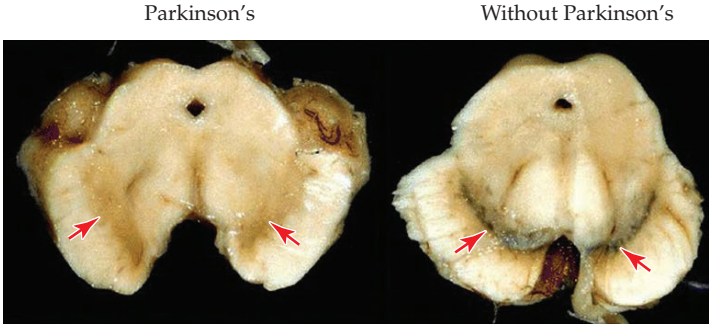


FIGURE 20.3 Selective loss of pigmented dopaminergic cells occurs in the substantia nigra The brain of a person with Parkinson's disease is compared with that of a healthy control. The substantia nigra is marked with red arrows. (© 2010, European Association for Predictive and Personalised Medicine.)

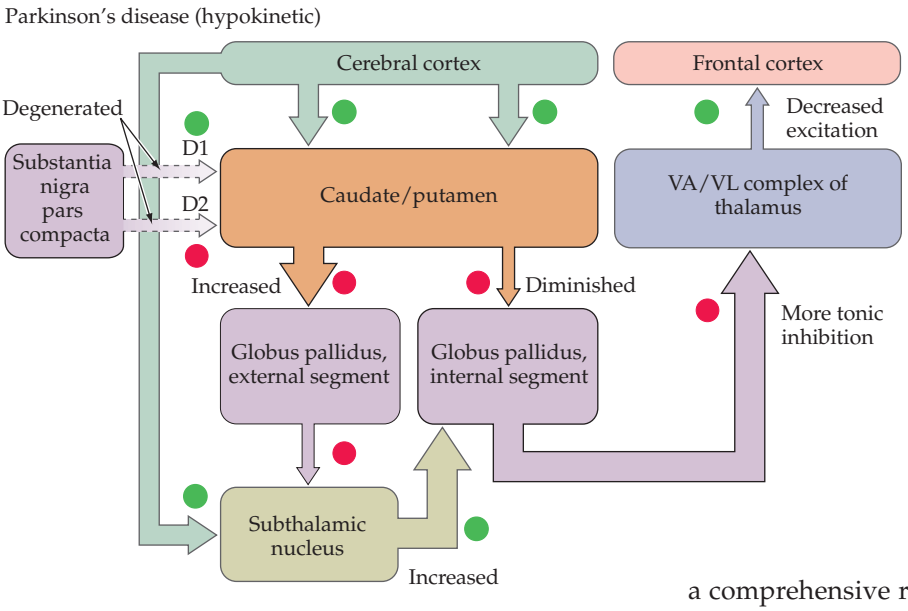


FIGURE 20.4 The effects of Parkinson's disease on motor control pathways With the degeneration of the substantia nigra, the balance of signals in the direct and indirect pathways is changed such that there is less inhibition from the caudate and putamen into the globus pallidus. This keeps the thalamus inhibited by the globus pallidus, thus decreasing activation of the motor cortex by the thalamus. VA, ventral anterior nucleus; VL, ventral lateral nucleus. (From Purves et al., 2018 after DeLong, 1990.)

GSH peroxidase (Kish et al., 1985). Additionally, in an experimental model, the use of **6-hydroxydopamine** (**6-OHDA**) destroys dopaminergic neurons by producing reactive oxygen species (see Chapter 5 for discussion of DA neurotoxins). This destruction produces symptoms of Parkinsonism. A caveat is required when discussing oxidative stress, because antioxidants such as tocopherol, more commonly known as vitamin E, (The Parkinson Study Group, 1993) and coenzyme Q (Shults et al., 2002; Storch et al., 2007) have not been shown to be consistently effective against the degeneration and symptomatology of PD.

The roles of inflammation and excitotoxicity in the pathology of PD are primarily supported by the fact that therapeutic effects of agents block them in some animal models and in some clinical trials. In particular, some evidence suggests a protective effect of nonsteroidal anti-inflammatory drugs (NSAIDs) (Gagne and Power, 2010). Glutamate excitotoxicity can also be blocked with some therapeutic benefit in both animal models and patients. *N*-methyl-D-aspartate (NMDA) receptor modulators and antagonists can be beneficial because of their glutamatergic connections to the DA-containing neurons of the substantia nigra (Koutsilieri and Riedererer, 2007).

One of the key components of PD degeneration, though, is protein aggregation, which makes PD similar in pathology to AD. In particular, Lewy bodies are formed in the cells that are affected by PD (**FIGURE 20.5**). Lewy bodies are primarily composed of the **α-synuclein** protein, along with associated proteins such as ubiquitin, neurofilament protein, and alpha B crystallin. The purported role of α-synuclein in healthy cells involves interactions with membranes, particularly those of vesicles. In these interactions, the protein mediates vesicle movement at the axon terminal (the name *synuclein* comes from its role at the *syn*apse). For a comprehensive review of α-synuclein function and dysfunction, see Auluck et al., 2010. There can also be tau proteins and surrounding neurofibrillary tangles that are more often associated with Alzheimer's disease. These Lewy body protein accumulations result from a combination of pathological processes, including protein misfolding and proteosomal dysfunction. Proteins require a precise three-dimensional conformation to function correctly. Those that are misfolded should be destroyed by proteosomes in the cells, but this process does not happen effectively, resulting in aggregation of these proteins and the formation of Lewy bodies. These protein aggregations interrupt normal cellular functions and trigger apoptotic cell death. In fact, the Braak stages discussed earlier are based on the accumulation of α-synuclein in the cells of the listed brain areas, not on neuronal death. Selective vulnerability has been noted in low-speed, long, nonmyelinated neurons in the brain.

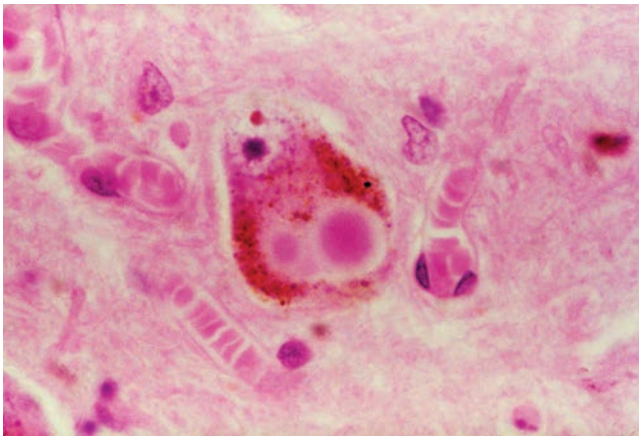


FIGURE 20.5 Lewy bodies are formed by abnormal accumulations of proteins They are composed mostly of α-synuclein but also contain ubiquitin, tau, and other proteins. (© Biophoto Associates/Science Source.)

Animal models of PD have strengths and limitations

We've already mentioned a couple of useful animal models: the use of MPTP (in mice or nonhuman primates) or 6-OHDA to make lesions, and the genetic model of the MitoPark mouse. These represent two classes of animal models: toxins and genetic manipulations. Another substance that falls into the category of toxin administration is rotenone. Rotenone is a compound obtained from the roots, seeds, and stems of several plants and is used as a pesticide, both as an insecticide and as a piscicide (fish poison). When administered to a mammal, this compound produces a pattern of pathology that is very similar to PD in humans. It triggers the accumulation and aggregation of α-synuclein and ubiquitin and causes oxidative damage and apoptosis (Li et al., 2003). It is thought that it produces this effect by blocking complex I in the mitochondrial respiratory chain. Blocking mitochondrial respiration results in the production of reactive oxygen species (particularly superoxide and hydrogen peroxide), which cause membrane lipid peroxidation and cell death (Lin and Beal, 2006). This provides further evidence for mitochondrial dysfunction as a mechanism in PD.

A pharmacological model uses the drug reserpine, an antihypertensive drug that depletes stores of three key neurotransmitters (DA, norepinephrine [NE], and serotonin [5-HT]), to mimic the lack of DA release and effects on NE neurons in PD, including cell death in the locus coeruleus. Lesion models include intentional damage of the nigrostriatal tract (cutting the dopaminergic axons as they run from the substantia nigra to the striatum), usually at the level of the medial forebrain bundle (a collection of ascending fiber tracts that include the nigrostriatal tract). Generally, these are good models of the motor dysfunction that occurs with PD, but there are certainly some limitations. Some of the models have problems with specificity. It is impossible to target only the DA neurons in the substantia nigra with 6-OHDA or reserpine. Many of these models are not degenerative in a progressive way as PD is, particularly the drug and lesion models. Most of the animal models are limited in the scope of the PD phenotype they exhibit and may not mimic the specific cellular changes seen in PD (e.g., Lewy bodies, α-synuclein accumulation, apoptosis).

Pharmacological treatments for PD are primarily symptomatic, not disease altering

Generally, there are five categories of drugs for PD:

1. Drugs that prevent clinical progression of the disease
2. Drugs that are symptomatic monotherapies (i.e., given alone for treatment of a particular symptom)
3. Drugs that are given as adjunct treatments to levodopa (L-DOPA) therapy
4. Drugs that prevent motor complications
5. Drugs that treat motor complications

We are going to address primarily those that are symptomatic treatments given alone or in conjunction with the most common pharmacological treatment, levodopa.

LEVODOPA **Levodopa** (**L-DOPA**), a metabolite of the amino acid tyrosine, immediately precedes the production of DA in its metabolic pathway (see Chapter 5). Because the pathway continues on to form NE and epinephrine (EPI), it is also a precursor to those neurotransmitters. The reason L-DOPA is given as a treatment, rather than DA itself, is that L-DOPA is capable of crossing the blood–brain barrier, where DA does not cross. Once L-DOPA has reached the brain, it can be converted to DA by cells that contain the enzyme **aromatic amino acid decarboxylase**. Because aromatic amino acid decarboxylase is also present in the periphery, L-DOPA is often administered with a peripheral inhibitor of this enzyme such as **carbidopa**, which allows more of the compound to reach the brain. Some drugs on the market are a combination of these two compounds (e.g., Rytary). L-DOPA is very effective, even compared with other dopamine agonist drugs (**FIGURE 20.6**), but this treatment is prone to several side effects. In the short term, L-DOPA therapy can result in nausea,

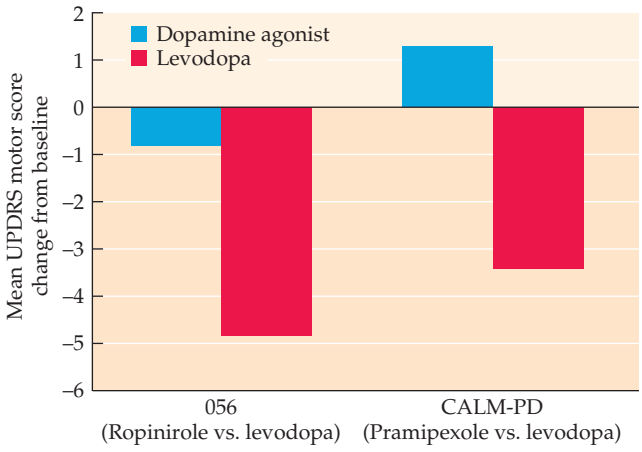


FIGURE 20.6 One measure of the severity of PD symptoms is the Unified Parkinson's Disease Rating Scale (UPDRS) Two large-scale studies—the Comparison of Agonist Pramipexole versus Levodopa on Motor Complications in Parkinson's Disease (CALM-PD) and the 056 study—compared long-term UPDRS scores (a higher rating on this scale denotes more severe disability) in patients receiving levodopa or the dopamine agonists pramipexole (CALM-PD) and ropinirole (056). Levodopa was more effective over time than either ropinirole or pramipexole. (After Poewe et al., 2010.)

hypotension, and neuropsychiatric side effects (e.g., hallucinations, confusion, and anxiety). The long term can bring motor fluctuations (e.g., "off periods," described in the chapter opener, fluctuating with "on periods") and **dyskinesias**. Dyskinesias are generally unwanted movements like severe tics or choreic movements. You've heard this term before in the context of side effects of antipsychotics that result in tardive (delayed) dyskinesias (see Chapter 19). Safinamide (Xadago) is a mono-amine oxidase inhibitor given as an adjuct therapy to carbodopa/levodopa treatment to help minimize the "off period" motor symptoms.

MAOIS, COMT INHIBITORS, AND DA AGONISTS Other treatments are also aimed at increasing dopamine signaling in the brain. Three classes of drugs used for this purpose are the **monoamine oxidase inhibitors** (**MAOIs**), the **catechol-*O*-methyltransferase** (**COMT**) inhibitors, and dopamine agonists. The two most common MAOIs used for this purpose are selegiline (Eldepryl) and rasagiline (Azilect). These can be given as monotherapies or as adjuncts to levodopa treatment. As in their use in depression, MAOIs prevent the breakdown of DA, NE, and EPI before their repackaging into vesicles, allowing these neurotransmitters to be released in greater quantities with cell stimulation, which increases the opportunity to connect with postsynaptic receptors and bring about signaling between neurons. Because PD is often comorbid with depression, it is important to watch for interactions between MAOIs and other antidepressants, such as **selective serotonin reuptake inhibitors** (**SSRIs**) or **tricyclic antidepressants** (**TCAs**), and to adhere to the strict dietary restrictions that come with MAOIs to avoid side effects such as severe hypertension (see Chapter 18). COMT inhibitors also prevent the breakdown of DA in the synapse but are given only as adjuncts to L-DOPA. The most common COMT inhibitor used is a drug called entacapone. There is a single-drug treatment (Stalevo) that combines carbidopa, levodopa, and entacapone into one pill for ease of dosing. Several side effects are associated with entacapone, including dyskinesia, dizziness, nausea, diarrhea, and urine discoloration.

Dopamine receptor agonists may also be used for the treatment of PD. The three most popular options are pramipexole (Mirapex), ropinirole (Requip), and rotigotine (Neupro). These drugs all have longer half-lives than L-DOPA (5 to 8 hours versus 0.75 to 1.5 hours for L-DOPA); however, with carbidopa coadministration, the half-life is on the longer end of the range These drugs also carry lower risks for dyskinesias and off periods. However, the usefulness of these medications is decreased by several side effects, including nausea, sedation, insomnia, orthostatic hypertension, hallucinations, leg edema, and impulse control disorders.

AMANTADINE Another approach to pharmacological treatment comes in the use of the drug amantadine (Symmetrel, Gocovri) as monotherapy or to decrease dyskinesias related to the use of L-DOPA. Amantadine was originally approved and used to treat and prevent infection caused by influenza A virus. The mechanism of the drug is anticholinergic and antiglutamatergic (an NMDA receptor antagonist). These actions lead to an increase in dopamine release in the nigrostriatal pathway and in other areas of the brain.

STATIN DRUGS Newer evidence has shown that people may be able to reduce the risk of developing Parkinson's by taking statin drugs for lowering cholesterol. This protection is likely provided by a decrease in cholesterol that subsequently improves heart health and increases anti-inflammatory effects. Additionally, evidence suggests that DA receptors are up-regulated by statin therapy (Q. Wang et al., 2011).

ANTIPSYCHOTIC MEDICATION As you read in Chapter 19, typical (and many atypical) antipsychotics have a mechanism of action that decreases DA signaling. That can exacerbate PD symptoms. But as many as 50% of patients with PD experience hallucinations and/or delusions that can be caused by medications that increase DA, Parkinson's dementia, or delirium. In 2016, pimavanserin (Nuplazid) was approved by the FDA, specifically for the treatment of hallucinations and delusions in PD. It is a selective serotonin inverse agonist. It reduces psychosis without directly affecting DA levels and therefore does not increase motor symptoms.

There are several unmet needs in PD diagnosis and treatment

Although researchers have made great strides in understanding the etiology of PD and in developing new treatments, more research and knowledge is needed in several areas. Since a definitive diagnosis of PD is currently not possible until death, a biomarker or diagnostic test that reliably indicates PD pathology that could be done with minimal expense would be of great use in determining treatment options and regimens. New imaging technologies for DA transporter visualization (Seibyl et al., 2012; Stoessl, 2012) may fill this need and increase diagnostic and treatment efficiency (**FIGURE 20.7**), but they are currently cost prohibitive for regular use. Although symptomatic treatments are available, none have yet convincingly showed slowing of the disease process. A treatment that could interrupt this process would be most useful. Treatment options for gait freezing to help patients avoid falls and for nonmotor symptoms, particularly the cognitive and behavioral changes, of PD should be explored. Finally, treatment options that promote regeneration and restoration of structure and function should also be actively pursued.

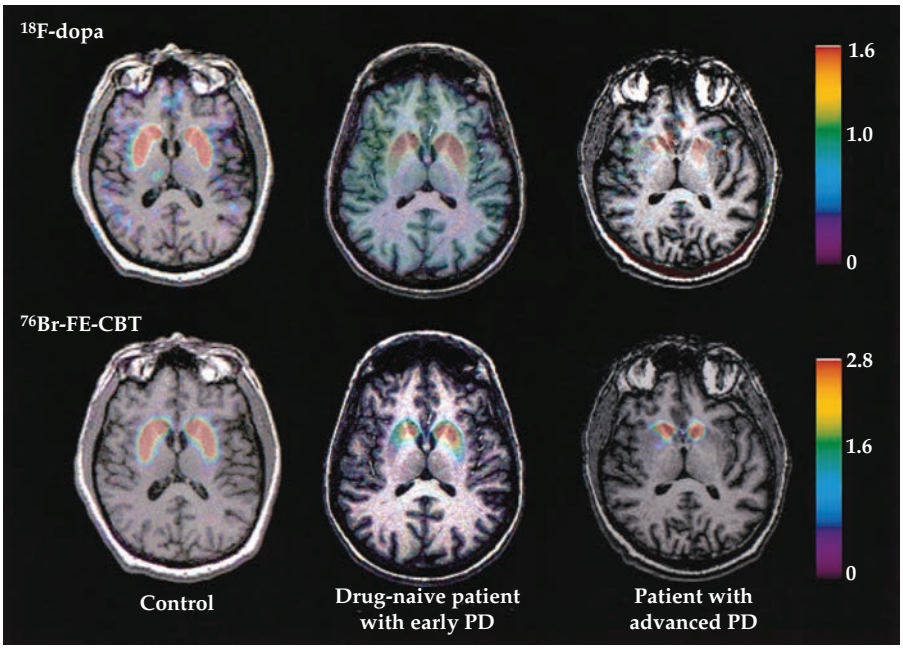


FIGURE 20.7 PET scan visualization of ^{18}F-dopa and ^{76}Br-FE-CBT uptake
The scans are at the level of the striatum in a control subject, a drug-naive patient with early Parkinson's disease (PD), and a patient with advanced PD. Uptake of both tracers is asymmetrically decreased in patients with PD and is less in the posterior than in the anterior striatum, indicating decreased dopaminergic cells. The decrease is more severe in more advanced disease. (From Ribeiro et al., 2002.)

Section Summary

- Parkinson's disease is a chronic, progressive neurodegenerative disorder.
- The primary symptoms of PD are motor disturbances that result in a visible resting tremor and slowing of movement (bradykinesia). Other motor symptoms of the disorder include rigidity and postural instability.
- Nonmotor effects of the disorder start with loss of the sense of smell and include REM behavior disorder and mood disorders.
- Cognitive disturbance can be a result of PD and is called Parkinson's disease dementia (PDD). The slowing of thought and verbal responses (bradyphrenia) is a cardinal symptom.
- These motor and cognitive symptoms are traced to loss of dopaminergic neurons in the substantia nigra, a major input pathway to the basal ganglia.
- Onset of PD is most commonly a sporadic event, although about 10% of cases have an inherited genetic cause.
- Pathology in PD is likely due to mitochondrial dysfunction and resulting oxidative stress. Additionally, protein aggregation in cells causes the formation of Lewy bodies, which trigger apoptotic cell death.
- Several animal models of PD allow investigation of various aspects of PD pathology, including the MPTP model, 6-OHDA lesions, and administration of the pesticide rotenone or the drug reserpine.
- The primary therapies in PD aim to increase DA signaling and include L-DOPA, a DA precursor, MAOIs, COMT inhibitors, and DA agonists such as pramipexole, ropinirole, and rotigotine.

Alzheimer's Disease

Alzheimer's disease (**AD**) is a chronic, progressive dementia disorder that is much more widespread than PD. Among the 25 million patients worldwide who suffer from dementia, 50% to 70% are afflicted with AD. The disorder affects approximately 4.4% of people over the age of 65 and 9.7% of those over 70 and is said to roughly double in prevalence for each 5 years over age 65 (Qui et al., 2009). With the aging of the population, incidence of AD will continue to grow (**FIGURE 20.8**). Although the typical course of the disease includes an increase in risk as one ages, there is an early-onset form of the disease that begins before age 60, progresses more quickly, and has a genetic basis. Alzheimer's disease is diagnosed as mild or major neurocognitive disorder (NCD) and possible or probable Alzheimer's disease. Mild NCD is marked by decline in cognitive functioning from previous levels of performance in one or more domains such as complex attention, executive function, learning and memory, language, perceptual, motor or social cognition. The difficulties must be noticed by the person experiencing them, a close observer, or a clinician, and they are often confirmed with standardized neuropsychological or clinical assessment. These difficulties, though, do not interfere with a person's ability to function independently.

As mild NCD progresses to major NCD, the deficits interfere with independent self-care and other

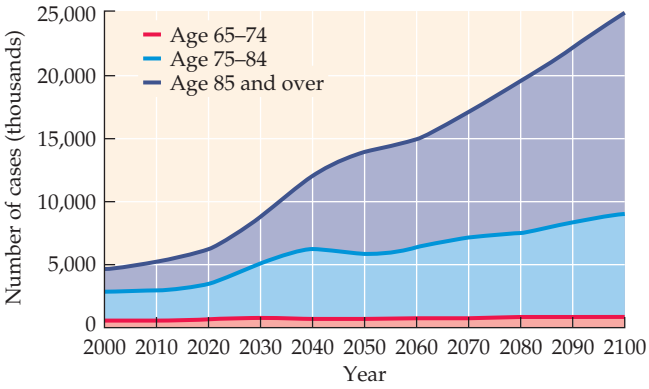


FIGURE 20.8 Past and projected rates of Alzheimer's disease (AD) With the aging population, about a fivefold increase in cases of AD is expected over the next 90 years. (After Hebert et al., 2013 and U.S. Census Bureau.)

tasks. The neuropsychological and clinical tests indicate substantial impairment in one or more of the cognitive domains mentioned above. To attribute mild or major NCD to Alzheimer's disease, the onset must be insidious and symptoms must progress gradually. Possible Alzheimer's disease is indicated if there is no evidence of contributing genetic mutation, while probable Alzheimer's is diagnosed with confirmed deterministic gene variants. Patients and caregivers may notice that tasks that used to be relatively easy are now more difficult, particularly more complex cognitive tasks. Anhedonia (loss of enjoyment of previously pleasurable things) is also common. Some anomia or anomic aphasia may be experienced, in which the ability to name familiar objects or people is impaired. Additionally, problems with misplacing or not finding items or getting lost on familiar routes occur with increasing frequency. For a comprehensive discussion of the symptoms of AD, see the Alzheimer's Association website (www.alz.org).

As the disease progresses, these issues get worse, and other behavioral and cognitive changes occur. Changes in physiological processes, such as disrupted sleep, incontinence, and difficulty swallowing, are seen. Psychiatric symptoms such as delusions, hallucinations, depressed mood, and agitation (including violent outbursts) may occur. Tasks that allow for basic self-sufficiency may suffer, including the ability to prepare food, to choose appropriate clothing, and, particularly, to drive. Additionally, the person's ability to recognize danger and to accurately and appropriately judge a situation is diminished. Reading and writing become more difficult, and strategies such as leaving lists and notes as memory cues may become less effective. Verbal communication also suffers as the disease progresses, and language becomes confused, with incorrect word usage and mispronunciation of words. Much of our sense of "self" comes from our memory and cognitive function, and this commonly is lost in those with advancing AD. The loss of personal episodic memories contributes to this. With loss of these functions comes withdrawal from social contact with family and friends. AD will eventually take away completely the ability to use language, interact with or even recognize family or friends, and live independently.

AD is defined by several pathological cellular disturbances

The cardinal cellular pathologies in AD are amyloid plaques and neurofibrillary tangles. These changes, described in detail in the following sections, result in degeneration of cells throughout the cortex but primarily in the frontotemporal association cortex (Perl, 2010), as seen in **FIGURE 20.9**. Additionally, a significant loss of synapses (up to 45%) may be the basis for the significant cognitive deficits seen in AD.

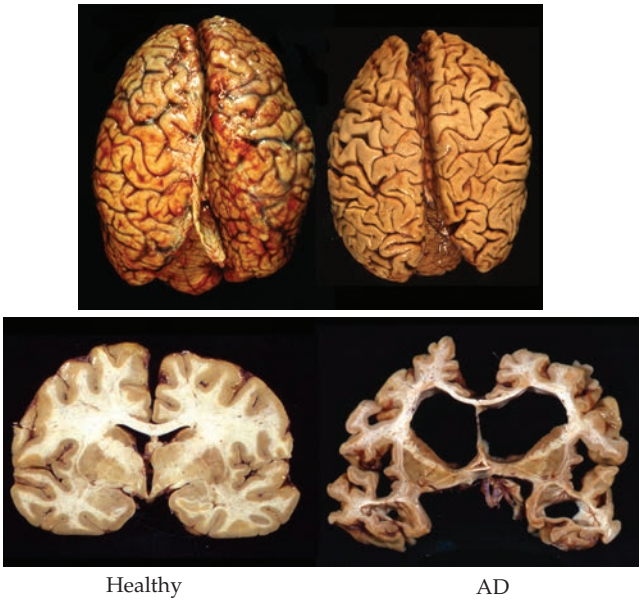


FIGURE 20.9 Pathological changes in the brain with advanced Alzheimer's disease (AD) The brain of an individual with AD is compared with a healthy brain from an age-matched individual. The brain of the individual with AD shows significant atrophy, narrowing of the gyri, widening of the sulci, and enlargement of the ventricles. (Courtesy of Ann C. McKee, MD, Boston University School of Medicine/VA Boston Healthcare System.)

AMYLOID PLAQUES The accumulation of the **beta-amyloid protein** (**β-amyloid**, or **A-beta** [**Aβ**]) between neurons in the brain results in the formation of amyloid plaques. Aβ is a protein fragment normally produced by the brain by enzymatic cleavage of **amyloid precursor protein** (**APP**). APP is first cleaved by a β-secretase, most commonly BACE1, although BACE2 may also play a role in AD pathology. The cleavage product, APP-CTFβ, is then further cleaved by γ-secretase to generate either the 40-amino-acid (Aβ40) or the 42-amino-acid (Aβ42) form of Aβ (Goedert and Spillantini, 2006; Zhang et al., 2011) (**FIGURE 20.10**). In healthy brains, there are several proposed functions of the fragments of APP (including soluble APPα, which is formed by APP cleaving by α-secretase), including kinase activation, facilitation of gene transcription, cholesterol transport regulation, and pro-inflammatory actions and antimicrobial activities. After use, these fragments are degraded and cause no harm. In the brains of those with AD, these protein fragments, particularly Aβ42, accumulate to form plaques. Several different subtypes of plaques exist, and they include the senile or neuritic plaque, which has a core of the amyloid protein surrounded by abnormal neurites (dendrites or axons). Often, microglial cells or reactive astrocytes are found in the periphery of these plaques. A second form of the plaque has focal diffuse deposits of amyloid with no neurites surrounding the core. The third form has a dense core of amyloid without neurites. These are generally considered the long-term outcome of neuritic plaques, after the neurites have died off.

NEUROFIBRILLARY TANGLES **Neurofibrillary tangles** (**NFTs**) are fibrous inclusions that are abnormally located in the cytoplasm of neurons. The neurons particularly susceptible to NFTs are pyramidal neurons—those with a pyramid-shaped cell body. The primary component of these tangles is the protein **tau**, which is a protein associated with microtubules (long filaments that help

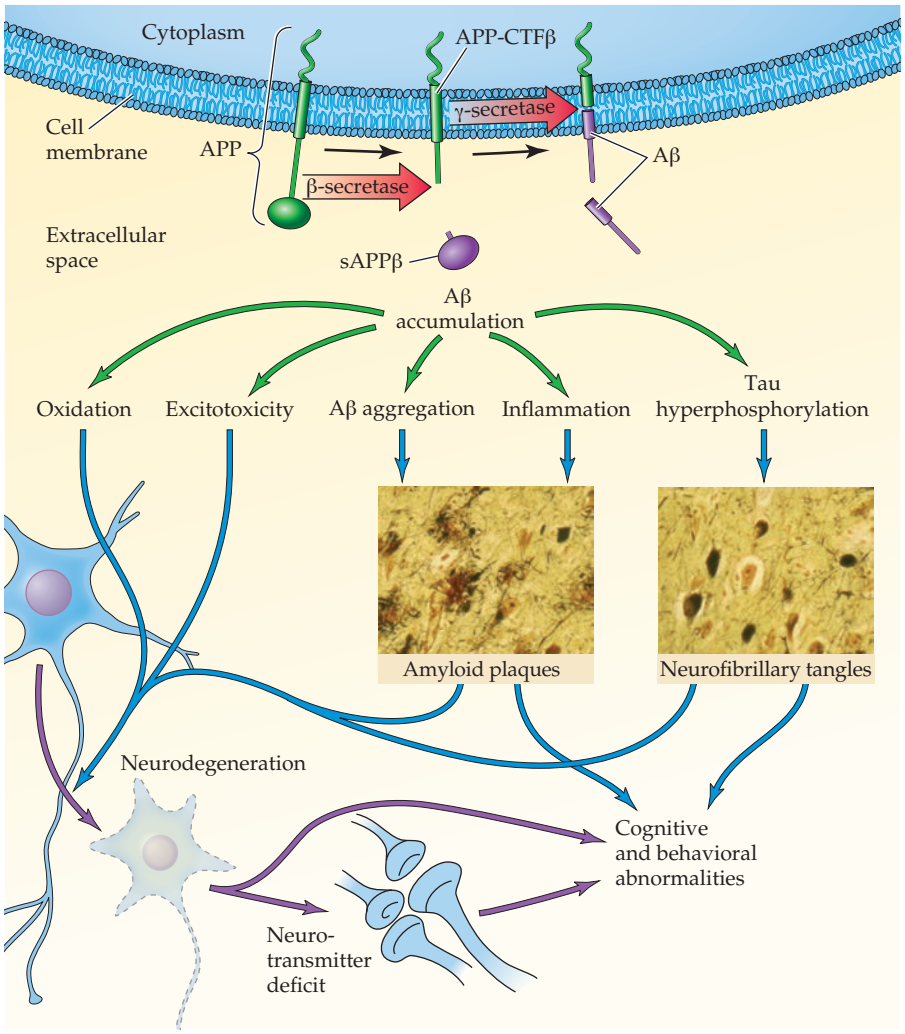


FIGURE 20.10 Formation of amyloid plaques β-amyloid (Aβ) is formed by the sequential cleavage of amyloid precursor protein (APP) by β-secretase and γ-secretase. The accumulation of Aβ results in several different pathological changes, which, in turn, result in neural degeneration and cognitive and behavioral symptoms. APP-CTFβ, cleavage product; sAPPβ, soluble APPβ. (After Cummings, 2004; photos © Dr. Thomas Deerinck/Visuals Unlimited, Inc.)

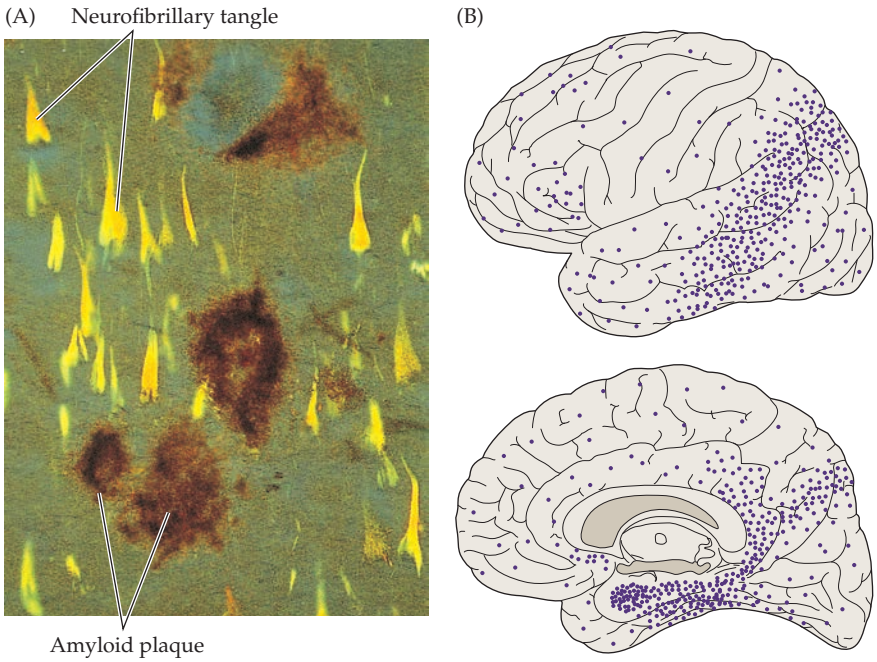


FIGURE 20.11 Cellular pathology in Alzheimer's disease Amyloid plaques and neurofibrillary tangles (A) accumulate in the brain (B). Dot density here indicates degree of pathology. (A courtesy of Gary W. Van Hoesen; B after Blumenfeld, 2002, based on Brun and Englund, 1981.)

maintain cellular structure and also participate in axonal transport; see Chapter 2). As a component of these tangles, the tau is abnormally phosphorylated. Other proteins, including ubiquitin, are also found in NFTs. The accumulation of these tangles follows a relatively typical pattern of trans-synaptic spread. In early stages of the disease (Braak and Braak, 1995), these tangles are found in the entorhinal cortex, with progression to the hippocampus and neocortex as the disease process continues (**FIGURE 20.11**). Additionally, neurons in the basal forebrain cholinergic and monoaminergic systems are susceptible to damage by AD pathological processes.

There are several behavioral, health, and genetic risk factors for AD

Several risk factors for dementia in general, and for AD specifically, are known. The most basic of these risk factors are advancing age and a family history of dementia or AD. Several of these risks are similar to those for the development of heart disease (e.g., diabetes, obesity, untreated hypertension, high cholesterol, stress, and a sedentary lifestyle). In addition, a history of head trauma or hypoxic brain injury, depression, bipolar disorder, or post-traumatic stress disorder (PTSD) can increase the risk for dementia.

Genetic contributors to AD consist of risk genes and deterministic genes (Zetzsche et al., 2010). Deterministic genes are those that can directly cause disease. Three genes are known to directly cause AD. These include the genes for APP, found on chromosome 21; for **presenilin-1** (**PS-1**), on chromosome 14; and for **presenilin-2** (**PS-2**), on chromosome 1. Mutations of these genes result in **autosomal dominant Alzheimer's disease** (**ADAD**). In this familial version of the disease, symptom onset is likely to occur before age 60 (it can occur as early as the 30s). Although ADAD is of concern, only about 5% of AD cases are familial.

The risk gene with the greatest influence on disease development is the gene for **apolipoprotein E** (**ApoE**). ApoE is normally a component of very-low-density lipoproteins (VLDLs). These lipoproteins remove excess cholesterol from the blood and carry it to the liver for degradation. The presence of the gene for the E4 form of this (*APOEε4*) increases risk; inheritance of this form from both parents increases risk further and may lead to earlier onset of the disease. Other risk genes produce protein products that normally interact with the proteins listed previously, or with their products. The protease produced by the alpha-2 macroglobulin gene (*A2M*) would normally contribute to the degradation and clearance of the Aβ protein produced by APP. *UBQLN1*, the gene that codes for the protein ubiquitin 1, is associated with AD because ubiquitin 1 promotes the accumulation of uncleaved PS-1 and PS-2 proteins, which are part of the structure of γ-secretase. SORL1 (sortilin-related receptor 1) is the neuronal receptor for ApoE. In the brains of those with AD, there can be a marked reduction of this receptor. In addition, this receptor is associated with the activity of APP such that decreased SORL1 production is correlated with higher Aβ load in the brain (**FIGURE 20.12**).

Perhaps surprisingly, AD is also closely linked to trisomy 21, the genetic variant that causes Down syndrome. By the age of 30 to 40, most patients with Down

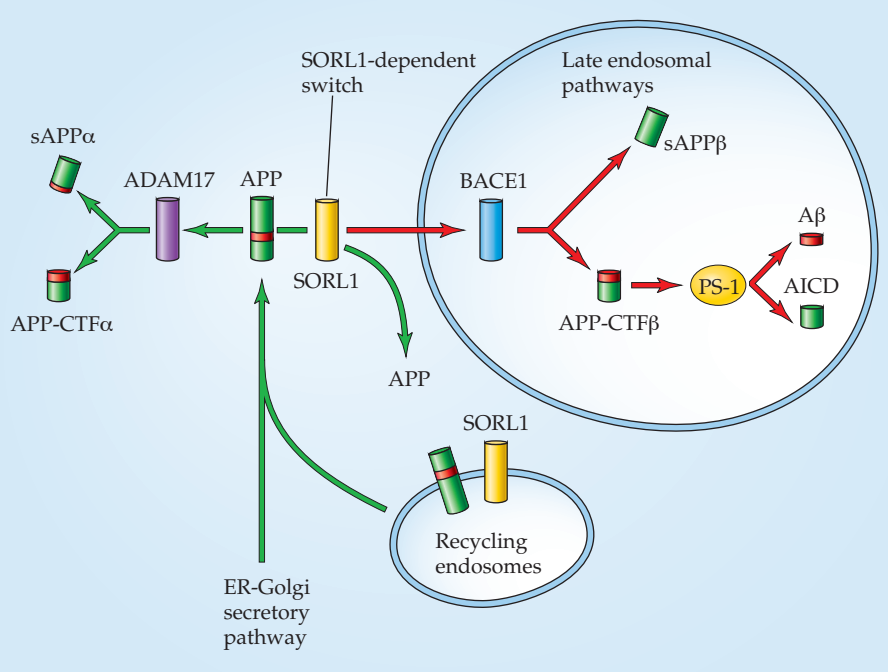


FIGURE 20.12 The amyloid precursor protein (APP) processing pathway Sortilin-related receptor 1 (SORL1) plays an important role as a switch in this process. After synthesis in the endoplasmic reticulum (ER), APP can be cleaved by ADAM17 (an α-secretase) to form soluble APPα (sAPPα) and a cytoplasmic fragment (APP-CTFα). Alternatively, SORL1 can bind APP and sort it into a recycling pathway. If SORL1 is absent, the APP is directed into β-secretase processing (e.g., by BACE1). BACE1 cleavage forms an N-terminal fragment (sAPPβ) and APP-CTFβ, which is cleaved again by presenilin-dependent γ-secretase (PS-1) to form Aβ and amyloid intracellular domain (AICD). A similar effect can be produced by inhibition of APP recycling from endosomes within the cell. (After Rogaeva et al., 2007.)

syndrome will develop the plaques and tangles that are associated with AD. These changes are nearly universal among patients with Down syndrome who reach this age, and although the severity of plaque and tangle accumulation mimics that found in AD, not all such individuals will develop AD. One of the possibilities for the connection is that patients have three copies of the *APP* gene, which is located on chromosome 21.

Alzheimer's disease cannot be definitively diagnosed until postmortem analysis

Alzheimer's disease is defined by changes that happen in the brain during degenerative processes, as in the plaques and tangles described previously. These processes generally are not visible without direct examination of the brain. So AD is a diagnosis of elimination rather than confirmation. There is no definitive test to rule *in* AD, but there are several tests that will rule *out* other sources of dementia. By performing physical and neurological exams, taking a thorough medical history, and doing a mental state exam (generally the Mini Mental State Examination, 2nd edition [MMSE-2]) along with other tests, doctors can rule out anemia, brain tumor, chronic infection, medication intoxication, severe depression, stroke, thyroid disease, and vitamin deficiency. Only when other causes of dementia have been ruled out can a differential diagnosis of AD be given to a living patient. Several newer technologies allow visualization of the plaques formed around deposits of Aβ protein. One, called florbetapir (Amyvid), is a ^{18}F-tagged small molecule that binds β-amyloid; it was developed by Eli Lilly and is used with a positron emission tomography (PET) scanner to examine those who already show signs of cognitive decline. It is a tool of elimination rather than confirmation for AD, because amyloid plaques can be present without AD. Another imaging technique with a similar mechanism, also used with a PET scanner, uses florbetaben. This technique has a small but significant false-positive rate. The risk of a false-positive ("You might have Alzheimer's") when a person doesn't have the disease is the reason for its use exclusively in those in whom cognitive decline has already occurred. A third technique, based on work by Wolk et al. (2009), uses [^{18}F]flutemetamol (Pittsburgh compound B [PiB]), which accumulates in the amyloid plaques (**FIGURE 20.13**). Despite these advancements, definitive diagnosis still requires postmortem analysis to look for the trademark neuronal changes that happen in AD.

Postmortem analysis of brain tissue is performed to look for the presence of two key indicators: NFTs and amyloid plaques. If these two pathologies are present, a diagnosis of AD is made; if these plaques are not detected, another explanation is put forth for the cognitive deficits seen in the patient. While postmortem diagnosis can confirm AD in the deceased, it is not useful in developing treatment strategies in those who are still alive. It is only through advancement of the amyloid imaging techniques discussed above that more informed treatment decisions can be made.

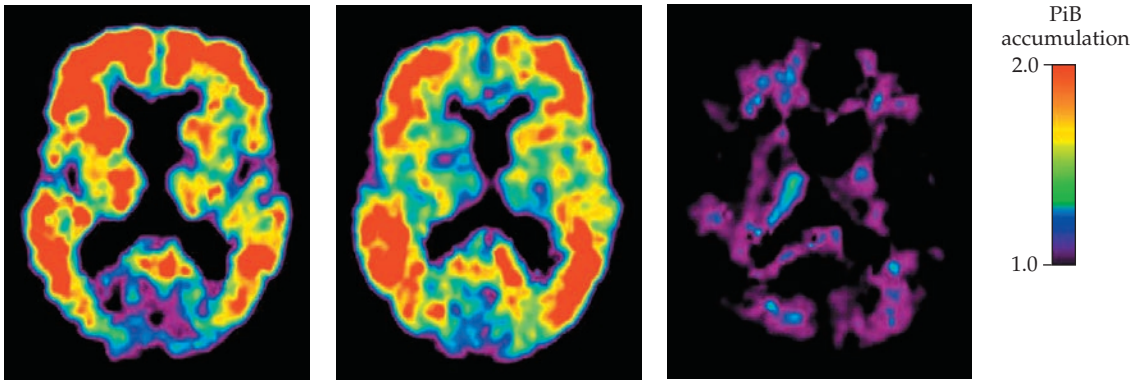


FIGURE 20.13 Visualization of amyloid plaques
One of several new methods for visualizing amyloid plaques in living brains, Pittsburgh compound B (PiB) dye accumulates in the plaques and can be visualized using PET scanning. Presence of these plaques is more common in those individuals with Alzheimer's or significant cognitive impairment. (From Wolk et al., 2009; courtesy of the University of Pittsburgh Amyloid Imaging Group.)

Several different animal models contribute to our understanding of AD

As with other disorders, the primary goal of AD animal models is to closely approximate one or more aspects of the human condition of AD. Several transgenic mouse models aim to get the progressive neuropathology that is seen with AD. Two common models (the APP/PSδE9 and APPswe/PS1δE9 models) are mutations on the *APP* gene. Each of these mutations results in memory deficits, high levels of Aβ42 in the brain, and amyloid deposits. The APPswe/PSδE9 model also shows the formation of neuritic plaques. The senescence-accelerated prone (SAMP8) mouse shows early learning and memory deficits along with the accumulation of Aβ protein, oxidative damage, and tau phosphorylation. Even *Drosophila* can be used to model some of these issues. These transgenic flies produce Aβ42 in their neurons. They show age-dependent short-term memory impairment and neurodegeneration. For further discussion of the models in this section, as well as others, see *Handbook of Animal Models in Alzheimer's Disease* (2011), edited by Casadesus.

A more natural (nontransgenic) model may be found in the beagle. Aged beagles can develop learning and memory deficits, as well as problems with executive function. This naturally occurring pathology shows similarity to the human condition in cortical atrophy, neuron loss, lack of neurogenesis, β-amyloid plaques, and other damage. The pathological changes correlate with the deficits in function. The progressive nature of the disorder in these animals is beneficial for longitudinal study.

Sometimes, models of other pathological processes can inadvertently bring about neuropathology. Originally models of coronary artery disease, cholesterol-fed rabbits also developed Aβ deposits, neurofibrillary tangles, and cognitive deficits at rates higher than those seen in rabbits that ate a normal diet. If the cholesterol feeding was paired with the administration of copper, outcomes were even worse. This is likely a result of copper interfering with clearance of Aβ from the brain by LDL receptor–related protein 1 (LRP1). Like humans with AD, these rabbits are deficient in eyeblink conditioning and show neuronal loss in the frontal cortex, hippocampus, and cerebellum.

Symptomatic treatments are available, and several others are under study for slowing disease progression

Current treatments fall into two categories: **cholinesterase inhibitors** and an **N-methyl-D-aspartate** (**NMDA**) glutamate receptor antagonist. AD seems to target cholinergic cells involved in cognition, and the cholinesterase inhibitors improve cognition by increasing the presence of acetylcholine in the synapse by lessening its breakdown. The first cholinesterase inhibitor approved for use in AD was tacrine (Cognex) for the treatment of mild to moderate AD. Soon after (in 1996) came donepezil (Aricept). Like Cognex and the cholinesterase inhibitors that followed, it was initially prescribed for mild to moderate AD, but newer evidence suggests that staying on the drug through the moderate stage (when previously the drug would have been stopped) may slow memory decline. After donepezil (in 2000) came rivastigmine (Exelon), which can be given in pill or transdermal patch form, and (in 2001) galantamine (Razadyne).

After it was observed that damaged neurons release significant amounts of glutamate, the drug **memantine** (**Namenda**) was introduced to prevent further excitotoxic neuron damage. Approved in 2003, memantine works by blocking current flow through the NMDA

subtype of glutamate receptor. This prevents the drastic increase in cell firing that leads to excitotoxicity, without producing significant side effects. As a result of binding to two sites on the receptor, modulation of the receptor is less severe than with other NMDA receptor antagonists such as MK-801 and ketamine (Johnson and Kotermanski, 2006). Memantine and donezepil were combined into a single drug with the FDA approval of Namzaric in 2014. Namenda and Namzaric are the only two drugs approved for treatment of moderate to severe AD.

Because of the devastating effects of AD, its increase in incidence and prevalence among those over age 65, and the increasing age of the population (at least in the United States), there is an urgent need for more effective AD therapies. Several recent studies have indicated promise for new types of therapies. In what certainly must have started as an anecdotal observation in patients with Alzheimer's who had comorbid cancer, researchers at Case Western Reserve University in Cleveland, Ohio, found that the chemotherapy drug epothilone D (EpoD) can reduce the presence of tau protein tangles in mice (Zhang et al., 2012).

Two antibodies have recently been the focus of clinical trials in humans. The first antibody, bapineuzumab, decreases levels of phosphorylated tau protein in the brains of mice, although its target is β-amyloid (which it does not decrease). The phase 3 trials for this antibody were terminated after clinical efficacy was not seen in the trials. The second antibody, crenezumab, is still in U.S. trials (phase 3) as well as a clinical trial that started in 2013 in a large Columbian family of 3000 people who carry the *PSEN1* gene. Early indications from preclinical work and from early-stage clinical trials are that it may be useful in preventing the onset of familial AD (Adolfsson et al., 2012).

A potential therapy that is in a much earlier stage of development consists of a class of compounds called spin-labeled fluorene compounds. These compounds reduce amyloid plaque formation in cultured neurons and can cross the blood–brain barrier (Petrlova et al., 2012). The next step is to test these compounds in animal models of AD. Another area of research involves the potential use of antibiotics as a treatment for AD. This is tied to the relationship between the gut microbiome and neuroinflammation. Learn more about this relationship in **BOX 20.1**.

BOX 20.1 The Cutting Edge

Alzheimer's Disease: It's all in your gut???

For most of the history of neuroscience, the central nervous system was thought to be "immunoprivileged," meaning that the brain and spinal cord have minimal interaction with the immune system, that they are not monitored by the immune system as is the rest of the body. And while there are certainly specializations of the immune system in the brain, current research indicates that there is actually a great deal of interaction between the CNS and the immune system, particularly with systemic immune activation and inflammation mediated by the gut microbiome. That's right, the bacteria that live in your gastrointestinal tract may influence whether or not you develop Alzheimer's disease.

In particular, increased levels of Bacteroidetes and decreased Firmicutes and Actinobacteria (primarily *Bifidobacterium*) in the gut microbiome are associated with AD (Vogt et al., 2017). Vogt and colleagues (2017) reported the first quantification of the microbiome comparing patients with AD to those without AD. They found that, in general, there is less diversity of microbiota in patients with AD and that there are specific differences in several genera of bacteria in these groups. Changes in Firmicutes and Bacteroidetes bacteria have also been associated with disorders such as type 2 diabetes, obesity, and Parkinson's disease. The effects of these bacteria on levels of inflammation (e.g., several species of bifidobacteria generally, and perhaps counterintuitively, exert anti-inflammatory effects) are likely the way that they influence the development of AD pathologies in the brain. The association with type 2 diabetes and obesity is interesting in that insulin resistance is associated with decreased cerebral glucose metabolism and with increased amyloid deposition in at-risk adults.

There's even some evidence that AD could be treated with antibiotics, that changing the gut microbiome with antibiotics may alter the process of AD. Hefendehl and colleagues (2016) found that the antibiotic ceftriaxone (Rocephin) can decrease the glutamate transport and subsequent glutamate accumulation (and resulting damage) in neurons. This study was done in mice. In another mouse model, Minter and colleagues (2016) found that antibiotics change the diversity of the gut microbiome. These changes then result in decreased Aβ deposition and decreased neuroinflammation at the location of existing plaques.

Section Summary

- Alzheimer's disease is a dementia disorder that affects increasing numbers of people in the United States.
- The onset of AD is preceded by neurocognitive disorder (NCD), but not all cases of NCD develop into AD.
- Early symptoms of AD include forgetfulness and impairment in other cognitive functions, including language, thinking, and judgment. Physiological changes include difficulty swallowing and disrupted sleep. Psychiatric difficulties come in the form of delusions, hallucinations, depression, and agitation. As the disease progresses, communication becomes increasingly difficult because of reading, writing, and verbal communication problems.
- Two pathological findings hallmark disease progression in AD: amyloid plaques and neurofibrillary tangles (NFTs). Amyloid plaques are formed by accumulation of Aβ (primarily Aβ42) after production by secretase cutting of the amyloid precursor protein (APP). NFTs are composed primarily of abnormally phosphorylated tau, a microtubule-associated protein, and other proteins like ubiquitin. Accumulation of NFTs results in disruption of cellular processes and eventually apoptotic cell death.
- Risk factors for development of AD include advancing age and poor cardiovascular health. Previous head injury or psychopathology can also increase risk.
- Several genes are associated with the development of autosomal dominant Alzheimer's disease (ADAD) and include the genes for APP, presenilin-1, and presenilin-2.
- Several other genes impart increased risk for AD, including the *APOEε4* allele, *A2M*, *UBQLN1*, and *SORL1*.
- Because of the location of risk genes on chromosome 21, AD has a strong association with Down syndrome (trisomy 21).
- AD generally is not diagnosed until after death, although newer imaging technologies may allow earlier diagnosis in the future.
- Animal models for AD include transgenic mice with alterations in the genes listed above, a natural aged-beagle model, and a cholesterol-fed rabbit model.
- Current treatments for AD are primarily cholinesterase inhibitors and an NMDA receptor modulator.
- Research indicates that Aβ antibodies, chemotherapy drugs, and perhaps even antibiotics may be effective treatments that will become available in the future.

Other Major Neurodegenerative Diseases

Huntington's Disease

Mr. R. was working for a large international bank in England—his dream job. When he forgot his password to the company banking system, he feared the worst. While most of us would attribute that slip to a rough day, he suspected that it was the beginning of a long, painful process that he had seen in his mother and grandmother. Anger and time management issues followed the memory lapses. These eventually led to loss of his job and the need to move back to the United States. He started a roofing business, but soon physical symptoms such as loss of balance prevented him from doing the job, and cognitive and psychiatric symptoms interfered with his ability to run the business. A genetic test for a clinical trial confirmed fears that Mr. R. had inherited the **Huntington's disease** (**HD**) gene from his mother. His focus became raising awareness of issues surrounding HD, receiving treatment for his increasing symptoms, and recognizing his fear for the future of his children, who have a 50% chance of developing the disorder, just as he and his siblings had. Mr. R. now faces progressive loss of cognitive and physical abilities and ongoing psychiatric problems. This will end with his death from Huntington's disease.

Huntington's disease is one of a few neurodegenerative disorders with a clear, singular genetic cause. A **trinucleotide repeat** (a CAG sequence) results in a gain-of-function mutation in the ***huntingtin* gene**. *Huntingtin* is a highly penetrant mutation (meaning that if the gene is a certain form, the chance of developing the disease is very high or is guaranteed). In this case, the likelihood of developing HD is dependent on the number of CAG repeats in the gene. There is essentially complete **penetrance** at 40 repeats, but there is lesser penetrance at smaller numbers of repeats, and no development of the disease at fewer than 35 copies. There is 90% penetrance at 39 copies, meaning that 90% of those with 39 copies of the CAG repeat will develop the disease, and 10% will not. There is 75% penetrance at 38 copies, 50% at 37, and 25% at 36 (Myers, 2011, personal communication). Those with 27 to 35 copies do not develop the disease themselves, but because of the instability of the gene, men can pass on a longer version of the gene to their offspring and increase their risk of developing the disorder. The number of repeats generally correlates with the age of onset, such that

more repeats result in earlier disease development. Age of onset can range from 4 to 65 years of age but generally comes in middle age. The "normal" version of the gene has about 20 CAG repeats at this location. The extra repeats in the gene cause a toxic gain of function resulting in protein aggregation and cell death.

Symptoms

Huntington's disease tends to be grouped with motor disorders when it is classified, but significant cognitive and psychiatric symptoms have been noted as well (Novak and Tabrizi, 2011). The motor symptoms result from degenerative effects on the basal ganglia (**FIGURE 20.14**) and reduced ability to suppress unwanted movement. Originally named "**Huntington's chorea**," these involuntary movements are jerky or writhing, "dance-like" movements of the limbs. In addition to these gross motor function changes, fine movement is significantly impaired in speed and coordination. Other issues in motor function include rigidity, dystonia, problems with speech and swallowing, and gait problems. Generally, the cause of death in HD is associated with these changes in motor function. Many with HD die from pneumonia and other complications of the inability to swallow or of injuries related to falls.

Motor symptoms, however, are not the only consequences of the significant degeneration in this disorder. Cognitively, patients with HD often find that they have difficulty with higher-order functions like planning and organizing. There are perseveration issues, where thoughts or behaviors are repeated over and over. There are issues with learning and memory, attention, and language usage. While this may seem unrelated to a movement disorder, these cognitive issues also result from damage to basal ganglia circuits. In addition to circuits initiating movement, the basal ganglia initiate cognitive and emotional processes through connections to the dorsolateral prefrontal cortex, the orbitofrontal cortex, and the cingulate cortex (Middleton and Strick, 2000). One of the more interesting consequences of this disorder is the patient's unawareness or denial of the symptoms, despite their obvious nature to others.

HD is also comorbid with several psychiatric symptoms and disorders. These comorbidities include obsessive-compulsive disorder (OCD), bipolar disorder, and mania. Several symptoms of depression, including changes in sleep patterns and energy, sadness, and thoughts of death, are also common in those with HD. Personality characteristics may change in HD, bringing about irritability, impulsivity, and anxiety.

Only symptomatic treatments are available for HD; none alter disease progression

No treatments yet tested can alter the course of Huntington's disease; all of the treatments are symptomatic in nature. Tetrabenazine (Xenazine) is the only drug specifically approved by the FDA for HD. This drug is useful in decreasing the excessive movement found in the disorder. The mechanism of this decrease in motor function is a decrease in monoamine vesicle packaging. Tetrabenazine is a reversible antagonist of the type 2 vesicular monoamine transporter (VMAT2; see Chapter 5). The half-life of tetrabenazine is 4 to

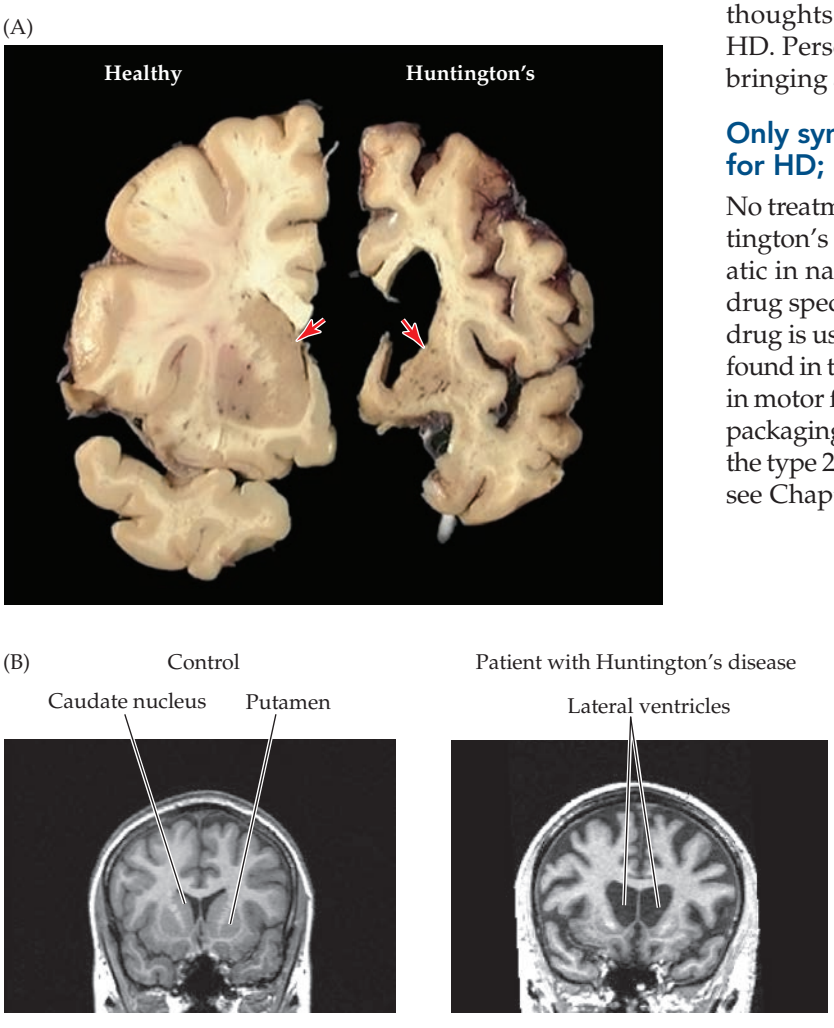


FIGURE 20.14 Neuropathology in Huntington's disease (A) Significant degeneration is seen in the brain of a patient with Huntington's disease compared with a healthy control. In particular, the size of the striatum (the caudate and putamen) is decreased by neurodegeneration of cells found there. (B) On MRI, the degeneration of the caudate and putamen can be seen in the enlargement of the lateral ventricles in patients with HD (right) compared with those of a healthy control (left). (A courtesy of Harvard Brain Tissue Resource Center; B courtesy of Terry L. Jernigan and C. Fennema Notestine.)

8 hours, and the duration of effect of a dose is approximately 5.5 hours. Because of the depletion of monoamines, the drug may increase psychiatric symptoms, particularly those associated with depression. Other side effects include Parkinsonism and sedation.

A similar compound, deutetrabenazine (Austedo), was approved for use to treat both HD and tardive dyskinesia. The side effects are similar to those for tetrabenazine and are particularly problematic for those with depression. It can also cause irregular heartbeat, neuroleptic malignant syndrome, restlessness, and Parkinsonism in those with HD. Interestingly, while sedation is one of the most common side effects in those with HD treated with deutetrabenazine, those with tardive dyskinesia report high levels of insomnia while using this drug.

Dopamine antagonist drugs, such as those usually used to treat schizophrenia, are also used to suppress the unwanted movements. Anticonvulsants and anxiolytic drugs are often used to combat both the choreic movements and the dystonia or rigidity that may accompany HD. To combat the psychiatric symptoms of the disorder, antidepressants, particularly the SSRIs, are used, as are the typical antipsychotics and mood stabilizers. More information about the mechanisms of these drugs is available in Chapters 18, 17, and 19, respectively.

Nondrug treatments that may be included in a treatment plan for someone with HD include physical and occupational therapy to combat changes in fine motor control, speech therapy, and psychotherapy.

Amyotrophic Lateral Sclerosis

Certainly the most famous patient with amyotrophic lateral sclerosis (ALS) was Lou Gehrig, a New York Yankee who, in 1939, retired from baseball after he was diagnosed with the disorder. Today, the disorder often bears his name. ALS is another degenerative disorder with principally motor symptoms. The degeneration of upper and lower motor neurons results in a progressive loss of fine and then gross motor function (Hardiman et al., 2011). The disorder leads to death from respiratory failure, generally within 5 years.

The incidence of ALS is about 1 to 3 cases per 100,000 people. Both familial and nonfamilial cases have been reported. Those that are nonfamilial in origin show more men affected than women. Risk factors may include exposure to some chemicals (such as insecticides and pesticides, which may also contribute to the development of PD). Those who smoke are at higher risk, as are those who have served in the military.

The symptoms and disease progression in ALS are devastating

In the early stages of ALS, with degeneration of spinal motor neurons, focal muscle weakness may manifest as tripping, clumsiness (particularly dropping things), and abnormal fatigue in the arms or legs. As the muscles are denervated, fasciculations (muscle twitches) and cramping may be noted. If the motor neurons most affected early on are those that give rise to the corticobulbar tract (the tract that runs from the cortex to the brainstem to control cranial nerve functions), the disease may start with difficulty chewing and swallowing and general facial weakness. Strangely, this degeneration may also be associated with involuntary exaggeration of emotional reflexes, including uncontrolled laughing or crying. Other manifestations of loss of these cells include slurred speech and difficulty projecting the voice.

Despite the start of symptoms in focal, distinct areas, eventually nearly all of the motor neurons are affected. This will lead to difficulty in breathing, which eventually will require ventilatory support for survival. Motor neurons are spared in only a couple of systems: eye movements are not compromised, nor is bladder and bowel function.

The loss of motor neurons in ALS is complicated and poorly understood

The neurons lost in ALS are particularly motor neurons. Within these neurons there is disruption of the cytoskeleton and aggregation of the component neurofilaments with other proteins to form "spheroids." This pathology leads to the activation and proliferation of astrocytes and microglia, resulting in neuroinflammation. The term *lateral sclerosis* comes from the scarred appearance of the spinal cord caused by the loss of the lateral corticospinal tract neurons (**FIGURE 20.15**).

How and why these cells die is a subject of much research. Several mechanisms seem to contribute to this loss (Joyce et al., 2011). Excitotoxicity from excessive glutamate signaling is one component of the damage.

FIGURE 20.15 Amyotrophic lateral sclerosis
ALS is characterized by scarring of the spinal cord (dark pink areas) that is caused by the loss of lateral corticospinal tract neurons. (From Bramwell, 1886.)

Other mechanisms include aggregation of proteins, breakdown of axonal transport with loss of neurofilament structure, reduced production of adenosine triphosphate (ATP), neuroinflammation, and triggering of cell death pathways. Indications suggest that some RNA binding proteins are mutated in some ALS cases. How problems in these proteins might lead to the death of motor neurons is not clear.

Two medications exist that are approved for ALS treatment

For years, the only treatment approved by the FDA specifically for the treatment of ALS was riluzole (Rilutek). Riluzole has a modest disease-modifying effect such that the average survival time in treated patients is 2 to 3 months longer than in those who are untreated. The drug acts as a presynaptic inhibitor of glutamate release. Because excitotoxic damage to neurons occurs in the pathological processes of ALS, this drug is thought to provide benefit by blocking this glutamate-mediated excitotoxicity (**FIGURE 20.16**). Some small benefit is associated with riluzole therapy in terms of symptom severity (including bulbar and limb function), but no benefit has been noted for muscle strength. Other indications suggest that the drug may delay the need for intubation and ventilatory support.

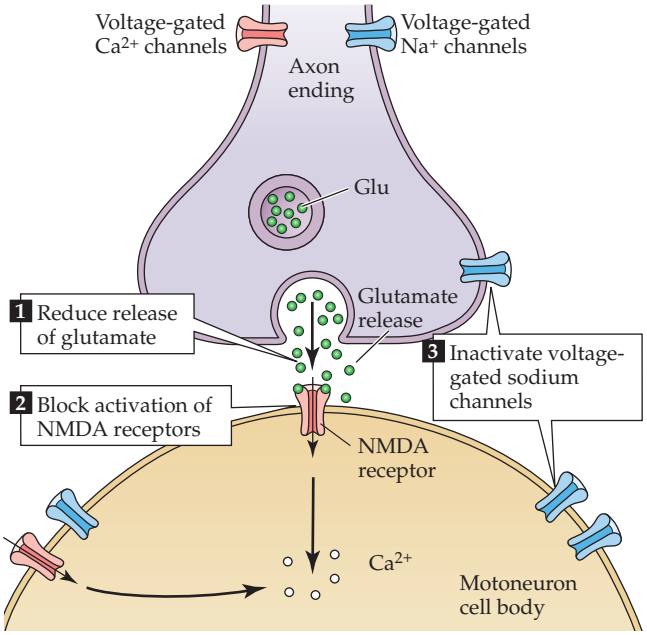


FIGURE 20.16 Riluzole is effective in preventing glutamate transmission by three mechanisms It can (1) reduce release of glutamate (Glu), (2) block activation of NMDA receptors, and (3) inactivate voltage-gated sodium channels, thereby preventing the transmission of action potentials. (After Doble, 1996.)

Following a grassroots fund-raising effort called the Ice Bucket Challenge, a new drug was approved in 2017 for the treatment of ALS. In July and August 2014, an online challenge started by Pete Frates and Pat Quinn (ALS patients) and Frates's friend Corey Griffin asked those challenged to either dump a bucket of ice water on their heads or donate to the ALS society. The challenge resulted in more than $100 million in donations to the ALS Association and millions more to ALS charities in other countries. That funding led directly to new drug development. Edaravone (Radicava) is a free-radical scavenger that is thought to be neuroprotective by preventing oxidative stress damage to neurons. It is administered in an intravenous infusion daily for 14 days, with a break of 2 weeks that is followed by 10 days of infusion. The drug slows physical decline in patients on the infusion, as measured by the revised ALS functional rating scale (ALSFRS-R). There are side effects to the infusion, most commonly bruising, gait (walking) disturbance, and headache. Long-term effects on disease progression and survival are not yet available, because the drug is so new on the market. The cost, however, is steep, with a yearlong, undiscounted cost of over $145,000.

Other treatments for ALS are largely symptomatic and are not pharmacological. These might include splinting of affected limbs and, at the patient's choice, measures to augment breathing function. When swallowing function is lost, patients might be fed through a gastrostomy tube.

Multiple Sclerosis

Multiple sclerosis (**MS**) is perhaps the least predictable of the disorders discussed in this chapter. Thought to be primarily an autoimmune disorder, MS is the result of a chronic attack on the brain, spinal cord, and optic nerves. In particular, the autoimmune target is the protein in the myelin produced by **oligodendrocytes,** as opposed to that produced by **Schwann cells** in the peripheral nervous system (see Chapter 2). Multiple sclerosis might take one of four courses: (1) relapsing-remitting MS (RRMS), (2) primary progressive MS (PPMS), (3) secondary progressive MS (SPMS), or (4) progressive-relapsing MS (PRMS) (**TABLE 20.1**). Disease progression is evaluated in several ways:

- Lesions in the central nervous system (CNS) can be visualized on magnetic resonance imaging (MRI); doctors will look for scarred lesions and gadolinium-enhanced new lesions (when given during an MRI, gadolinium is a contrast material that indicates areas of active inflammation).
- Sensory-evoked potentials can indicate deficits in signaling through sensory pathways.
- Neurological and functional exams can be used to assess physical and cognitive function.

TABLE **20.1** MS Can Take One of Four Courses

Type	Incidence	Course
Relapsing-remitting (RRMS)	85%	Clearly defined attacks of neurological deficits, which are followed by times of partial or complete recovery. During the remissions, disease progression is halted.
Primary progressive (PPMS)	10%	Neurological deficits are experienced slowly but progressively worsen over time. The rate of progression varies across cases and even for a given patient such that there can be plateaus or brief periods of minor improvement.
Secondary progressive (SPMS)	Approximately 50% of those with RRMS developed this before disease-modifying medications were available. Long-term data are not yet available on whether this number has declined.	After first presenting with RRMS, these patients start a period of steady decline. There may still be periods of relapse and minor improvement (remissions) or plateaus in progression.
Progressive-relapsing (PRMS)	5%	The worst of the possibilities, this course includes steady decline from disease onset, along with clear attacks of worsening function. While there may be some recovery from these attacks, there are no periods of remission from disease symptoms.

For more information about these and other aspects of MS, see the National MS Society website (www.nationalmssociety.org).

The symptoms of MS are variable and unpredictable

The symptoms experienced by any one person with MS vary widely across the disease course and vary to an even greater extent across patients. The more common symptoms include **fatigue**, numbness, walking/balance/coordination problems, bladder and/or bowel dysfunction, vision problems, dizziness and vertigo, sexual dysfunction, pain, cognitive dysfunction, emotional changes, and **spasticity**. Other, less common symptoms include speech disorders, swallowing problems, persistent headache, hearing loss, seizures, tremor, breathing problems, and itching.

The symptoms listed here can lead to secondary and tertiary symptoms of the disease. If a person with MS is experiencing bladder dysfunction, she may be at significant risk for repeated urinary tract infections (UTIs)—an example of a secondary symptom. Other examples of secondary symptoms include prolonged periods of inactivity due to motor symptoms that may cause muscle weakness, pressure sores, and decreased bone density. Tertiary symptoms are those that are a result of the disease's impact on the person's life; job loss or limitations, stress, failure of relationships, and social isolation are some examples.

Depression is more common in those with MS than in the general population; the lifetime risk is approximately 50% in people with MS versus about 12% to 15% in those without MS (Siegert and Abernethy, 2005). It is likely that this condition can be either a primary symptom or a tertiary symptom of MS. Studies of lesion location and symptom severity reveal variation in the association of depression with lesion location, although there may be a relationship between some frontal and temporal region lesions and depression. Significant concern arises when rates of suicidal ideation are investigated in those with MS, along with the impact of depression, in general, on quality of life and on participation in therapeutic interventions. **TABLE 20.2** summarizes the symptoms of MS and the other neurodegenerative diseases discussed in this chapter.

Diagnosis

The diagnosis of MS is an inexact science and often takes some time. This is the case because the symptoms of MS can result from several disorders. To receive a diagnosis of MS, one must have lesions in at least two distinct areas of the CNS (or optic nerves). Evaluation must indicate that these lesions happened at least 1 month apart. Perhaps most important, other causes of the symptoms (such as viral infections, exposure to toxic chemicals, vitamin B_{12} deficiency, and Guillain-Barré syndrome) must be ruled out.

Lesions are visualized using MRI. To differentiate between older and more current lesions, gadolinium can be used as a contrast agent to reveal active inflammation. This can help with the timing of lesions for diagnosis. However, about 5% of those who have MS do not have lesions visible on MRI in the early stages of

TABLE 20.2 Neurodegenerative Disorders Can Have a Variety of Symptoms

	Symptoms			
Disorder	**Motor**	**Sensory**	**Cognitive**	**Psychiatric**
Parkinson's disease (PD)	Resting tremor; akinesia; bradykinesia; rigidity; postural instability; retropulsion; anteropulsion; festination; dystonia; akathisia	Loss of smell	PD dementia; bradyphrenia; mental inflexibility; visuospatial deficits	PD dementia; hallucinations
Alzheimer's disease (AD)	Inability to perform self-care activities such as bathing, cooking, and driving	—	Forgetfulness; progressive memory loss; verbal communication deficits; anomia; poor judgment; slowed thinking	Altered emotional processing; personality changes; anhedonia; sleep disturbance; agitation; delusions; hallucinations
Huntington's disease (HD)	Choreic movements; rigidity; dystonia; swallowing and speech difficulties; gait problems	—	Planning and organization difficulties; perseveration; attention deficits; memory difficulties; language use issues	Obsessive-compulsive disorder; bipolar disorder; mania; depression; irritability; impulsivity; anxiety; other personality changes
Amyotrophic lateral sclerosis (ALS)	Focal and progressive muscle weakness; tripping; clumsiness; abnormal fatigue; muscle fasciculation; slurred speech; difficulty with voice projection, breathing, and chewing and swallowing	—	—	Exaggerated emotional responses such as uncontrolled laughing or crying
Multiple sclerosis (MS)	Fatigue; problems with coordination and gait; sexual, bladder, and bowel dysfunction; spasticity; difficulty swallowing	Vision loss; dizziness/vertigo; persistent headache; pain	Memory and attention deficits	Emotional changes

symptoms. Another test that can be done to support a diagnosis of MS is measurement of responses to visual evoked potentials (VEPs). These potentials measure the speed at which information passes through the visual system to reach the appropriate processing area of the brain. Brief visual stimuli are presented, and electrodes on the scalp overlying the visual cortex in the occipital lobe measure resulting brain activity. Another indicator of MS, but unfortunately also of other immune-activating issues in the brain, is the presence of **oligoclonal bands** in the cerebrospinal fluid without presence in the blood). These bands, visualized with protein electrophoresis methods, are actually immunoglobulins that indicate inflammatory processes within the CNS. When combined with MRI and VEP data, they strengthen confidence in a diagnosis of MS.

Causes of MS

The causes of MS are still unknown, but researchers have identified several factors that may contribute to the development of the disease.

IMMUNOLOGICAL MS is generally thought of as an autoimmune disease because of the presence of autoreactive **T cells**, which recognize oligodendrocyte myelin-specific antigens. Although the exact antigens have yet to be definitively identified, it is known that T cells cross the blood–brain barrier and secrete molecules that are damaging to neurons and release pro-inflammatory cytokines. While the brain is generally considered immunoprivileged (meaning that there is little interaction with the immune system), some immune cells enter the brain to surveil the environment. If they do not find a target,

they die in the unwelcoming brain environment. If they do find their target, they recruit more cells to the area.

ENVIRONMENTAL Some of the evidence for an environmental contributor to the development of MS is the interesting trend toward a geographical risk map (Simpson et al., 2011). Generally, the risk for MS is very much greater in those who live above 40° latitude in the northern hemisphere (**FIGURE 20.17**). The risk for development of MS seems to be determined by the location of residence up to about age 15. Those who move to a temperate climate from a tropical one before the age of 15 take on the increased risk of a temperate climate. Those who make this move after age 15 keep the risk level from the tropical climate. There are several risk factors for MS that might also be linked to this geographical anomaly. One is that vitamin D may be protective. Those who live in more tropical climates tend to spend more time in the sun and therefore produce more vitamin D.

Interestingly, the warmer months in both the United States and Italy tend to increase relapses of MS in diagnosed patients. The risk in the United States, particularly in Massachusetts, was found to be highest between May and August (Meier et al., 2010). In the study conducted in Italy, the highest risk was between May and June, with a smaller peak in November and December. Although these studies are small, they do indicate an interesting seasonal pattern that may invite further study.

As with PD and ALS, there may be a role for environmental toxins and metals in disease onset. There are clusters, or areas, where the rate of MS is higher than would normally be expected that are currently being investigated for the presence of a toxin or toxins that may influence the development of the disease.

INFECTIOUS Infectious processes can also increase the likelihood of developing MS. Infection with measles, human herpesvirus 6, Epstein-Barr (EB) virus, and *Chlamydia pneumoniae* may increase one's risk (about 90% of all those with MS have also had an EB virus infection such as mononucleosis). These infections are more likely (and spread more easily) in colder climates, where residents are likely to spend more time inside.

GENETIC MS is not a strictly hereditary disease. There has been no identified "MS gene," as there is for Huntington's disease. But indications suggest that some genes may increase a person's risk for developing this disorder. This conclusion comes from the fact that first-degree relatives of those with MS are at greater risk for development of MS than are people in the general population. It is likely that there are gene variants that make one more likely to react to certain environmental or immune triggers with the development of autoimmunity.

Treatments fall into several categories for MS and can be very effective

Treatments for MS may be disease modifying or intended to treat an acute exacerbation. Still others may be used to treat specific symptoms.

DISEASE-MODIFYING TREATMENTS In contrast to many of the other disorders discussed in this chapter, MS can be treated with several drugs that can reduce the frequency and severity of relapses and the accumulation of lesions and that appear to slow the accumulation of disability.

The first class of drugs approved for use in MS was the interferons. **Interferon beta-1a** and **-1b** likely work in MS by increasing the function of **suppressor T cells**. These T cells modulate the immune system and maintain tolerance to self-antigens in contrast or opposition to the myelin-reactive T cells described previously. Thus, they decrease the self-attack in MS. The

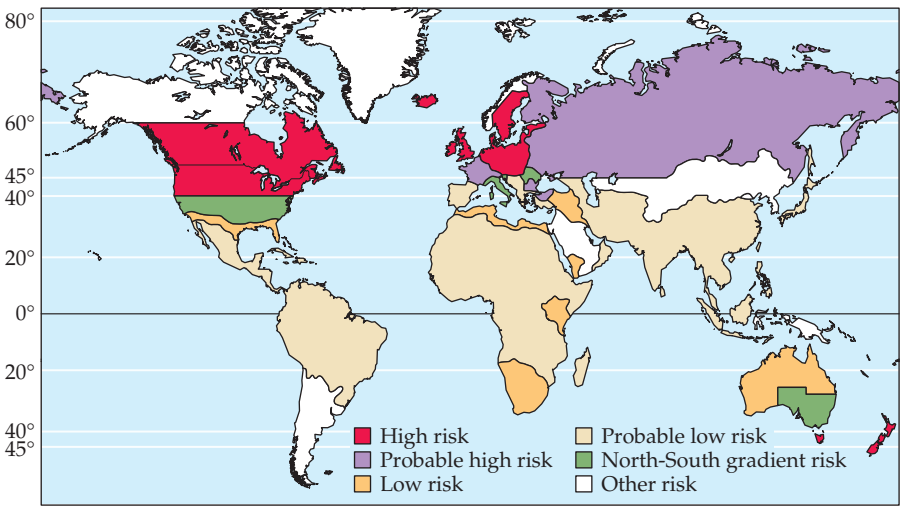


FIGURE 20.17 Geographic patterns to the distribution of multiple sclerosis (MS) cases In general, colder climates bring increased risk for the development of MS. (After Rose et al., 1996 and McAlpine et al., 1965.)

four interferon drugs are Avonex and Rebif (interferon beta-1a) and Betaseron and Extavia (interferon beta-1b). Each is administered by injection—Avonex once per week by IM injection, the rest every other day or three times per week by SC injection. The side effects of these drugs are flu-like symptoms and injection site reactions. Evidence suggests the possibility of a relationship between therapy with these drugs and depression, but it remains unclear whether this is an increased risk for depression above the effect of MS itself.

Glatiramer acetate (Copaxone) is a synthetic protein that simulates myelin basic protein, a component of myelin that may be one of the antigenic targets of autoimmunity. It is thought to work by blocking myelin-damaging T cells, but it is unclear how it achieves this effect. Side effects of Copaxone generally resolve on their own and include injection site reactions, runny nose, tremor, unusual tiredness or weakness, and weight gain. About 13% of patients taking the drug have a one-time (although not necessarily a first-time) reaction, which includes very short-lived (about 15 minutes) flushing, chest tightness, palpitations, anxiety, and difficulty breathing.

Mitoxantrone (Novantrone) is the only MS drug currently available as a generic drug. It is an antineoplastic agent, and before its use for MS, it had been used only to treat cancers. Novantrone works in MS because it suppresses several components of the immune system, including T cells, B cells, and macrophages. This is the only drug that is approved for subtypes of MS other than RRMS. It is approved for use in worsening RRMS, SPMS, and PRMS, but not in PPMS. Four injections are administered across a year (injected once every 3 months). Risks are similar to those of other chemotherapy drugs and include cardiotoxicity and secondary acute myelogenous leukemia.

Following its original approval, natalizumab (Tysabri) was briefly removed from the market by the FDA because of an increased incidence of progressive multifocal leukoencephalopathy (PML). An IV infusion that is administered four times per year, Tysabri is a monoclonal antibody that hampers movement of damaging immune cells from the bloodstream across the blood–brain barrier. Further study revealed that the risk for PML is increased in those who have been exposed to **John Cunningham (JC) virus** and have JC virus antibodies. JC virus causes PML in those with suppressed immune systems (e.g., individuals with acquired immunodeficiency syndrome [AIDS] or those receiving treatment with immunosuppressants). After this discovery, Tysabri was reapproved but contraindicated for those at particular risk for development of PML.

The goal in the field of MS therapy research has long been to develop a disease-modifying medication that can be administered orally. Recent advancements have actually led to two such medications. Fingolimod (Gilenya) is a sphingosine 1-phosphate receptor modulator. This therapy causes retention of lymphocytes in lymph nodes, thereby preventing their entry to the CNS through the blood–brain barrier. This, in turn, prevents inflammatory damage to neurons. The side effects are relatively mild and include headache, flu, diarrhea, back pain, and cough. The side effect of abnormal liver tests is a little more serious.

Another recent MS disease-modifying medication is also a drug that is administered orally. Teriflunomide (Aubagio) is a pyrimidine synthesis inhibitor that inhibits the function of specific immune cells. It is closely related to a therapy for another autoimmune disorder, rheumatoid arthritis. Aubagio reduces the proliferation of both T and B cells. It also reduces T-cell cytokine release. The side effects are similar to those seen with Gilenya and include diarrhea, nausea, flu, alopecia (thinning/loss of the hair), and abnormal liver tests.

TREATING EXACERBATIONS The most common mechanism for treating acute exacerbations that are affecting someone's ability to perform at home or at work is the administration of corticosteroids. Three are commonly used on a short-term basis, generally for 3 to 5 days, to treat a relapse: prednisone (administered PO), methylprednisolone (MP; given as an IV), and dexamethasone (also administered by IV means). MP is also used for acute optic neuritis. These drugs work through their powerful anti-inflammatory effects. The side effects of these drugs prohibit their long-term use in the management of MS and include stomach irritation, elevated blood sugar, water retention, restlessness, insomnia, and mood swings.

MANAGING SYMPTOMS Many medications are used to minimize the symptoms of MS, in addition to the disease-modifying and exacerbation-treating medications. Antidepressants, pain medications, and drugs for bladder and bowel dysfunction are common. Dalfampridine (Ampyra) has been approved for the treatment of walking dysfunction in those with MS. A potassium channel blocker, Ampyra, allows transmission through demyelinated fibers in motor pathways. This medication has several side effects, including UTI, difficulty sleeping, dizziness, headache, nausea, weakness, back pain, problems with balance, MS relapse, burning, tingling/itching of the skin, irritation of the nose and throat, constipation, indigestion, and throat pain. Recently, the FDA added a notice to the prescribing information for Ampyra, indicating risk for seizures associated with this medication in those who have reduced kidney function.

TREATMENT OF PROGRESSIVE MS Prior to 2017, all of the MS therapies on the market were focused on RRMS, not because of lack of interest in the progressive

forms, but because of a lack of efficacious therapies. That changed with the approval of ocrelizumab (Ocrevus) for PPMS. The *mab* at the end of the generic drug name indicates that the drug is a monoclonal antibody, a biologic treatment. The antibody targets CD20-positive B cells, which contribute to the autoimmune activity that results in MS symptoms. Ocrevus is administered as an infusion—initially two infusions that are 2 weeks apart and then one infusion every 6 months. As with all MS drugs that affect the immune system, infections are a possible side effect, including reactivation of hepatitis B and potentially PML (though no PML infections were seen in clinical trials). Ocrevus may also increase a patient's risk for cancer, particularly breast cancer. Ocrevus reduces relapses in RRMS and slows progression in about a quarter of patients with PPMS. It costs approximately $60,000 per year, which is below the cost of many MS treatments.

Several nonmedication treatments are also used in the management of MS symptoms. These include participation in physical or occupational therapy, visits to a speech/language pathologist, cognitive rehabilitation, and assistive devices used inside and outside the home. In addition to the therapies currently used to try to prevent damage, much research is being focused on repairing damage, particularly through remyelination of affected neurons. See **BOX 20.2** for more about these potential treatments.

BOX 20.2 Pharmacology in Action

Can We Repair or Replace Myelin?

The consequences of demyelination are seen quite clearly when one looks at the symptoms and course of multiple sclerosis. There are other disorders, though, which would also benefit from drugs or biologic therapies that bring about remyelination of axons, such as spinal cord injury and the leukodystrophies. Remyelination happens naturally, at least to some extent, after demyelinating events like those in MS. This accounts for the most common pattern of MS, relapsing-remitting. But also true is that for most patients with MS, their RRMS will transition to a chronic, progressive form of the disease. This happens because of changes in regeneration capacity of cells in the human body as we age. It is, in fact, the basis of aging. Our tissues and cells are less able to regenerate themselves. When myelin is not regenerated, the underlying axons are damaged, contributing to permanent disability.

The mechanisms and potential therapies for demyelination are reviewed extensively by Franklin and Ffrench-Constant (2017). Remyelination happens through a series of stages. First, the oligodendrocyte progenitor cells (OPCs) are activated by damage to myelin and the resulting immune response to the damage. Once activated, they divide (proliferate) and migrate to the areas of demyelination. Differentiation into mature, myelinating oligodendrocytes is the final stage. Despite the similarity of these stages to those that happen during development, the myelin that results from these remyelinating processes is thinner than the myelin made during development that was lost to disease or injury.

Remyelination efficiency is affected by both local and systemic factors. Locally, there are inhibitory signals (in the myelin itself) that prevent the activation of the remyelination process. Systemically, the immune system must respond to clear the myelin debris that inhibits remyelination, in order for remyelination to occur. The immune cells themselves may also offer signals for remyelination. Activated macrophages and T cells have been implicated in the remyelination process. Animal models of demyelination indicate that regulatory T cells actively promote oligodendrocyte differentiation and remyelination (Dombroski et al., 2017).

Alternatively, it may be possible to accelerate the naturally occurring remyelination by increasing the presence of receptors like the retinoic acid receptor gamma RXRγ. One of the fascinating ligands for this receptor is vitamin D, which has been shown to be a susceptibility factor for the development of MS (see Figure 20.17 and information about geographic susceptibility). This implies that a lack of vitamin D may not only make one more likely to get MS, but also prevent remyelination. The addition of vitamin D either through natural means (exposure to sunlight) or as a supplement may help prevent and treat the disease.

Drug development for remyelination is proceeding on two fronts (Franklin and Ffrench-Constant, 2017). In one, the targets are signals that regulate the remyelination process at its various stages. Some of these, like a monoclonal antibody against an inhibitory molecule, LINGO-1, have progressed to the clinical trial stage. While this had early success in optic neuritis, it failed in an MS trial. Preclinical work has identified other potential targets such as the protein tyrosine phosphatase receptor type Z (PTPRZ) (Kuboyama et al., 2015). Normally, the activation of PTPRZ prevents the differentiation of oligodendrocytes, keeping

(*Continued*)

BOX 20.2 Pharmacology in Action (*continued*)

them in the precursor state. By using pleiotrphin to block the activity of PTPRZ, researchers increased the differentiation of oligodendrocytes in culture.

In the second prong of drug development for remyelination, high-throughput screening of existing drugs is occurring. This has brought about several potential therapies, for which the path to approval is faster given the prior approval by the FDA for other conditions. One, the drug clemastine, an antihistamine approved for the treatment of allergies, has shown promise in optic neuritis (a common MS symptom) in clinical trials.

While there is promising evidence that remyelination can be affected by drug intervention, there are several major challenges to the progress of these therapies. One is the lack of a consistent preclinical model. None of the demyelinating models in animals (e.g., experimental autoimmune encephalomyelitis, toxin-induced demyelination) reliably model the time course and process of demyelination and remyelination seen in human MS. In addition, the lesions in patients with MS show variability. In some, remyelination happens; in others, there are not enough OPCs generated or migrated to the site of the lesion to allow for differentiation into myelinating oligodendrocytes. It should be expected, then, that there will be several failures of clinical trials, as translation from imperfect animal models to approved human therapies is challenging.

Section Summary

- Huntington's disease (HD) is an inherited, single-gene, neurodegenerative disorder that leads to significant motor, cognitive, and psychiatric symptoms.
- The *huntingtin* gene contains a CAG trinucleotide repeat, and HD is tied to an abnormal number of repeats (generally 40 repeats).
- Motor function deficits are characterized by choreic movements, which are jerky, writhing movements that are present during waking hours. Additionally, speech, swallowing, and gait problems are noted in patients. Psychiatric symptoms associated with HD include OCD, bipolar disorder, depression, and mania.
- No disease-modifying treatments are available for HD, but one drug, tetrabenazine, is approved for treatment of the excessive movement found in HD. Tetrabenazine works by decreasing monoamine vesicle packaging, thereby reducing dopamine signaling. The decreased DA signaling, however, can bring about depressive and Parkinsonian side effects.
- Amyotrophic lateral sclerosis (ALS) is a degenerative disorder that affects motor function but spares most other cognitive and mood function.
- Degeneration of upper and lower motor neurons results in a progressive loss of motor function that generally starts with the far extremities and moves "inward" until muscles of breathing are finally affected. Some motor groups are spared, including bladder and bowel function and eye movements. A strange side effect of this degeneration is an exaggeration of emotional behavioral responses, including laughing and crying.
- The mechanism of degeneration of the motor neurons is still poorly understood but seems to involve protein aggregation in the cells and inflammation. These processes cause the triggering of apoptotic cell death pathways.
- Two FDA-approved therapies are available for ALS: riluzole has modest disease-modifying effects, generally increasing life expectancy by about 2 to 3 months, and may delay reliance on mechanical breathing support; Radicava is a disease-modifying drug that slows progression of motor symptoms.
- Multiple sclerosis is widely believed to be the result of an autoimmune attack on central nervous system myelin.
- Four subtypes of MS are defined by their course: relapsing-remitting MS, primary progressive MS, secondary progressive MS, and progressive-relapsing MS.
- Symptoms of MS vary widely and are largely unpredictable. Depending on the location of the immune attack, symptoms may include motor, sensory, gait, emotional, cognitive, or vision problems. Depression is often comorbid with MS, at rates that are not explained entirely by the stress of having a neurological disorder.
- MS is diagnosed by MRI, neurological exam, and evidence of neuroinflammation.
- Immunological, environmental, infectious, and genetic contributors may play a role in the

development of MS, but no clear-cut cause has been identified.

- Treatments fall into disease-modifying and symptomatic categories.
- Disease-modifying treatments include several interferon drugs (Avonex, Rebif, Betaseron, and Extavia), Copaxone (a protein that stimulates myelin basic protein), Novantrone (a cancer drug), Tysabri (an antibody that prevents immune cells from entering the brain), Gilenya (a drug that keeps immune cells from leaving the lymph system), and Aubagio (which inhibits immune cell function). Recently, Ocrevus was approved for treatment of both RRMS and PPMS; it is the first available therapy for the progressive form of the disease.
- Symptomatic treatments include corticosteroids for treating acute exacerbations, antidepressants, drugs for bowel and bladder function, and Ampyra (a drug that treats gait and walking dysfunction).

STUDY QUESTIONS

1. What are the primary cell types lost in Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS)?
2. Describe the primary symptoms of Parkinson's disease.
3. What are the known genetic risks for Parkinson's disease?
4. What are the primary categories of drugs for Parkinson's disease? How does levodopa alleviate some Parkinson's disease symptoms?
5. What would the diagnosis be, according to the *DSM*, for Alzheimer's disease?
6. What are the cardinal cellular disturbances in Alzheimer's disease? How are they thought to come about?
7. What are the risk genes for Alzheimer's disease? What are the deterministic genes?
8. What neurotransmitter is the primary target of the drug therapies for Alzheimer's disease? How is that neurotransmitter affected by the drugs?
9. What is the cause of Huntington's disease? How does this differ from the other diseases in this chapter?
10. What are the primary symptoms of Huntington's disease?
11. What symptoms are targeted by the currently available Huntington's disease medications?
12. What are the major motor symptoms of ALS?
13. What are the two medications approved by the FDA for treatment of ALS? How do they work?
14. What are the subtypes of MS?
15. Why are the symptoms of MS so unpredictable?
16. What is thought to be the primary pathological mechanism of MS? What is the evidence that supports this?
17. Although it is not the case for many of the disorders in this chapter, there are disease-modifying treatments for MS. What are the different mechanisms of these drugs?
18. What are the symptomatic treatments for MS?

Go to the *Psychopharmacology* Companion Website at oup-arc.com/access/meyer-3e for animations, web boxes, flashcards, and other study aids.

Glossary

Numbers in brackets refer to the chapter(s) in which the term is introduced.

A

AADC See *aromatic amino acid decarboxylase*. [5, 6]

AAS See *anabolic–androgenic steroids.* [16]

absolute refractory period Short period of time after an action potential when Na^+ channels close and are refractory until repolarization to resting potential occurs. [2]

absorption Movement of a drug from the site of administration to the circulatory system. [1]

abstinence syndrome Condition characterized by unpleasant symptoms when an individual tries to cease drug use. [9, 11]

acamprosate (Campral) Partial antagonist at NMDA receptors used for the treatment of alcoholism. [10]

acetyl coenzyme A (acetyl CoA) Precursor necessary for ACh synthesis. [7]

acetylcholine (Ach) Neurotransmitter involved with the central and peripheral nervous system and synthesized by the cholinergic neurons. It is the target of many of the deadliest neurotoxins. [7]

acetylcholinesterase (AChE) Enzyme that controls levels of ACh by breaking it down into choline and acetic acid. [7]

Ach See *acetylcholine*. [7]

AChE See *acetylcholinesterase*. [7]

ACTH See *adrenocorticotropic hormone*. [3]

action potential Major depolarization generated in the axon hillock that is transmitted down the axon. [2]

active zones Areas along the axon terminal, near the postsynaptic cell, that are specialized for neurotransmitter release. [3]

acute tolerance Rapid tolerance formed during a single administration of a drug, as is the case with alcohol. [1, 10]

additive effects Drug interactions characterized by the collective sum of the two individual drug effects. [1]

adenosine Blockade of receptors for this substance is responsible for caffeine's stimulant effects. [13]

adoption studies Studies used to understand how heredity contributes to a disorder by comparing the incidence of the disorder in the biological and adoptive parents of people adopted at an early age who have the disorder. A higher incidence of the disorder among biological parents than adoptive parents suggests a hereditary influence. [18]

adrenal cortex Outer portion of the adrenal gland that secretes glucocorticoids. [3]

adrenal glands Endocrine glands that are located above the kidney and secrete EPI, NE, and glucocorticoids. An adrenal gland is composed of the adrenal medulla and the adrenal cortex. [3]

adrenal medulla Inner portion of the adrenal gland that secretes the catecholamines EPI and NE. [3]

adrenergic Adjectival form of adrenaline, also called epinephrine (EPI). May be used broadly to include both NE- and EPI-related features. [5]

adrenoceptors Receptors to which NE and EPI bind; part of the metabotropic receptor family. Also known as an adrenergic receptors. [5]

adrenocorticotropic hormone (ACTH) Hormone secreted by the anterior pituitary that stimulates glucocorticoid synthesis and release from the adrenal cortex.

affinity Ability of a molecule to bind to a receptor, which then determines potency. [1]

afterglow Desired subjective state that may follow the peak experience produced during a hallucinogenic drug-assisted therapeutic session. [15]

agoraphobia Fear of public places. [17]

akathisia Constant urge to move certain body parts. [20]

akinesia Difficulty in initiating movement. [20]

albuterol (Ventolin) Drug that selectively stimulates the β-adrenoceptor. It is used in asthma treatments. [5]

alcohol Ethyl alcohol is an organic compound that is a product of fermentation and belongs to the class of sedative–hypnotics. [17]

alcohol dehydrogenase Enzyme in the liver and stomach that oxidizes alcohol into acetaldehyde. [10]

alcohol or **barbiturate withdrawal** Symptoms associated with the termination of alcohol, barbiturate, or benzodiazepine use. The three drugs are cross dependent; administration of one alleviates withdrawal symptoms of the other. [17]

alcohol poisoning Toxic effects associated with the ingestion of excess alcohol, characterized by unconsciousness, vomiting, irregular breathing, and cold, clammy skin. [10]

alcohol use disorder A form of substance abuse characterized by compulsive alcohol seeking and use despite damaging social and health effects. Formerly called *alcoholism*. [10]

alcohol-induced cirrhosis Condition seen in chronic alcohol abusers caused by scar tissue formation that promotes cell death as scar tissue cuts off blood supplies. [10]

alcohol-induced hepatitis Condition seen in chronic alcohol abusers caused by death of liver cells and characterized by inflammation of the liver, fever, jaundice, and pain. [10]

aldehyde dehydrogenase (ALDH) Enzyme in the liver that metabolizes the acetaldehyde intermediate formed by alcohol oxidation into acetic acid. [10]

ALDH See *aldehyde dehydrogenase*. [10]

allodynia Condition characterized by painful responses to stimuli that do not normally cause pain. [6]

allostasis Adaptive biological process in which an organism's response to repeated threats or challenges results in long-lasting physiological or behavioral changes. This concept is distinguished from homeostasis, which refers to the tendency of an organism to maintain physiological or behavioral stability in the face of threats or challenges (i.e., to remain unchanged). [9]

allosteric modulators Compounds that bind to a receptor site distinct from the main agonist binding site, may or may not have an effect on the receptor when administered alone, and either enhance (in the case of a positive allosteric modulator) or reduce (in the case of a negative allosteric modulator) the effectiveness of an agonist on the receptor. They have potential therapeutic use because of their ability to tune receptor activity more subtly than either a receptor agonist or an antagonist. [3, 7]

allosteric sites Binding sites on a receptor protein that modulate the receptor's response, either positively or negatively, to a receptor agonist. [3]

allylglycine Drug that blocks GABA synthesis, inducing convulsions. [8]

alosetron (Lotronex) Drug that inhibits 5-HT_3 receptors. It is used to treat the diarrhea-predominant form of irritable bowel syndrome. [6]

α-methyl-para-tyrosine (AMPT) Drug that inhibits TH activity, thereby reducing catecholamine synthesis. [5]

α-PVP Cathinone derivative (α-pyrrolidinovalerophenone) that is an abused stimulant drug. It is a member of a group of compounds sometimes called "bath salts," "plant food," "pond water cleaner," and "legal highs." [12]

5α-reductase Enzyme that converts testosterone to 5α-dihydrotestosterone. [16]

α-synuclein A protein that is found primarily in neurons and accumulates to form Lewy bodies in people affected with Parkinson's disease and some forms of dementia. [20]

ALS See *amyotrophic lateral sclerosis*. [8]

altered states of consciousness (ASC) rating scale Psychometric scale developed to quantify the subjective effects of hallucinogenic agents. [15]

Alzheimer's disease Neurodegenerative disorder, almost always occurring in the elderly, which is characterized behaviorally by progressive loss of cognitive function and histologically by the presence of extracellular plaques containing beta-amyloid protein and intracellular neurofibrillary tangles containing hyperphosphorylated tau protein. It is the most common cause of dementia. [7, 20]

amino acids Essential building blocks of proteins, some of which also act as neurotransmitters. [3]

amotivational syndrome Symptoms of cannabis use that relate to poor educational achievement and motivation. [14]

AMPA receptor An ionotropic glutamate receptor selective for the synthetic amino acid agonist AMPA. [8]

ampakines Class of cognitive enhancing drugs that work by inhibiting the desensitization of glutamate AMPA receptors. [8]

amphetamine-induced stereotypy Model for schizophrenia induced by giving animals high doses of amphetamine to produce repetitive, stereotyped behavior. [19]

amphetamine Psychostimulant that acts by increasing catecholamine release in nerve cells. [5, 12]

AMPT See *α-methyl-para-tyrosine*. [5]

amygdala Part of the limbic system that helps to modulate emotional behavior and coordinates the various components of emotion. [2]

amyloid precursor protein (APP) Transmembrane protein. Cleavage by secretases forms Aβ. [20]

amyotrophic lateral sclerosis (ALS) Neurological disorder characterized by degeneration of the motor neurons of the spinal cord and cortex. Also known as Lou Gehrig's disease. [8]

anabolic–androgenic steroids (AAS) Group of performance enhancers characterized by their ability to increase muscle mass and produce masculine qualities. The name may be shortened to anabolic steroids. [16]

analeptics Drugs that act as circulatory, respiratory, or general CNS stimulants. [15]

anandamide Common chemical name of the arachidonic acid derivative that functions as an endogenous ligand for cannabinoid receptors in the brain. [14]

androgen receptor Target site of testosterone and other androgens, located within the cytoplasm of the cell and present in many tissues. [16]

androgens Male sex hormones secreted by the testes. [3]

anesthetics General anesthetics are substances that depress the CNS, decreasing all sensations in the body and causing unconsciousness. Local anesthetics do not cause unconsciousness, but prevent pain signals by blocking Na^+ channels. Some anesthetics such as nitrous oxide (also known as laughing gas), chloroform, and ether comprise a class of abused inhalant substances. [11, 16]

anhedonia Difficulty or lack of the ability to experience pleasure. Such a state is characteristic of many depressed patients and may also occur during drug withdrawal in an addicted person. [9]

anorectic Having the effect of reducing appetite. [6]

anosmia Inability to perceive odors. [20]

Antabuse See *disulfiram*. [10]

anterior pituitary Portion of the pituitary gland that secretes the hormones TSH, ACTH, FSH, LH, GH, and PRL. [3]

anterior Located near the front or head end of an animal. [2]

anteropulsion Feeling of being pushed forward. [20]

antibody Protein produced by the immune system for the purpose of recognizing, attacking, and destroying a specific foreign substance (i.e., an antigen). [4]

anticipatory anxiety Feeling of extreme worry over the possibility that a certain unpleasant event will occur in a particular, often public, situation. [17]

anticonvulsants Drugs, such as benzodiazepines, that prevent or control seizures. They are used to treat epilepsy. [17]

antireward system System within the brain that is recruited during the withdrawal/negative affect stage of the addiction spiral and plays an important role in the transition from controlled to compulsive drug use. Key components include the central amygdala and the neurotransmitters NE and CRF. [9]

antitussives Drugs that suppress the coughing reflex. [15]

anxiolytics Drugs that alleviate feelings of anxiety in humans and that reduce anxiety-related behaviors in animals. [17]

apnea Cessation of breathing. [6]

apolipoprotein E (ApoE) Protein that helps break down amyloid. Individuals carrying the E4 allele of the gene encoding this protein develop AD. [20]

apomorphine Drug that is a D_1 and D_2 receptor agonist and causes behavioral activation. It may also be used to treat erectile dysfunction by acting through DA receptors in the brain to increase penile blood flow. [5]

apoptosis Cell death resulting from a programmed series of biochemical events designed to eliminate unnecessary cells. It may also be called programmed cell death. [8]

APP See *amyloid precursor protein*. [20]

arachidonoyl ethanolamide (AEA) Formal chemical name of *anandamide*. [14]

2-arachidonoylglycerol (2-AG) An arachidonic acid derivative that functions as an endogenous ligand for brain cannabinoid receptors. [14]

arachnoid Membrane consisting of a weblike sublayer that covers the brain and spinal cord and contains CSF. One of the three meninges. [2]

area postrema Area in the medulla of the brain stem that is not isolated from chemicals in the blood. It is responsible for inducing a vomiting response when a toxic substance is present in the blood. [2]

arecoline Chemical from the seeds of the betel nut palm *Areca catechu* that stimulates muscarinic receptors. [7]

aromatase Enzyme that converts testosterone to estradiol. [16]

aromatic amino acid decarboxylase (AADC) Enzyme that catalyzes the removal of a carboxyl group from certain amino acids. It is responsible for the conversion of DOPA to DA in catecholaminergic neurons and the conversion of 5-HTP to 5-HT in serotonergic neurons. [5, 6, 20]

Artane See *trihexyphenidyl*. [7]

ASC See *Altered States of Consciousness rating scale.* [15]

aspartate Ionized form of aspartic acid. It is an excitatory amino acid neurotransmitter of the CNS. [8]

association analysis Determination of whether a particular genetic polymorphism is associated with a particular disease or trait. [10]

astrocytes Star-shaped glial cells that have numerous extensions and that modulate the chemical environment around neurons, metabolically assist neurons, and provide phagocytosis for cellular debris. [1, 2]

astroglia The collective terms for astrocytes. [1]

atomoxetine (Strattera) Selective norepinephrine uptake inhibitor used in the treatment of ADHD. [5]

atropine Drug found in nightshade, *Atropa belladonna*, and henbane, *Hyoscyamus niger*, that blocks muscarinic receptors. [7]

attentional set-shifting task Rodent version of the WCST in which the animals must choose the bowl with the food reward based on either odor or surface texture. [19]

autoimmune disorder Condition in which the immune system attacks part of one's own body. [7]

autoradiography Process used to detect the amount and location of bound radioligand by using a specialized film to create an image of where the radioligand is located within a tissue slice. [4]

autoreceptors Neuronal receptors in a cell that are specific for the same neurotransmitter released by that cell. They typically inhibit further neurotransmitter release. [3]

autosomal dominant Alzheimer's disease (ADAD) Familial version of AD caused by mutations in the genes for presenelin-1, presenilin-2, and amyloid precursor protein. [20]

axoaxonic synapses Junctions used for communication between the axon terminals of two neurons, permitting the presynaptic cell to control neurotransmitter release from the postsynaptic cell at the terminals. [3]

axodendritic synapses Junction used for communication between the axon terminal of a presynaptic neuron and a dendrite of a postsynaptic neuron. [3]

axon Long tubular extention from the soma of the nerve cell that conducts electrical signals away from the cell body and toward the axon terminals. [2]

axon collaterals Branches formed when an axon splits, giving the neuron the ability to signal more cells. [2]

axon hillock The segment of axon adjacent to the soma where the action potential is first generated. [2]

axoplasmic transport Method of transporting proteins along the microtubules of the cytoskeleton to designations throughout a neuron. Anterograde transport moves newly synthesized proteins from the soma. Retrograde transport moves waste back to the soma from the terminals. [2]

axosomatic synapses Junctions used for communication between a nerve terminal and a nerve cell body. [3]

ayahuasca Hallucinogenic mixture originating in the Amazon rain forest. It typically contains stalks from the *Banisteriopsis caapi* vine and leaves from *Psychotria viridis* and/or *Diplopterys cabrerana*. Together, these plants

provide the hallucinogenic tryptamine DMT along with β-carbolines that inhibit MAO and permit the DMT to reach the brain when the ayahuasca is consumed orally. [15]

Azilect See *rasagiline*. [5]

B

BAC See *blood alcohol concentration*. [10]

baclofen (Lioresal) Drug that is a selective agonist for the $GABA_B$ receptors. It is used as a muscle relaxant and an antispastic agent. [8]

barbiturates Drugs that act as a CNS depressant, in part by enhancing $GABA_A$ receptor activity. [8]

basal forebrain cholinergic system (BFCS) Collection of cholinergic nerve cells that innervates the cerebral cortex and limbic system structures. Damage to this system contributes to the symptoms of Alzheimer's disease. [7]

basal ganglia Nuclei of the telencephalon that includes the caudate, putamen, and globus pallidus. The structures help regulate motor control. [2, 20]

BDNF See *brain-derived neurotrophic factor*. [18]

BDZs See *benzodiazapine*. [8]

beam walking Device resembling a human gymnastics balance beam used to evaluate rodent fine motor coordination and balance. [4]

behavioral addictions Uncontrolled behaviors not involving substance use but that have characteristics similar to those seen in substance-related disorders. [9]

behavioral desensitization Technique used to treat phobias by introducing the fear-inducing stimulus in increments, allowing the patient to maintain a relaxed feeling in its presence. [17]

behavioral despair Technique used to measure depression in animals by placing them in a cylinder of water from which they cannot escape and recording the time it takes for them to abandon attempts to escape. [4]

behavioral supersensitivity An increased response to a drug treatment as a direct result of previous drug history or drug intake. [5]

behavioral tolerance The reduced effectiveness of a drug administered chronically that involves learning: either instumental or classical conditioning. [1, 10]

benzodiazepines (BDZs) Drugs that act as a CNS depressants, in part by enhancing $GABA_A$ receptor activity. [8]

benzoylecgonine Major metabolite of cocaine. [12]

benztropine mesylate (Cogentin) Anticholinergic drug used to treat early symptoms of Parkinson's disease. [7]

beta-amyloid protein (β-amyloid or **A-beta [Aβ])** Protein fragment derived from enzymatic cleavage of amyloid precursor protein. Primary component of the plaques characteristic of Alzheimer's disease. Also called A-beta and Aβ. [20]

β-carboline An inverse agonist at the BDZ modulatory site on $GABA_A$ receptors that makes GABA less effective in producing hyperpolarization. It is anxiogenic and is a useful tool in studying the neurobiology of anxiety. [17]

BFCS See *basal forebrain cholinergic system*. [7]

bicuculline Drug that blocks the binding of GABA to the $GABA_A$ receptor and acts as a convulsant. [8]

binge drinking Consumption of five or more alcoholic drinks within a 2-hour period. [10]

bioactivation A metabolic process that converts an inactive drug into an active one. [1]

bioavailability Concentration of drug present in the blood that is free to bind to specific target sites. [1]

biogenic amine A transmitter that is made by a living organism and contains at least one amine group. [5]

biopsychosocial model Model of addiction that attempts to give a full account of addiction by incorporating biological, psychological, and sociological factors. [9]

biotransformation Inactivation of a drug through a chemical change, usually by metabolic processes catalyzed by enzymes in the liver. [1]

bipolar disorder Type of affective disorder characterized by extreme mood swings between depression and mania. [18]

blackout Amnesia directly associated with heavy alcohol consumption. [10]

blood alcohol concentration (BAC) The amount of alcohol in a given unit of blood, usually given as a percent representing milligrams of alcohol per 100 milliliters of blood. [10]

bradykinesia General slowing of movement that is characteristic of Parkinson's disease. Examples include slowed movement of facial muscles leading to "stone-faced" expression and reduced hand movement resulting in micrographia (smaller handwriting). [20]

bradyphrenia Slowed response to questioning. [20]

brain-derived neurotrophic factor (BDNF) Protein of the CNS that stimulates cell proliferation, aids in cell survival and synaptic restructuring. It is also implicated in the neurotrophic hypothesis of depression. [8, 18]

brainstem Portion of the brain, consisting of the medulla, pons, and midbrain. [2]

breaking point The point at which an animal will no longer expend the effort required to receive the reward (e.g., in a drug self-administration paradigm). [4, 9]

breakpoint See *breaking point*. [9]

bridging Pattern of anabolic–androgenic steroid use in which a low dose is used to bridge between each high dose of the steroid (also called "blast and cruise"). [16]

broad-spectrum antipsychotics Class of drugs used to treat schizophrenia by blocking a wide range of receptors in addition to the D_2 receptor. [19]

bufotenine Tryptamine hallucinogen present in the toxic secretions of an American desert toad, *Bufo alvarius*. [15]

bulk endocytosis Mechanism for retrieval of large amounts of synaptic vesicle membrane after very strong or prolonged neuronal firing. [3]

Buprenex See *buprenorphine*. [11]

buprenorphine (Buprenex) An opioid agonist–antagonist used in opioid treatment programs that may be substituted for methadone and yields similar treatment results. [11]

bupropion (Zyban) Drug that inhibits DA and NE uptake and is also a weak *nAChR* antagonist. It is used in the treatment of tobacco dependence. [13]

burst mode Mode of neuronal cell firing characterized by the production of bursts of action potentials. [5]

buspirone (BuSpar) Drug that is a partial agonist at 5-$HT1_A$ receptors. Symptoms include increased appetite, reduced anxiety, reduced alcohol cravings, and a lower body temperature. It is prescribed as an antianxiety medication that lacks sedation, mental clouding, potential for abuse, or physiological dependence. [6, 17]

C

c-fos Transcription factor that rises rapidly within cells during increased neural activity. [4]

caffeine Stimulant drug found naturally in coffee and tea. It is also consumed in tablet form and in various beverages such as such drinks and energy drinks. [13]

caffeine dependence syndrome Disorder produced by chronic high-dose caffeine use and characterized by caffeine craving, difficulty controlling caffeine consumption, caffeine tolerance, and withdrawal symptoms that occur following abstinence. It is a recognized disorder within *ICD-10* but not within *DSM-5*. [13]

caffeine intoxication Disorder produced by recent high-dose caffeine use and characterized by symptoms of restlessness, nervousness, insomnia, and physiological disturbances including tachycardia (increased heart rate), muscle twitching, and gastrointestinal upset. [13]

caffeine use disorder *DSM-5* category with features similar to those of caffeine dependence syndrome. It is not a recognized disorder but has been designated for additional research. [13]

calcium/calmodulin kinase II (CaMKII) Enzyme stimulated by calcium and calmodulin that phosphorylates specific proteins in a signaling pathway. [3]

cAMP See *cyclic adenosine monophosphate*. [3]

Campral See *acamprosate*. [10]

candidate gene analysis Analysis of a gene that is suspected of involvement in the development, progression, or manifestation of a disease. [9]

candidate genes Genes that are suspected of involvement in the development, progression, or manifestation of a disease. [19]

cannabidiol (CBD) Phytocannabinoid that lacks the intoxicating and dependence-producing effects of THC. [14]

cannabinoid receptor Receptor for cannabinoids, including THC and anandamide. In the CNS, they are concentrated in the basal ganglia, cerebellum, hippocampus, and cerebral cortex. [14]

Carbamazepine (Tegretol) An anticonvulsant drug used to treat bipolar disorder. [18]

carbidopa A decarboxylase inhibitor that cannot cross the blood–brain barrier. Increases the availability of L-DOPA to the brain. [6, 20]

case–control method Technique used to identify genes associated with a disorder by comparing the genes of unrelated affected and unaffected people to determine if those who are affected are more likely to possess a particular allele. [10]

catalepsy State characterized by a lack of spontaneous movement. It is usually associated with D_2 receptor blockers (a DA receptor subtype), but can also be induced with a D_1 blocker. [4, 5]

cataplexy Loss of muscle control in patients with narcolepsy that is usually triggered by a strong emotion such as laughing or becoming angry or frustrated. [3]

Catapres See *clonidine*. [5]

catechol-*O*-methyltransferase (COMT) One of the enzymes responsible for metabolic breakdown of catecholamines. [5, 20]

catecholamines Group of neurotransmitters and hormones characterized by two chemical similarities: a core structure of catechol and a nitrogen-containing amine. They belong to a wider group of transmitters called monoamines or biogenic amines. [5]

cathinone Psychostimulant that is the primary active ingredient in khat. [12]

caudal The tail end of the nervous system is caudal or posterior. [2]

CB_1 Cannabinoid receptor of the metabotropic receptor family located in the CNS. [14]

CB_2 Cannabinoid receptor located primarily in the immune system. [14]

CBD See *cannabidiol*. [14]

central canal Channel within the center of the spinal cord filled with CSF. [2]

cerebellar peduncles Large bundles of axons that connect the cerebellum to the pons. [2]

cerebellum Large structure of the metencephalon that is located on the dorsal surface of the brain and that is connected to the pons by the cerebellar peduncles. It is an important sensorimotor control center of the brain. [2]

cerebral ventricles Cavities within the brain filled with CSF. [2]

cerebrospinal fluid (CSF) Fluid that surrounds the brain and spinal cord, providing cushioning that protects against trauma. It also fills the cerebral ventricles and the central canal of the spinal cord. [1]

cGMP See *cyclic guanosine monophosphate*. [3]

Chantix See *varenicline.* [13]

ChAT See *choline acetyltransferase.* [7]

chemogenetics The activation or suppression of a genetically engineered receptor with a designer ligand in order to study behavioral effects of longer duration compared to optogenetic manipulation. Sometimes referred to as DREADD. [4]

chlorogenic acids Class of chemicals present within brewed coffee that are hypothesized to confer a protective effect against the development of type 2 diabetes among people who drink at least three to four cups of coffee per day. [13]

choline acetyltransferase (ChAT) Enzyme that catalyzes the synthesis of ACh from acetyl CoA and choline. [7]

choline transporter Protein in the membrane of the cholinergic nerve terminal involved with the uptake of choline from the synaptic cleft. [7]

choline Precursor necessary for ACh synthesis. [7]

cholinergic Adjectival form of ACh. [7]

cholinesterase inhibitors Drugs that improve cognitive symptoms by increasing the presence of acetylcholine in the synapse by lessening breakdown of ACh. One of two categories of treatment for AD. [20]

chromaffin cells The cells of the adrenal medulla. [3]

chromatin remodeling One type of environmentally-induced epigenetic modification that increases or decreases gene transcription. [2]

chromosomes Double helical strands of DNA that carry genes. [2]

chronic mild unpredictable stress Rodent model of depression created by exposing animals to a series of stressful events in an unpredictable fashion. [4]

chronic obstructive pulmonary disease (COPD) Disorder of the respiratory system characterized by shortness of breath, wheezing, chronic coughing, and chest tightness. Two main conditions comprise COPD, namely emphysema and bronchitis. [13]

chronic social defeat stress Rodent model of depression created by the intense stress of being repeatedly placed as an intruder in a cage with a resident animal. [4]

classical conditioning Repeated pairing of a neutral stimulus with an unconditioned stimulus. Eventually the neutral stimulus becomes a conditioned stimulus and elicits a (conditioned) response that is similar to the original unconditioned response. This type of learning has a role in drug use and tolerance. [1]

clathrin Protein that participates in synaptic vesicle recycling, either in the endocytotic step of retrieving vesicle membrane from the axon terminal membrane or in the budding of new vesicles from endosomes in the axon terminal. [3]

clathrin-mediated endocytosis Process of synaptic vesicle membrane recovery that relies on the protein clathrin. [3]

clonidine (Catapres) An α_2-adrenergic agonist that stimulates autoreceptors and inhibits noradrenergic cell firing. It is used to reduce symptoms of opioid withdrawal. [5]

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cloning Method used to produce large numbers of genetically identical cells. [4]

closed State of a receptor channel in which the channel pore is closed, thereby preventing ion flow across the cell membrane. [7]

clozapine (Clozaril) Drug that inhibits 5-HT_{2A} and D_2 dopamine receptors. It is used to treat schizophrenia. [6]

club drug Street name for GHB, as well as MDMA and ketamine, coined as a result of their popularity at nightclubs. [16]

CNS depressants Large category of drugs that inhibit nerve cell firing within the central nervous system. They include sedative–hypnotics and are used to induce sleep and to treat symptoms of anxiety. [17]

co-agonists Substances needed simultaneously to activate a specific receptor. [8]

cocaethylene Metabolite formed from the interaction of cocaine and alcohol. It produces biological effects similar to those of cocaine. [12]

cocaine Stimulant drug that blocks reuptake of DA, NE, and 5-HT by neurons, thereby increasing their concentration in the synaptic cleft. [5, 12]

cocaine binges Periods of cocaine use lasting hours or days with little or no sleep. [12]

coding region Portion of the gene that codes for the amino acid sequence of a protein. [2]

Cogentin See *benztropine mesylate*. [7]

cognitive behavioral therapy (CBT) Type of psychotherapy that can be used to treat drug addiction by restructuring the drug user's cognitive (thought) processes and training the user either to avoid high-risk situations that might cause relapse or to employ appropriate coping mechanisms to manage such situations when they occur. [12]

cognitive symptoms A category of symptoms of schizophrenia that includes impaired working memory, poor executive function, and attention deficits. [19]

common disease–common variant hypothesis Hypothesis that genetically based susceptibility to a particular neuropsychiatric disorder stems from a pool of risk-conferring gene alleles that are possessed in common throughout the population. Each of these "risk alleles" confers a small increase in susceptibility to developing the disorder. [9]

common disease–rare variant hypothesis Hypothesis that genetic risk for a neuropsychiatric disorder stems from rare mutations or other genetic anomalies such as copy number variations (variable numbers of repeated stretches of DNA). It is alternative to the common disease–common variant hypothesis. [9]

comorbidity Diagnosis of simultaneous but distinct disease processes in an individual, such as the propensity for drug abusers to be diagnosed with other psychiatric problems. [9]

competitive antagonist Drug that binds to a receptor but has little or no efficacy. When it competes with an agonist for receptor sites, it reduces the effect of the agonist. [1]

compulsions Repetitive tasks that an individual feels obligated to complete in an effort to quell the anxiety caused by obsessive thoughts. [17]

computerized tomography (CT) X-ray based technique that provides computer-generated "slices" through the brain or body part that can be computer reconstructed into 3-dimensional images. [4]

COMT See *catechol*-O-*methyltransferase*. [5]

Comtan See *entacapone*. [5]

concentration gradient Difference in the amount or concentration of a substance on each side of a biological barrier, such as the cell membrane. [1]

conditioned emotional response Learned response to a neutral stimulus presented just prior to a negative stimulus (e.g., an electric shock) in an effort to create a fear association to the neutral stimulus. [4]

conditioned place preference Method used to determine the rewarding effects of a drug by allowing an animal to associate the drug with a specific environment and measuring its subsequent preference for that environment. [4]

conflict procedure Method that creates a dilemma for an animal by giving it the choice of selecting a reward that is accompanied by a negative stimulus. Conflict procedures screen drugs for antianxiety effects. [4]

construct validity Term that represents the extent to which the animal measurement tool actually measures the human characteristic of interest. [4]

contingency management program Type of addiction treatment program in which the client's drug taking is monitored by regular urine testing and abstinence is reinforced with vouchers redeemable locally for consumer products or services. [12]

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convergence Process by which neurons receive and integrate the numerous signals from other cells. [2]

COPD See *chronic obstructive pulmonary disease*. [13]

core Following an ischemic stroke, this is the inner region of damage within which the dying neurons cannot be rescued. [8]

coronal Sections cut parallel to the face. [2]

corpus callosum Large pathway connecting corresponding areas of the two brain hemispheres, allowing communication between each half of the brain. [2]

correlational relationship Connection between two events that appear related, but cannot be assumed to be cause and effect. [4]

corticosterone Glucocorticoid secreted by the adrenal cortex of rats and mice. [3]

corticotropin-releasing hormone (CRH) Hormone synthesized by neurons of the hypothalamus that stimulates ACTH release. Also known as corticotrophin-releasing factor (CRF). [3]

cortisol Specific glucocorticoid secreted by the adrenal cortex of primates. [3]

cotinine Principal product of nicotine metabolism by the liver. [13]

COX-2 See *cyclooxygenase-2.* [14]

crack Form of cocaine made by adding baking soda to a solution of cocaine HCl, heating the mixture, and drying the solid. [12]

craving Strong urge addicts feel, compelling them to take a drug. [9]

CRF_1 antagonists A drug that binds to CRF_1 receptors and produces little or no conformational change. In the presence of a CRF agonist, the agonist effect is reduced. [10]

CRH See *corticotropin-releasing hormone*. [3]

CRISPR Acronym for *c*lustered *r*egularly *i*nterspaced *s*hort *p*alindromic *r*epeats. A faster and less expensive way to create genetically engineered mice. The technique uses a "guide RNA" to locate a specific gene sequence that is then cut out or replaced. [4]

cross dependence Withdrawal signs occurring in a dependent individual can be terminated by administering drugs in the same class. [10, 11]

cross-tolerance Tolerance to a specific drug can reduce the effectiveness of another drug in the same class. [1, 10, 11]

CSF See *cerebrospinal fluid*. [1]

CT See *computerized tomography*. [4]

cyclic adenosine monophosphate (cAMP) Second messenger that activates PKA and is controlled by DA, NE, 5-HT, and endorphins. [3]

cyclic guanosine monophosphate (cGMP) Second messenger that activates PKG and is controlled in part by NO. [3]

cycling Pattern of steroid use characterized by 6 to 12 weeks of drug use, followed by a period of abstinence before repeating the drug use pattern. [16]

cyclooxygenase-2 (COX-2) Enzyme that can metabolize endocannabinoids and that plays an important role in the process of inflammation. [14]

CYP450 See *cytochrome P450*. [1]

cytochrome P450 (CYP450) Class of liver enzymes, in the microsomal enzyme group, responsible for both phase 1 and phase 2 biotransformation of psychoactive drugs. [1]

cytochrome P450 Class of liver enzymes, in the microsomal enzyme group, responsible for both phase 1 and phase 2 biotransformation of psychoactive drugs. [10]

cytochrome P450 2A6 (CYP2A6) Specific type of cytochrome P450 that metabolizes nicotine into cotinine. [13]

cytoplasm Salty gelatinous fluid of the cell, outside of the nucleus and bounded by the cell membrane. [2]

cytoskeleton Structural matrix of a cell that is composed of tubular materials such as microtubules and neurofilaments. [2]

D

D-serine Amino acid that is a co-agonist with glutamate for the NMDA receptor. [8]

D-tubocurarine Poison that targets muscle nicotinic receptors, blocking cholinergic transmission. [7]

DA imbalance hypothesis Theory that excessive DA function of mesolimbic neurons produces positive symptoms and insufficient DA function of mesocortical neurons produces negative and cognitive symptoms of schizophrenia. [19]

DA transporter Protein in the membrane of dopaminergic neurons that is responsible for DA uptake from the synaptic cleft. [5]

DA See *dopamine*. [5]

dabbing Form of high-potency cannabis consumption. It typically involves extraction of cannabis with butane, evaporation of the solvent, and then smoking the resulting waxy residue. [14]

DAG See *diacylglycerol*. [3]

DBH *See dopamine β-hydroxylase*. [5]

delayed match-to-sample Behavioral task used to test short-term memory. [8]

delirium tremens (DTs) Severe effects of alcohol withdrawal characterized by irritability, headaches, agitation, hallucinations, and confusion. [10]

δ-receptors A type of opioid receptor primarily in the forebrain that may help regulate olfaction, motor integration, reinforcement, and cognitive function. [11]

Δ^9-tetrahydrocannabinol (THC) Psychoactive chemical found in cannabis plants; a cannabinoid. [14]

ΔFosB Member of the Fos family of transcription factors. This protein accumulates in some brain areas after repeated exposure to various drugs of abuse and is hypothesized to contribute to the development of an addicted state. [9]

dendrites Projections from the soma that receive signals and information from other cells. [2]

dendritic spines Projections from dendrites that increase the receiving surface area. [2]

2-deoxyglucose autoradiography A research tool that visually identifies neurons that are active by measuring glucose uptake. [4]

Depakote See *valproate*. [18]

depolarization Change in membrane potential making the inside of the cell more positive, increasing the likelihood that the cell will have an action potential. [2]

depolarization block Process in which the resting potential across the cell membrane is lost. The neuron cannot be excited until the membrane is repolarized. [7]

depot binding Type of drug interaction involving binding to an inactive site, such as to proteins in the plasma, to bone, or to fat. [1]

deprivation reversal model Theory that smoking is maintained by mood enhancement and increased concentration that occur when nicotine withdrawal symptoms are alleviated. [13]

descending modulatory pathways Bundles of nerve fibers originating at higher brain regions that influence lower brain or spinal cord function. One arises from the PAG in the midbrain and influences pain signals carried by the spinal cord neurons. [11]

desensitization Process by which an ionotropic channel receptor remains closed despite the presence of an agonist bound to the receptor. The receptor cannot respond again until it leaves the desensitized state. [3]

desensitized Altered receptor state characterized by a lack of response to an agonist. [7]

detoxification Procedure used to treat addicted individuals in which the drug is stopped and withdrawal symptoms are treated until the abstinence syndrome has ended. [10]

detoxified A drug user undergoing detoxification is considered to be detoxified when signs of the abstinence syndrome end. [11]

developmental origins of health and disease Hypothesis that postulates that characteristics of the intrauterine environment, such as nutrient availability and the presence of drugs, environmental toxins, or infectious agents, "program" the fetus in a way that determines the vulnerability for developing chronic diseases in adulthood. [13]

dexamethasone A synthetic corticosteroid used to test the function of the negative feedback mechanism regulating the HPA axis. [18]

dexmedetomidine (Precedex) Drug that stimulates α_2-receptors, characterized by its sedative, anxiolytic, and analgesic effects. It is used to treat surgical patients in intensive care. [5]

dextromethorphan Opioid-like drug that is the major antitussive agent in most over-the-counter cough medicine. [15]

dextrorphan Biologically active metabolite of dextromethorphan. [15]

5,7-DHT See *5,7-dihydroxytryptamine*. [6]

diacylglycerol (DAG) Second messenger generated by the phosphoinositide second messenger system; stimulates protein kinase C (PKC). [3]

diazepam (Valium) A BDZ that binds to the BDZ receptor, increasing the effectiveness of GABA to open the $GABA_A$ receptor channel. [8]

diffusion tensor imaging (DTI) An imaging technique that allows visualization of axonal connections and evaluates the integrity of the neuronal pathway. [4]

dihydro-beta-erythroidine (DhβE) Blocks high-affinity nAChRs (i.e., receptors containing α4 and β2 subunits). [13]

5,7-dihydroxytryptamine (5,7-DHT) Neurotoxin that selectively damages serotonergic neurons. [6]

1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane (DOI) Drug that stimulates 5-HT_{2A} receptors, producing "head-twitch" in rodents and hallucinations in humans. [6]

dimethyltryptamine (DMT) Hallucinogenic drug found in several South American plants. [15]

Diprivan See *propofol*. [8]

***DISC1* gene** *DISC1* variants may increase the probability of developing schizophrenia. [19]

disease model Model of addiction that treats addiction as a distinct medical disorder or disease. [9]

dissociative anesthesia An unusual type of anesthetic state characterized by environmental detachment. It is produced by certain noncompetitive NMDA receptor antagonists such as ketamine and PCP. [15]

disulfiram (Antabuse) A drug used to treat alcoholism by causing the buildup of toxic metabolites producing illness after alcohol ingestion. [10]

divergence Process by which neurons transmit their integrated signals back out to many neurons. [2]

dizocilpine See *MK-801.* [8]

DMT See *dimethyltryptamine*. [15]

DNA methylation Environmentally-induced epigenetic covalent attachment of methyl groups to a gene decreases its expression. [2]

DNA microarray Method used to screen tissue or cell extracts for changes in the expression of many genes at the same time. [19]

DOI See *1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane*. [6]

domoic acid Amino acid found in certain seafoods that causes excitotoxicity in organisms that consume it. [8]

donepezil (Aricept) Drug that blocks AChE activity. It is used in the treatment of Alzheimer's disease. [7]

dopamine (DA) Neurotransmitter, related to NE and EPI, that belongs to a group called catecholamines. [5]

dopamine hypothesis of schizophrenia Theory that altered DA function leads to the symptoms observed in individuals with schizophrenia. [19]

dopamine system stabilizers Antipsychotic drugs that are DA partial agonists which increase DA function where it is too low and reduce DA function where it is excessive. [19]

dopamine β-hydroxylase (DBH) Enzyme that catalyzes the third step of NE synthesis in neurons, the conversion of DA to NE. [5]

dopamine-deficient (DD) mouse Mutant strain of mouse lacking dopamine from embryonic development onward. It is produced by genetically knocking out the tyrosine hydroxylase gene but restoring the gene in dopamine β-hydroxylase-expressing (i.e., noradrenergic) cells. [5]

dopaminergic Adjectival form of dopamine. [5]

doping agents Substances such as an anabolic steroids that are used to enhance athletic performance despite being banned by sports organizations. [16]

dorsal raphe nuclei Structure located in the area of the caudal midbrain and rostral pons that contains a large number of serotonergic neurons. In conjunction with the median raphe nucleus, it is responsible for most of the serotonergic fibers in the forebrain. Together they regulate sleep, aggression, impulsiveness, and emotions. [2]

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dorsal Located toward the top of the brain and back of the body in humans. [2]

dose–response curve Graph used to display the amount of biological change in relation to a given drug dose. [1]

double-blind experiment Type of experiment in which neither the patient nor the observer knows the treatment received by the patient. [1]

down-regulation Decrease in the number of receptors, which may be a consequence of chronic agonist treatment. [1]

DREADD See *chemogenetics*. [4]

drug action Molecular changes associated with a drug binding to a particular target site or receptor. [1]

drug competition A factor that modifies biotransformation capacity. When two drugs share a metabolic system and compete for the same metabolic enzymes bioavailability of one or both increases. [1]

drug depots Inactive sites where drugs accumulate. There is no biological effect from drugs binding at these sites, nor can they be metabolized. [1]

drug detoxification Process whereby an individual eliminates a drug from the body and goes through an abstinence syndrome. [9]

drug disposition tolerance See *metabolic tolerance*. [1]

drug effects Alterations in physiological or psychological functions associated with a specific drug. [1]

drug priming Delivery of a small dose of a drug by the experimenter for the purpose of eliciting drug-seeking behavior, typically in an animal whose drug self-administration responding was previously extinguished. See *reinstatement of drug-seeking behavior*. [9]

drug redistribution Transfer of drug molecules from organs of high drug concentration to those of low concentration until equilibrium is reached in all tissues. [1]

drug reward A positively-motivating subjective response to a drug, often experienced by humans as a euphoric feeling or "high." [9]

drug-seeking behavior Performance of an operant response such as a lever-press or a nose-poke with the expectation of receiving delivery of a drug dose. [12]

dry mouth effect Lack of salivation that is a side effect of drugs with muscarinic receptor blocking activity. [7]

DTs See *delerium tremens*. [10]

dual NE/5-HT modulators Antidepressants that enhance both NE and 5-HT function. [18]

dura mater The outer layer of the meninges. It is the strongest of the three meninges layers. [2]

dyskinesias Abnormal or impaired movement such as severe tics or choreic movements. [20]

dystonia Persistent invuluntary muscle contractions. [20]

E

EAATs See *excitatory amino acid transporters*. [8]

early LTP (E-LTP) Type of long-term potentiation that lasts no longer than a few hours. [8]

Edronax See *reboxetine*. [5]

EEG See *electroencephalography*. [4]

effector enzymes Enzymes of the cell membrane that may be regulated by G proteins and that cause biochemical and physiological effects in postsynaptic cells (e.g., by means of second messengers). [3]

efficacy The extent to which a ligand-receptor binding initiates a biological action (e.g., the ability of an agonist to activate its receptor). [1]

Eldepryl See *selegiline*. [5]

electrical self-stimulation A procedure whereby an animal self-administers a weak electrical shock to a specific brain area due to the reinforcing properties of the stimulation. [4, 9]

electroencephalography (EEG) Technique used to measure brain activity by using electrodes taped on the scalp to obtain electrical recordings in humans. [4]

electrostatic pressure Force drawing an ion to either side of the cell membrane in an attempt to balance or neutralize ionic charges. [2]

elevated plus-maze Maze type that involves a cross-shaped maze that has two open arms, two enclosed arms, and has been raised 50 cm off the floor. It is used to test a rodent's level of anxiety. [4]

endocannabinoid membrane transporter Hypothesized membrane carrier protein that binds anandamide and transports it into the cell. [14]

endocannabinoids Lipid-like substances that activate CB receptors. They are produced from arachidonic acid in the body. [14]

endocrine glands Specialized organs that secrete hormones into the bloodstream. [3]

endomorphins Group of endogenous opioid peptides in the CNS that selectively bind to the opioid receptor, and eliminate pain. [11]

endorphins Group of endogneous peptides in the brain that stimulate mu and delta opioid receptors, reducing pain and enhancing one's general mood. [11]

endosomes Membranous structures present in axon terminals that play a role in one type of vesicle recycling. [3]

entacapone (Comtan) COMT inhibitor used in conjunction with L-DOPA to treat Parkinson's disease. [5]

enteral Drug administration by oral or rectal routes. [1]

enteric nervous system Large system of ganglia located in the muscle walls of the intestines. Some of the neurons within this system use 5-HT as a neurotransmitter. [6]

enterochromaffin cells Specialized secretory cells within the walls of the intestines that synthesize and secrete 5-HT. [6]

enzyme induction Increase in liver drug-metabolizing enzymes associated with repeated drug use, which leads to drug tolerance. [1]

enzyme inhibition In drug metabolism, reduction in liver enzyme activity associated with a specific drug may cause more intense or prolonged effects of other drugs taken at the same time. [1]

enzyme-linked immunosorbent assay (ELISA) An immunoassay used to quantify a specific protein that does not rely on radioactivity for detection but instead uses a colored reaction product. [4]

ephedrine Psychostimulant that is a constituent of the herb *Ephedra vulgaris*. [12]

EPI See *epinephrine*. [3, 5]

epidural Method that involves administration of a drug into the cerebrospinal fluid surrounding the spinal cord. [1]

epinephrine (EPI) Hormone related to NE that belongs to a group called catecholamines. It is secreted by the chromaffin cells of the adrenal medulla, and it produces the "fight-or-flight" response by regulating the diversion of energy and blood to muscles. Also known as adrenaline. [3, 5]

EPSP See *excitatory postsynaptic potential*. [2]

equilibrium potential for potassium Point at which the electrostatic pressure and the concentration gradient for potassium are balanced. [2]

ergot Fungus, *Claviceps purpurea*, that infects certain grains and that contains several important alkaloids from which the structure of LSD was derived. [15]

ergotism Disease caused by ergot-contaminated grains that can lead to death. [15]

ERK Extracellular signal-regulated kinase, a major component of the MAP kinase system. [3]

eserine See *physostigmine*. [7]

estradiol Specific estrogen and a powerful female sex hormone. [3]

estrogens Female sex hormones secreted by the ovaries. [3]

ethanol Proper chemical name for the type of alcohol consumed by humans. Similar to BDZs, it acts as a CNS-depressant in part by enhancing $GABA_A$ receptor activity. [8]

event-related potentials (ERPs) Electrical changes in neuron activity in response to a sensory stimulus. [4]

excitatory amino acid neurotransmitters Transmitters, including glutamate, aspartate, and some other amino acids, that cause an excitatory response in most neurons of the brain or spinal cord. [8]

excitatory amino acid transporters (EAATs) Protein that transports glutamate and aspartate across the plasma membrane. There are five such transporters, designated **EAAT1** to **EAAT5**. [8]

excitatory postsynaptic potentials (EPSPs) Small localized membrane depolarizations of a postsynaptic neuron that result from neurotransmitters binding to specific receptors that open ion channels. EPSPs move the membrane potential closer to the threshold for firing. [2]

excitotoxicity hypothesis Theory that excessive glutamate or other excitatory amino acid exposure results in prolonged depolarization of receptive neurons, leading to their damage or death. [8]

executive function Collection of higher-order cognitive abilities including planning, organization, problem solving, mental flexibility, and valuation of incentives. The prefrontal cortex plays an important role in executive function. [9]

exocytosis Method by which vesicles release substances and neurotransmitters, characterized by fusion of the vesicle and the cell membrane, specifically the axon terminal membrane in the case of neurotransmitters. The vesicle opens toward the synaptic cleft allowing neurotransmitter molecules to diffuse out. [3]

expectancy Term used to describe the anticipated effect of a drug and its role in drug action or perceived drug action. [10]

expression phase The period of time after a tetanic stimulation is given, characterized by enhanced synaptic strength (i.e., LTP). [8]

expression Process that leads to manifestation of a sensitized response and that requires enhanced reactivity of DA nerve terminals in the nucleus accumbens. [12]

extracellular fluid Salty fluid surrounding nerve cells that provides oxygen, nutrients, and chemical signals, and that removes secreted cell waste. [2]

extracellular recording Method of taking measurements of cell firing by inserting a fine-tipped electrode into the extracellular fluid surrounding the cell. [4]

F

face validity Term used to describe the relationship between a testing procedure done on animals and its direct correlation to human test results or behavior. [4]

FAS See *fetal alcohol syndrome*. [10]

FASD See *fetal alcohol spectrum disorder.* [10]

fatigue State of weariness that diminishes an individual's energy and mental capacity. [20]

fatty acid amide hydrolase (FAAH) Enzyme that metabolizes endocannabinoids. [14]

fatty liver Damaging effect of alcohol characterized by the accumulation of triglycerides inside liver cells. [10]

fear-potentiated startle Enhancement of a startle response when the stimulus is preceded by the presentation of a conditioned fear stimulus. [4]

fenestrations Large pores in endothelial cells allowing rapid exchange of materials between blood vessels and tissue. [1]

fenfluramine Drug similar in structure to amphetamine that stimulates 5-HT release. It is an appetite suppressor formerly used as a treatment for obesity. [6]

festination Uncontrollable acceleration of gait. [20]

fetal alcohol spectrum disorders (FASD) A cluster of disorders due to developmental abnormalities caused by prenatal exposure to alcohol. Features vary but may include learning disabilities, poor impulse control, and attention deficits. [10]

fetal alcohol syndrome (FAS) The damaging developmental effects of prenatal alcohol exposure. [10]

fetal solvent syndrome Group of symptoms, typically including cognitive deficits and craniofacial (i.e., head and face) abnormalities, seen in some newborn infants of inhalant-abusing women. [16]

first-order kinetics Term used to describe exponential elimination of drugs from the bloodstream. [1]

first-pass metabolism Phenomenon in which the liver metabolizes some of a drug before it can circulate through the body, particularly when the drug has been taken orally. [1]

fissures Deep grooves of the cerebral cortex. [2]

flashbacks Reexperience of the perceptual drug effects, specifically those of a hallucinogen, following termination of drug use. [15]

fluoxetine (Prozac) Drug that selectively blocks the 5-HT transporter. It is used as an antidepressant. [6]

fMRI See *functional MRI*. [4]

focused stereotypies Behaviors produced by high doses of psychostimulants (e.g., cocaine and amphetamine) and characterized by repetitive and aimless movement. [12]

follicle-stimulating hormone (FSH) Hormone secreted by the anterior pituitary that helps control gonad growth and function. [3]

forced swim test Technique used to measure depression in animals by placing them in a cylinder of water from which they cannot escape and recording the time it takes for them to abandon attempts to escape. [4]

***fragile X mental retardation 1* gene (*FMR1*)** Gene that codes for the fragile X mental retardation protein (FMRP). [8]

fragile X mental retardation protein (FMRP) Protein encoded by the *FMR1* gene that regulates local protein synthesis at postsynaptic sites. Deficiency of FMRP due to *FMR1* mutations is the cause of fragile X syndrome. [8]

fragile X syndrome Congenital disorder that is a leading cause of intellectual disability and autistic symptoms. It is caused by a mutation in the *fragile X mental retardation 1* gene (*FMR1*). [8]

freebasing Smoking the freebase form of cocaine obtained by dissolving cocaine HCl in water, adding an alkaline solution, and then extracting with an organic solvent. [12]

frontal Tissue sections cut parallel to the face. [2]

frontal lobe One of four lobes of the cerebral cortex. It is responsible for movement and executive planning. [2]

FSH See *follicle-stimulating hormone*. [3]

fuels Class of inhalants composed of volatile liquids or gases that serve many purposes, including automobile fuel, fuels for lamps and heating appliances, lighter fluids, and propellants used in many kinds of spray cans. [16]

functional MRI (fMRI) Technique used to regionally visualize brain activity by detecting the increase in blood oxygen levels through magnetic resonance measurements of oxygenated and oxygen-depleted hemoglobin. [4]

G

G protein–coupled receptor Slow acting receptor type composed of a single large protein in the cell membrane that activates G proteins. It may also be called a metabotropic receptor. [3]

G proteins Specific membrane proteins that are necessary for neurotransmitter signaling by metabotropic receptors. They operate by regulating ion channels or effector enzymes involved in the synthesis or breakdown of second messengers, ultimately causing biochemical or physiological changes in the postsynaptic cell. [3]

GABA See *γ-aminobutyric acid*. [8]

GABA aminotransferase (GABA-T) Enzyme that breaks down GABA in GABAergic neurons and astrocytes. [8]

$GABA_A$ receptor Ionotropic receptor for GABA that allows Cl^- ions to enter the cell, thereby inhibiting cell firing. [8]

$GABA_B$ receptor Metabotropic receptor for GABA. [8]

Gabitril See *tiagabine*. [8]

GAD See *generalized anxiety disorder* or *glutamic acid decarboxylase*. [8, 17]

gait Pattern of limb movement during locomotion over a solid surface. [4]

galantamine (Reminyl) Drug that blocks AChE activity. It is used in the treatment of Alzheimer's disease. [7]

γ-aminobutyric acid (GABA) Amino acid that is the principal inhibitory neurotransmitter in the CNS. [8]

γ-hydroxybutyrate (GHB) Chemical similar in structure to GABA that produces sedative and anesthetic effects in users and that is used medicinally as well as recreationally. [16]

ganglia A cluster of cell bodies outside the CNS. [2]

gaseous transmitters Substances in the gas phase that acts as neurotransmitters in the body. [3]

GAT-1 Member of the family of plasma membrane GABA transporters that is expressed in both neurons and astrocytes. [8]

GAT-2 Member of the family of plasma membrane GABA transporters that is expressed in both neurons and astrocytes. [8]

GAT-3 Member of the family of plasma membrane GABA transporters that is expressed only in astrocytes. [8]

gated channels Ion channels that are normally in a closed configuration that can be opened momentarily by specific stimuli. [2]

gateway theory Theory proposing that use of certain drugs of abuse, particularly during childhood or adolescence, increases the risk of progressing to other substances. For example, tobacco or alcohol have been proposed as gateways to marijuana use, and in turn marijuana has been proposed as a gateway to so-called "hard drugs" like cocaine or heroin. [9]

gene therapy Application of DNA that encodes a specific protein to increase or block expression of the gene product to correct a clinical condition. [1]

generalized anxiety disorder (GAD) An anxiety disorder characterized by excessive worrying that does not have a specific cause. [17]

genes Portions of a chromosome that code for particular proteins. [2]

genetic models Creation of knockout, knockin, or transgenic mice to produce a phenotype analagous to the human clinical disorder of interest. [19]

genetic polymorphisms Genetic variations in a population resulting in multiple forms of a particular protein. [1]

genome-wide association studies (GWAS) A modification of microarray technology used to compare the incidence of single-nucleotide polymorphisms and copy number variants in DNA samples from people with a given disorder and matched controls. [4, 9, 10]

GH See *growth hormone*. [3]

GHB See *γ-hydroxybutyrate*. [16]

glial cells Supporting cells of the nervous system that insulate, protect, and metabolically support neurons. [2]

gliotransmission Chemical transmission mediated by transmitter release from glial cells such as astrocytes. [8]

glucagon Hormone secreted by the islets of Langerhans that, along with insulin, regulates metabolic energy sources in the body. [3]

glucocorticoid hypothesis Theory that stress-induced elevation of glucocorticoid levels accelerate cell damage including decreases in dendritic branches and loss of dendritic spines and apoptosis in multiple brain regions, and failure of neurogenesis in the hippocampus and leads to the symptoms of depression. [18]

glucocorticoids Hormones belonging to the steroid family that are secreted by the adrenal cortex and help maintain blood glucose levels in the body. [3]

glutamate The ionized form of glutamic acid. It is an excitatory amino acid neurotransmitter of the CNS. [8]

glutamatergic neurons Neurons that use glutamate as a transmitter. [8]

glutamic acid decarboxylase (GAD) Enzyme that transforms glutamate into GABA. [8]

glutaminase Enzyme that transforms glutamine into glutamate. [8]

glutamine Precursor of the transmitter-related glutamate. [8]

glutamine synthetase Enzyme in astrocytes that converts glutamate into glutamine. [8]

glycine Amino acid characterized by the lack of a functional group. It is a co-agonist with glutamate for the NMDA receptor. [8]

GnRH See *gonadotropin-releasing hormone.* [3]

gonadotropin-releasing hormone (GnRH) Hormone that stimulates FSH and LH release. It is synthesized by neurons of the hypothalamus. [3]

gonads Glands that secrete sex-specific steroid hormones. [3]

granisetron (Kytril) Drug that inhibits 5-HT_3 receptors. It is used to treat the nausea and vomiting side effects of cancer chemotherapy. [6]

growth hormone (GH) Hormone secreted by the anterior pituitary that increases production of IGF-I in peripheral organs. [3]

guanfacine α_{2A}-adrenergic agonist prescribed for the treatment of ADHD. Guanfacine is believed to improve attention and memory by stimulating α_{2A}-adrenergic receptors in the prefrontal cortex. [5]

GWAS See *genome-wide association studies.* [4, 9, 10]

gyri (sing. gyrus) Bulges of tissue between the grooves in the cerebral cortex. [2]

H

half-life Time required to remove half of the drug from the blood. It is referred to as $t_{1/2}$. [1]

hallucinogen persisting perception disorder (HPPD) Disorder of hallucinogen use characterized by severe perceptual symptoms (i.e., flashbacks) that persist for a long period of time following drug use and are experienced sufficiently frequently to cause significant distress or impairment to the individual. [15]

hallucinogen rating scale Psychometric scale developed to quantify the subjective effects of hallucinogenic agents. [15]

hallucinogenic Adjectival form of hallucinogen. [6, 15]

halogenated hydrocarbons Class of inhalants composed of hydrocarbon molecules possessingone or more chlorine or fluorine atoms. [16]

haloperidol A D_2 receptor blocker that can induce catalepsy in animals when administered in high doses. It is used clinically as an antipsychotic agent. [5]

hangover Effect of heavy alcohol consumption that may be a sign of withdrawal, acute toxicity, or other negative effects on body regulation. [10]

hashish Type of cannabis derivative that is smoked or eaten. [14]

HD See *Huntington's disease*. [20]

hemicholinium-3 (HC-3) Drug that blocks the choline transporter in cholinergic nerve terminals. [7]

heritability The relative contribution of genetics to the variability of a trait within a population. [9]

heteroreceptors Axon receptors that are specific for neurotransmitters released by other cells at axoaxonic synapses. They may either decrease or increase further neurotransmitter release. [3]

5-HIAA See *5-hydroxyindoleacetic acid*. [6]

hippocampus Subcortical structure of the limbic system that helps to establish long-term, contextual, and spatial memories. The hippocampus is where LTP was first discovered and is also one of the brain areas damaged in Alzheimer's disease. [2]

homovanillic acid (HVA) Major metabolite formed in the breakdown of DA. [5]

horizontal Brain sections cut parallel to the horizon. [2]

hormones Chemical substances secreted by endocrine glands into the bloodstream, where they travel to target locations in the body. [3]

hot plate test Method used to evaluate analgesia by subjecting an animal to a heated metal plate and measuring the time it takes to make an avoidance response. [4]

5-HT See *serotonin*. [6]

5-HT transporter (SERT) Protein in the membrane that is responsible for 5-HT reuptake from the synaptic cleft. [6]

5-HTP See *5-hydroxytryptophan*. [6]

***huntingtin* gene** Gene containing a CAG trinucleotide repeat. A mutation resulting in more than 40 repeats of the CAG sequence results in Huntington's disease. [20]

Huntington's chorea Involuntary jerky, writhing movements of the limbs. [20]

Huntington's disease (HD) An inherited disorder caused by a genetic defect on chromosome 4. The defect results in the abnormal repetition of a CAG sequence. The disorder causes progressive degeneration of nerve cells in the brain resulting in movement, cognitive, and psychiatric symptoms. [20]

HVA See *homovanillic acid*. [5]

5-hydroxyindoleacetic acid (5-HIAA) Major metabolite of 5-HT that is produced by the action of MAO. [6]

6-hydroxydopamine (6-OHDA) Neurotoxin similar in structure to DA that damages catecholaminergic nerve terminals and is used to study catecholamine pathways. [5, 20]

2-hydroxysaclofen Chemical analog of baclofen that is a competitive antagonist at the GABAB receptor. [8]

5-hydroxytryptamine (5-HT) Neurotransmitter present in the central and peripheral nervous system and synthesized by the serotonergic neurons. Also known as serotonin. [6]

5-hydroxytryptophan (5-HTP) Intermediate formed in the synthesis of 5-HT. [6]

8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) Drug that stimulates 5-HT_{1A} receptors. Effects include increased appetite, reduced anxiety, reduced alcohol cravings, and a lower body temperature. [6]

hyperalgesia Condition characterized by an increased sensitivity to pain. [6, 14]

hypercapnia Elevated blood CO_2 levels. [6]

hyperpolarization Change in membrane potential making the inside of a cell more negative relative to the resting potential, reducing the likelihood that the cell will fire an action potential. [2]

hypnagogic hallucinations Vivid dreamlike sensations that occur during the daytime in some patients with narcolepsy. [3]

hypnotics Drugs, such as benzodiazepines, that help a patient to fall asleep and stay asleep. [17]

hypocretin 1 See *orexin-A*. [3]

hypocretin 2 See *orexin-B*. [3]

hypoglutamate model NMDA antagonists produce rodent behaviors analogous to the positive, negative, and cognitive symptoms of schizophrenia. [19]

hypophagia Decrease in food consumption due to loss of appetite. [6]

hypothalamic releasing hormones Neuropeptide hormones synthesized by neurons of the hypothalamus and carried by blood vessels to the anterior pituitary, where they control the release of many of the pituitary hormones. [3]

hypothalamus Structure of the diencephalon located at the base of the brain, ventral to the thalamus. It provides many functions important for survival, including the maintenance of body temperature and salt balance, regulation of hunger and thirst, control of the ANS and pituitary gland, and modulation of emotional responses. [2]

I

idalopirdine Drug that inhibits 5-HT_6 receptors. It has been tested for treating cognitive decline in patients with Alzheimer's disease. [6]

192 IgG–saporin Neurotoxin containing the monoclonal antibody 192 IgG, which binds to cholinergic neurons in the forebrain, and the cellular toxin saporin. It is used to selectively kill cholinergic neurons. [7]

IM See *intramuscular*. [1]

immunocytochemistry (ICC) Technique that uses antibodies to determine the brain areas or neurons that contain a specific antigen such as a protein, neuropeptide, or neurotransmitter. [4]

in situ hybridization (ISH) Technique used to locate cells that manufacture a specific protein or peptide by detecting the specific mRNA sequence coding for that substance. It can also be used to study changes in regional mRNA levels (i.e., gene expression). [4]

in vitro Refers to measurements performed outside the living body (traditionally in a test tube). [4]

in vivo Refers to measurements observed in the living organism. [4]

in vivo voltammetry Technique used to measure neurotransmitter release in the brain of an awake, freely moving animal by using a microelectrode to measure electrochemical responses to an applied electrical signal. [4]

25I-NBOMe Member of the NBOMe class of hallucinogens containing an iodine atom attached to the phenyl ring. [15]

incentive salience Psychological process by which drug-related stimuli gain increased prominence and attractiveness. It is an important component of the incentive-sensitization model of addiction. [9]

incentive sensitization theory Model of addiction based on the theory that repeated drug use leads to an increase in "wanting" the drug (i.e., craving) but no increase in drug "liking" (reward or euphoria) because only the neural system underlying drug "wanting" becomes sensitized. [9]

incubation Time-dependent increase in drug craving and drug seeking behavior during abstinence. [12]

Inderal See *propranolol*. [5]

indoleamine Indole derivative containing an amine group. Indoleamines include serotonin and the hallucinogens LSD, psilocybin, psilocin, DMT, and 5-MeO-DMT. [15]

induction 1. Increase in liver enzymes specific for drug metabolism in response to repeated drug use. 2. Process that establishes psychostimulant sensitization by activating glutamate NMDA receptors and, in some cases, D_1 receptors. [10, 12]

induction phase Phase of LTP, during and immediately after a tetanic stimulation is given, which requires activation of NMDA receptors. [8]

inferior Located toward the underside of the brain in humans. [2]

infusion pump Drug delivery via an implanted pump (e.g., subcutaneous) that delivers regular, constant doses to the body or into the cerebral ventricles. [1]

inhalants Group of volatile substances, such as glue, that may be abused as a drug by inhaling the fumes. [16]

inhalation Method that involves administration of a drug through the lungs. [1]

inhibitory postsynaptic potentials (IPSPs) Hyperpolarizing responses of a postsynaptic cell that result from neurotransmitters opening ion channels. [2]

inositol trisphosphate (IP_3) See *diacylglycerol* (*DAG*). [3]

insulin Polypeptide hormone that is secreted by the islets of Langerhans and, along with glucagon, regulates glucose and metabolic energy sources in the body. It regulates glucose uptake from the bloodstream into tissues and stimulates the uptake of certain amino acids. [3, 6]

integration Process at the axon hillock whereby several small depolarizations or hyperpolarizations will summate to create a larger change in membrane potential. Similarly, simultaneous depolarizations and hyperpolarizations will cancel each other out. [2]

intercellular clefts Small gaps between adjacent cells. These gaps between endothelial cells of typical blood vessels permit the passage of molecules to and from the blood. [1]

Interferon beta 1a Drug used in the treatment of relapsing forms of MS. [20]

Interferon beta 1b Drug used in the treatment of relapsing forms of MS. [20]

interictal period Time interval between seizures in an epileptic patient. [8]

interneurons Nerve cells in the CNS and spinal cord that form complex neural circuits providing sensorimotor integration. [2]

intracellular recording Method of taking measurements of cell firing by inserting a fine-tipped electrode into the cell. [4]

intracerebroventricular Method that involves administration of a drug into the cerebrospinal fluid of the ventricles. [1]

intracranial Method that involves administration of a drug into the brain tissue. [1]

intramuscular (IM) Method that involves administration of a drug by injection into a muscle. [1]

intranasal administration Topical administration of a drug to the nasal mucosa. [1]

intraperitoneal (IP) Injection technique that is the most common route of administration for small laboratory animals. The drug is injected through the abdominal wall into the peritoneal cavity—the space that surrounds the abdominal organs. [1]

intrauterine growth restriction (IUGR) Condition in which fetal growth is hampered, thereby resulting in a baby being born underweight for its gestational age. [13]

intravenous (IV) Method that involves administration of a drug directly into the bloodstream by means of injection into a vein. [1]

inverse agonists Substances that activate a receptor but produce the opposite effect of typical agonists at that receptor. [1, 8]

Inversine See *mecamylamine*. [7]

ionization Process involving the dissociation of an electrically neutral molecule into charged particles (ions). [1]

ionotropic receptor Fast acting receptor type comprised of several subunits that come together in the cell membrane. The receptor has an ion channel at its center, which is regulated by neurotransmitters binding to specific sites on the receptor causing the channel to open. It may also be called a ligand-gated channel receptor. [3]

iontophoresis Process by which electrically charged drug molecules stored in a reservoir inside a skin patch are driven into the skin by the passage of electrical current. This process can be used for constant administration of migraine headache medications. [6]

IP See *intraperitoneal*. [1]

ipsapirone Drug that stimulates 5-HT_{1A} receptors. Some of its effects include increased appetite, reduced anxiety, reduced alcohol cravings, and a lower body temperature. [6]

IPSP See *inhibitory postsynaptic potential*. [2]

irritable bowel syndrome (IBS) Gastrointestinal disorder characterized by abdominal pain, gas and bloating, frequent abnormal bowel movements (diarrhea, constipation, or an alternation between the two), and mucus in the stool. [6]

ischemia Condition characterized by an interruption of blood flow to the brain. [8]

ISH See *in situ hybridization*. [4]

islets of Langerhans Endocrine gland in the pancreas that secretes insulin and glucagons. [3]

isoproterenol β-adrenergic receptor agonist. [5]

IUGR See *intrauterine growth restriction*. [13]

IV See *intravenous*. [1]

J

John Cunningham (JC) virus Common virus that is present in more than 50% of the population. Most people acquire it sometime during childhood. It lives in a latent harmless state in the kidneys and the gastrointestinal tract in individuals with healthy immune systems, but becomes life-threatening in those whose immune systems are compromised. The virus causes progressive multifocal leukoencephalopathy (PML), a rare, but frequently deadly condition that destroys myelin, a protective covering of nerve cells in the brain. [20]

K

kainate receptor An ionotropic glutamate receptor selective for the agonist kainic acid. [8]

κ-receptors An opioid receptor located in the striatum, amygdala, hypothalamus, and pituitary gland that may help regulate pain, perception, gut motility, dysphoria, water balance, hunger, temperature, and neuroendocrine function. [11]

ketamine Drug that binds to the PCP site and acts as a noncompetitive antagonist of the NMDA receptor. It is a dissociative anesthetic used in both human and veterinary medicine, and it is also used recreationally. [8, 15]

ketanserin Drug that inhibits 5-HT_{2A} receptors. [6]

kiss-and-run Hypothesized type of exocytosis in which the synaptic vesicle does not collapse during the neurotransmitter release process, thus eliminating the need for an endocytotic mechanism to retrieve vesicle membrane components from the membrane of the axon terminals. [3]

knockin mice Mice that have a specific gene inserted into their DNA, so they produce a slightly different protein than is produced by wild-type mice. [4]

knockout mice Mice that are homozygous for the targeted deletion of a specific gene. They are used to study the normal function of that gene as well as the involvement of the gene in behavioral and physiological responses to various psychoactive drugs. [4]

L

L-2-amino-4-phosphonobutyrate (L-AP4) Synthetic amino acid that is an agonist selective for glutamate autoreceptors. [8]

L-DOPA Precursor necessary for the synthesis of DA. L-DOPA is formed by the addition of a hydroxyl group to tyrosine by the enzyme TH. It is used to treat Parkinson's disease by increasing DA formation. [5]

L-DOPA Precursor necessary for the synthesis of DA. L-DOPA is formed by the addition of a hydroxyl group to tyrosine by the enzyme TH. It is used to treat Parkinson's disease by increasing DA formation. [20]

late LTP (L-LTP) Type of LTP that is dependent on protein synthesis and that can last for much longer periods of time than early LTP. [8]

lateral Located to either side of the body or brain. [2]

laterodorsal tegmental nuclei (LDTg) Structure within the dorsal lateral pons containing cholinergic neurons that project to the ventral tegmental area (important for stimulating VTA dopamine neurons) and others that project to the brainstem and thalamus (important for behavioral arousal, sensory processing, and inititation of rapid-eye-movement sleep). [7]

LC See *locus coeruleus*. [5]

LDTg See *laterodorsal tegmental nucleus*. [7]

learned helplessness A classic screening device for antidepressant drugs. After being subjected to periods of unescapable foot shock, rodents fail to respond when given the opportunity to alter an aversive event. Antidepressant drugs increase appropriate responding.

lesioning Process whereby brain cells are destroyed using an electrode to administer a high radio frequency current, or by injecting a neurotoxin that kills cells. [4]

Levodopa See *L-DOPA*. [20]

Lewy body dementia (LBD) Progressive form of dementia that is similar in symptomology to Parkinson's disease and Alzheimer's disease. Characterized by abnormal accumulations of proteins (Lewy bodies) in the nuclei of neurons in the brain that control memory and movement. [20]

Lewy body An abnormal aggregate of protein that develop inside neurons in Parkinson's disease and Lewy body dementia. [20]

LH See *luteinizing hormone*. [3]

ligand-gated channel receptors See *ionotropic receptor*. [3]

ligand-gated channels Types of ion channels that are regulated by a ligand binding to a receptor site associated with the particular channel. [2]

ligand Molecule that selectively binds to a receptor. [1]

light–dark crossing task Test used to determine a rodent's level of anxiety by placing it in a two-compartment box, one side lit and the other side dark. Fewer crossings and less time spent in the lighted side indicate anxiety. [4]

limbic system Neural network that integrates emotional responses and regulates motivated behavior, reinforcement, and learning. Some major structures include the limbic cortex, amygdala, nucleus accumbens, and hippocampus. [2]

linkage analysis Genetic method that seeks to find chromosomal regions that tend to associate with a disorder (e.g., a substance-related disorder) that is being studied. [9]

linkage studies Methods used to locate genes responsible for a disorder, such as alcohol use disorder or schizophrenia, by comparing similarities in the genetic loci of families with affected members. [18]

Lioresal See *baclofen*. [8]

lipids Fatty molecules in the body. Lipids are a major component of cell membranes, and some of them also act as neurotransmitters. [3]

lithium carbonate Drug that stabilizes moods, preventing episodes of mania and depression, in people with bipolar disorder. [18]

local potentials Small localized short-lived change in voltage across the cell membrane following the opening of ligand-gated channels. [2]

loci (sing. locus) The location of genes on a chromosome. [19]

locus coeruleus (LC) Collection of noradrenergic neurons in the reticular formation of the pons that supplies most of the NE to the cortex, limbic system, thalamus, and hypothalamus. These cells cause arousal and increased attention when active. [2, 5]

long-term depression (LTD) Type of synaptic plasticity resulting in weakening of synaptic connections. [8]

long-term potentiation (LTP) Phenomenon whereby synaptic connections are strengthened for a period of at least an hour. It requires activation of NMDA receptors for its induction and AMPA receptors for its expression. [8]

Lopressor See *metoprolol*. [5]

lorcaserin (Belviq) Drug that selectively stimulates 5-HT_{2C} receptors. It is used in the treatment of obesity. [6]

LSD See *lysergic acid diethylamide*. [6, 15]

LTD See *long-term depression.* [8]

LTP See *long-term potentiation*. [8]

luteinizing hormone (LH) Hormone secreted by the anterior pituitary that helps control gonad growth and function, and increases estrogen and androgen secretion. [3]

lysergic acid Core structural unit of all ergot alkaloids. [15]

lysergic acid diethylamide (LSD) Hallucinogenic drug that is synthesized from lysergic acid and based on alkaloids found in ergot fungus. It is thought to produce its effects mainly by stimulating 5-HT_{2A} receptors in the brain. [6, 15]

lysis Bursting of a cell. [8]

M

macroelectrode Device used to electrically stimulate deep brain regions while monitoring behavior or recording the summated electrical response of thousands of neurons. [4]

MAGL See *monoacylglycerol lipase*. [14]

magnetic resonance imaging (MRI) Technique used to visualize in high resolution, detailed slices through the brain or other organ by taking computerized measurements of the signals emitted by atoms in the tissue as they are exposed to a strong magnetic field. Computer technology permits recreation of the structure in 3-dimensions. [4]

magnetic resonance spectroscopy (MRS) Using MRI-generated data, MRS calculates the quantity of brain chemicals such as glutamate. [4]

magnetogenetics Genetic manipulations that make cells sensitive to magnets. Remote magnetic stimulation of specific cells is used to trigger a behavioral response. [4]

major depression Type of affective disorder characterized by extreme recurring episodes of dysphoria and negative thinking that are reflected in behavior. [18]

male hypogonadism Condition involving deficient functioning of the testes, abnormally low secretion of testosterone, and reduced sperm production. [16]

MAO inhibitors (MAOIs) Class of drugs that inhibit monoamine oxidase (MAO), thereby causing an accumulation of catecholamines and serotonin in the brain. They are often used to treat clinical depression. [5, 18]

MAO See *monoamine oxidase*. [5]

MAP kinase system Family of mitogen-activated protein kinases known to play important roles in growth, development, and synaptic plasticity. [3]

marijuana Crude mixture of dried and crumbled leaves, small stems, and flowering tops of the cannabis plant. [14]

maternal separation Technique used to test the role of early-life stress as a factor in the development of depression, substance abuse, and other psychopathology. Week-old animals are separated from their mothers for brief periods daily. [4]

mavoglurant Selective mGluR5 antagonist that was developed and clinically tested for the treatment of fragile X syndrome. [8]

MDMA See *3,4-methylenedioxymethamphetamine*. [6]

MDPV Cathinone derivative (3,4-methylenedioxypyrovalerone) that is an abused stimulant drug. It is a member of a group of compounds sometimes called "bath salts," "plant food," "pond water cleaner," and "legal highs." [12]

mecamylamine (Inversine) Drug that is an antagonist for nicotinic ACh receptors. [7, 13]

medial Located near the center or midline of the body or brain. [2]

median eminence Area in the hypothalamus that is not isolated from chemicals in the blood and where hypothalamic-releasing hormones are secreted for transport to the anterior pituitary gland. [3]

median raphe nuclei (sing. nucleus) Structure located in the area of the caudal midbrain and rostral pons that contains a large number of serotonergic neurons. In conjunction with the dorsal raphe nucleus, it is responsible for most of the serotonergic fibers in the forebrain. Together they regulate sleep, aggression, impulsiveness, and emotions. [2, 6]

medical model See *disease model*. [9]

medulla Structure located in the caudal brain stem responsible for regulating heart rate, digestion, respiration, blood pressure, coughing, and vomiting. [2]

melatonin Hormone that regulates rhythmic functions in the body. It is secreted by the pineal gland. [3]

memantine (Namenda) Noncompetitive NMDA receptor antagonist that is used to treat moderate to severe Alzheimer's disease. [8, 20]

meninges Layers of protective tissue located between the bones of the skull and vertebrae and the tissue of the brain and spinal cord. [2]

MEOS See *microsomal ethanol oxidizing system*. [10]

mephedrone Cathinone derivative (4-methylmethcathinone) that is an abused stimulant drug. It is a member of a group of compounds sometimes called "bath salts," "plant food," "pond water cleaner," and "legal highs." [12]

3-mercaptopropionic acid Drug that blocks GABA synthesis, inducing convulsions. [8]

mescal button Crown of the peyote cactus, *Lophophora williamsii*, which can be dried and ingested to obtain the hallucinogenic drug mescaline. [15]

mescaline Hallucinogenic drug produced by several cacti species, especially that of the peyote cactus, *Lophophora williamsii*. [15]

mesocortical dopamine pathway Group of dopaminergic axons that originates in the VTA and travels to the cerebral cortex, including the prefrontal, cingulated, and entorhinal cortices. It may also be called the mesocortical tract. [5]

mesolimbic dopamine pathway Group of dopaminergic axons that originates in the VTA and travels to structures of the limbic system, including the nucleus accumbens, septum, amygdala, and hippocampus. It may also be called the mesolimbic tract. [5]

Mestinon See *pyridostigmine*. [7]

metabolic tolerance Type of tolerance to a drug that is characterized by a reduced amount of drug available at the target tissue, often as a result of more-rapid drug metabolism. It is sometimes also called drug disposition tolerance. [1, 10]

metabolites Byproducts of biochemical pathways, such as those involved in neurotransmitter or drug inactivation. [5]

metabotropic glutamate receptor theory Theory that loss of FMRP causes exaggerated group I mGluR-related functions, which results in the characteristic of fragile X syndrome. [8]

metabotropic receptor Slow-acting receptor type composed of a single large protein in the cell membrane that activates G proteins. It may also be called a G protein–coupled receptor. [3]

methadone A long-acting opioid drug that may be substituted for other opioids in order to prevent withdrawal symptoms. [11]

methadone maintenance program Most effective treatment program for opioid addicts that involves the substitution of the opioid with methadone to prevent withdrawal symptoms and craving to avoid a relapse. [11]

methamphetamine Psychostimulant that acts by increasing catecholamine release from nerve terminals. It can also cause neurotoxicity at high doses. [5, 12]

methoxetamine Potent ketamine analog that is an abused drug. [15]

5-methoxy-dimethyltryptamine (5-MeO-DMT) Hallucinogenic drug found in certain South American plants. Its street name is "foxy" or "foxy methoxy." [15]

3-methoxy-4-hydroxy-phenylglycol (MHPG) A metabolite of NE, formed primarily as a result of NE breakdown in the brain. [5]

3,4-methylenedioxymethamphetamine (MDMA) Drug similar in structure to amphetamine that stimulates 5-HT release and is neurotoxic at high doses. It is a recreational drug that is often abused. [6]

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) Dopamine neurotoxin sometimes used to produce an animal model of Parkinson's disease. [5]

methyllycaconitine (MLA) Blocks low-affinity nAChRs consisting only of $\alpha 7$ subunits. [13]

methylone Cathinone derivative (3,4-methylenedioxy-*N*-methylcathinone) that is an abused stimulant drug. It is a member of a group of compounds sometimes called "bath salts," "plant food," "pond water cleaner," and "legal highs." [12]

methylphenidate (Ritalin) Synthetic psychostimulant that is used to treat ADHD. [12]

methylxanthines Class of naturally occurring chemicals that include caffeine and theophylline. [13]

metoprolol (Lopressor) Drug that selectively blocks the β_1-receptor, limiting contraction of the heart muscles. It is useful for treating hypertension. [5]

Metrazol See *pentylenetetrazol*. [8]

mGluR1–mGluR8 Eight metabotropic glutamate receptors of the nervous system. They can inhibit cyclic adenosine monophosphate synthesis, activate the phosphoinositide second-messenger system, or inhibit glutamate release into the synaptic cleft. [8]

MHPG See *3-methoxy-4-hydroxy-phenylglycol*. [5]

microdialysis Technique used to measure neurotransmitter release in the brain of an awake, freely moving animal by collecting samples of extracellular fluid and then analyzing the samples biochemically using sensitive methods such as HPLC. Chemicals can also be applied to precise brain sites with the same technique. [4]

microelectrode Device used to electrically stimulate or record the response of a single cell intracellularly or extracellularly. [4]

microglia Small nonneuronal cells in the CNS that collect at points of cell damage or inflammation and demonstrate phagocytic behavior. [2]

microsomal enzymes Enzymes in liver cells responsible for metabolizing exogenous substances such as drugs. [1]

microsomal ethanol oxidizing system (MEOS) The cytochrome P450 enzyme CYP 2E1 that metabolizes ethanol and many other drugs. [10]

midsagittal Section taken of the brain that divides it into left and right symmetrical pieces. [2]

mini-mental state exam (MMSE) Cognitive test for measuring the severity of dementia. [20]

Minipress See *prazosin*. [5]

mitochondria (sing. mitochrondrion) Organelles of the cell that produce energy, in the form of ATP, from glucose. [2]

MK-801 (dizocilpine) Drug that binds to the PCP site and acts as a noncompetitive antagonist of the NMDA receptor. [8]

moclobemide Selective MAO-A inhibitor approved in several countries outside of the United States for the treatment of depression and social anxiety. [5]

modafinil (Provigil) Synthetic psychostimulant that is used to treat patients with narcolepsy who exhibit excessive daytime sleepiness, people with obstructive sleep apnea, and people with disordered sleep due to shift work. [12]

monoacyl-glycerol lipase (MAGL) Enzyme primarily responsible for metabolism of the endocannabinoid 2-arachidonoylglycerol. [14]

monoamine Refers to a compound or transmitter that contains a single amine group. [3, 5]

monoamine hypothesis Theory that a reduced level of monoamines in the CNS will cause depressed moods, including clinical depression. [18]

monoamine oxidase (MAO) Enzyme responsible for metabolic breakdown of catecholamines and serotonin. [5]

monoamine oxidase inhibitors (MAOIs) Class of drugs that inhibit monoamine oxidase (MAO), thereby causing an accumulation of catecholamines and serotonin in the brain. They are often used to treat clinical depression. [20]

moral model Model of addiction that treats addiction as a personal and moral problem. [9]

Morris water maze Maze type that involves repeatedly placing the animal in a pool of opaque water and testing its ability to use visual cues from outside the pool to find the escape platform. It is used to test spatial learning. [4]

motor efferents Nerve fibers originating in the CNS and traveling to the skeletal muscles, controlling voluntary movements. [2]

motor neurons Nerve cells that transmit electrical signals from the CNS to muscles. [2]

MPTP See *1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine*. [5, 20]

MRI See *magnetic resonance imaging*. [4]

MS See *multiple sclerosis*. [20]

multidimensional approach Treatment that involves a combination of methods to prevent drug abuse relapse, including detoxification, pharmacological support, and counseling. [11]

multiple sclerosis (MS) Disorder caused by autoimmune destruction of the myelin covering neurons in the brain and spinal cord. MS affects movement, sensation, and bodily functions. [20]

multiple T-maze Maze type that contains many alleys ending in a "T" shape, which gives the animal two possible directions at each choice point. [4]

μ-receptor A subtype of opioid receptor located in the brain and spinal cord that has a high affinity for morphine and certain other opiate drugs. [11]

muscarinic receptors Family of metabotropic cholinergic receptors that are selectively stimulated by muscarine. [7]

muscimol Drug found in the mushroom *Amanita muscaria* that is an agonist for the $GABA_A$ receptor. [8]

muscle dysmorphia Psychological disorder characterized by a false perception that the sufferer is weak and small, constant checking of one's appearance, concealing one's body shape, and a preoccupation with working out and using steroids to enhance muscle growth. [16]

muscle relaxants Drugs, such as benzodiazepines, that reduce muscle tension in a patient. [17]

mutant mice Genetically modified mice produced by gene disruptions: knockout, knockin, or transgenic manipulations. They are used to study genetic disorders. [18]

myasthenia gravis Neuromuscular disorder involving an attack on the muscle cholinergic receptors by one's own immune system. [7]

myelin A fatty insulating sheath surrounding many axons that increases the speed of nerve conduction. It is produced by oligodendrocytes in the CNS and by Schwann cells in the peripheral nervous system. [2]

N

***N*-benzylphenethylamines (NBOMes)** Relatively new class of potent synthetic hallucinogens. [15]

***N*-methyl-D-aspartate (NMDA)** An exogenous amino acid derivative that has high affinity for a specific subtype of ionotropic glutamate receptors. [20]

Na^+–K^+ pump An enzyme (Na^+-K^+ ATPase) that helps to maintain the resting membrane potential by removing Na^+ from inside the cell. Three Na^+ ions are exchanged for two K^+ ions, maintaining a negative charge inside the cell. It also forces the ions against their concentration gradients following an action potential. [2]

NAcc See *nucleus accumbens*. [2]

nAChRs See *nicotinic acetylcholine receptors*. [13]

nalmefene A dual κ/μ-opioid antagonist effective in reducing lever pressing for alcohol in rodent studies, particularly in alcohol-dependent animals. [10]

naltrexone A μ-receptor antagonist that reduces consumption and craving in some alcoholic individuals, perhaps by reducing the positive feeling caused by alcohol. [10]

Namenda See *memantine*. [8, 20]

narcolepsy Sleep disorder characterized by repeated bouts of extreme sleepiness during the daytime. Symptoms include sudden cataplexy, sleep paralysis, and dream-like hallucinations. [3]

narcotic analgesics Class of drugs originally derived from the opium poppy that reduce pain but do not cause unconsciousness. They create a feeling of relaxation and sleep in an individual, but in high doses can cause coma or death. [11]

Nardil See *phenelzine*. [5]

natural recovery Recovery from drug addiction without the aid of treatment. [9]

NBOMes See N-*benzylphenethylamines.* [15]

NBQX Antagonist that blocks both AMPA and kainate receptors, but has no effect on NMDA receptors. [8]

NE transporter Protein in the membrane of noradrenergic neurons that is responsible for NE reuptake from the synaptic cleft. [5]

NE See *norepinephrine*. [3, 5]

necroptosis See *programmed necrosis*. [8]

necrosis Cell death resulting from exposure to a chemical agent (such as glutamate), disease, or other injury. It differs in several important ways from apoptosis (programmed cell death). [8]

negative symptoms Characteristics of schizophrenia that are observed as a decline in normal function, such as reduced speech, loss of motivation, social withdrawal, and anhedonia. [19]

neonatal ventral hippocampal lesion model (NVHL) Neurodevelopmental model of schizophrenia that relies on early damage to the hippocampus in rodents. Hippocampal lesioning leads to some behaviors analogous to the early negative symptoms of schizophrenia. Behaviors similar to the positive symptoms of psychosis appear only at post-adolescence. [19]

neostigmine (Prostigmin) Synthetic analog of the drug physostigmine that cannot cross the blood–brain barrier. It is used to treat myasthenia gravis due to its ability to block AChE activity in muscle tissue. [7]

nerves Bundles of neurons outside the CNS that transmit electrical signals for nervous system function. [2]

neuraxis Imposed line through the body that starts at the base of the spinal cord and ends at the front of the brain. [2]

neuroadaptations Changes in brain functioning that attempt to compensate for the effects of repeated substance use. [9]

neurodevelopmental model Theory that genetic vulnerability in combination with environmental stressors alters the trajectory of brain development resulting in the symptoms observed in schizophrenics. [19]

neurofibrillary tangles (NFTs) Fibrous inclusions, composed primarily of tau protein, that are abnormally located in the cytoplasm of neurons. Pyramidal neurons are particularly susceptible to NFTs. [20]

neuroleptic malignant syndrome (NMS) Undesired response to antipsychotic drugs characterized by fever, instability of the autonomic nervous system, rigidity, and altered consciousness. [19]

neuroleptics Drugs useful in treating schizophrenia; an older term that refers to their ability to selectively reduce emotionality and psychomotor activity. Now more often called antipsychotics. [19]

neuromodulators Chemicals that don't follow the typical neurotransmitter model. They may regulate neurotransmitter activity or act at distant sites from their point of release. [3]

neuromuscular junction Connection point between neurons and muscle cells. It has some of the characteristics of a synapse. [3, 7]

neurons Nerve cells that form the brain, spinal cord, and nerves and that transmit electrical signals throughout the body. [2]

neuropathic pain Chronic pain caused by nerve tissue damage. Produced within the nervous system itself, not

in response to a nociceptive stimulus like a stab wound or burn. [6, 14]

neuropeptides Small proteins (3 to 40 amino acids long) in the nervous system that act as neurotransmitters. [3]

neuropharmacology Area of pharmacology specializing in drug-induced changes to the function of cells in the nervous system. [1]

neuropsychopharmacology Area of pharmacology focusing on chemical substances that interact with the nervous system to alter behavior, emotions, and cognition. [1]

neurosteroids Family of substances that are synthesized in the brain from cholesterol and that have a steroid structure. They act as local signaling agents. [8]

neurotoxin Chemical that damages or kills nerve cells without damaging axons in the area. [4, 5]

neurotransmitters Chemical substance packaged in synaptic vesicles and released by a neuron to communicate across a synapse with another neuron, muscle cell, organ, or a hormone-producing cell in an endocrine gland. [2, 3]

neurotrophic factors Proteins that encourage the growth, development, and survival of neurons. They are also involved in neuronal signaling. [3]

neurotrophic hypothesis Theory that low BDNF is responsible for the loss of dendritic branches and spines and reduced volume of brain areas responsible for clinical depression. [18]

NFTs See *neurofibrillary tangles*. [20]

nicotine replacement therapy (NRT) Method to stop smoking that involves giving the smoker a safer nicotine source, thereby maintaining a level of nicotine in the body and reducing nicotine withdrawal symptoms. [13]

nicotine resource model Theory that smoking is maintained due to positive effects of nicotine such as increased concentration and greater mood control. [13]

nicotinic acetylcholine receptors (nAChRs) Family of ionotropic receptors that are activated by ACh and selectively stimulated by nicotine. They may also be called nicotinic receptors. [13]

nicotinic cholinergic receptors (nAChRs) See *nicotinic receptors*. [7]

nicotinic receptors Family of ionotropic receptors that are activated by ACh and selectively stimulated by nicotine. They may also be called nicotinic cholinergic receptors. [7]

nigrostriatal tract Dopaminergic nerve tract originating at the substantia nigra and terminating in the stratum. It is important for regulation of movement and is severely damaged in Parkinson's disease. [5]

nitrites Class of inhalants that are characterized by the presence of an NO_2 group and that heighten sexual arousal and pleasure. [16]

nitrosamines Class of toxic chemicals contained in tobacco cigarette smoke that have been implicated in smoking-related carcinogenesis and other disease mechanisms. [13]

NMDA receptor Ionotropic glutamate receptor selective for the agonist NMDA. [8]

NMS See *neuroleptic malignant syndrome*. [19]

nocebo Substance that is pharmacologically inert, yet can produce negative therapeutic outcomes. [1]

nodes of Ranvier Gaps in the myelin sheath that expose the axon to the extracellular fluid. [2]

noncompetitive antagonists Drugs that reduce the effect of an agonist, but do not compete at the receptor site. The drug may bind to an inactive portion of the receptor, disturb the cell membrane around the receptor, or interrupt the intercellular processes initiated by the agonist–receptor association. [1]

nonspecific drug effects Physical or behavioral changes not associated with the chemical activity of the drug–receptor interaction but with certain unique characteristics of the individual such as present mood or expectations of drug effects. [1]

nonsteroidal anti-inflammatory drugs (NSAIDs) COX-2 inhibitors such as ibuprofen. [14]

nootropics Drugs that enhance cognitive function, especially memory. [8]

NOP-R One of the four opioid receptors. It is widely distributed in the CNS and the peripheral nervous system and is activated by the neuropeptide nociceptin/orphanin FQ. [11]

noradrenergic Adjectival form of noradrenaline (norepinephrine). [5]

norepinephrine (NE) Neurotransmitter related to DA that belongs to a group called catecholamines. It also functions as a hormone secreted by the chromaffin cells of the adrenal medulla. Also known as noradrenaline. [3, 5]

Norflex See *orphenadrine*. [7]

novelty suppressed feeding paradigm An experimental technique to evaluate anxiety that measures the latency to eat novel foods in a familiar environment or usual foods in a novel environment. [4]

NRT See *nicotine replacement therapy*. [13]

NSAIDs See *nonsteroidal anti-inflammatory drugs.* [14]

nuclei Localized cluster of nerve cell bodies in the brain or spinal cord. [2]

nucleus accumbens (NAcc) Structure of the limbic system that mediates the reinforcing and incentive salience effects of many activities, including the abuse of drugs. [2]

Nuedexta Dextromethorphan-containing medication prescribed for the treatment of pseudobulbar affect. [15]

NVHL See *neonatal ventral hippocampal lesion model*. [19]

O

obsessions Worrying thoughts or ideas that an individual cannot easily ignore. [17]

obsessive-compulsive disorder (OCD) Psychiatric anxiety disorder characterized by persistent thoughts of contamination, violence, sex, or religion that the individual cannot easily ignore, and that cause the individual anxiety, guilt, or shame, etc. and may be accompanied by compulsive repetitive behaviors. [17]

occipital lobe One of four lobes of the cerebral cortex. It contains the visual cortex and helps integrate visual information. [2]

OCD See *obsessive-compulsive disorder.* [17]

6-OHDA *See 6-hydroxydopamine.* [5]

8-OH-DPAT See *8-hydroxy-2-(di-n-propylamino) tetralin*. [6]

oligoclonal bands Immunoglobins that indicate inflammatory processes within the central nervous system. [20]

oligodendrocytes Glial cells that myelinate nerve axons of the CNS. Also known as oligodendroglia. [20]

oligodendroglia Glial cells that myelinate nerve axons of the CNS. Also known as oligodendrocytes. [2]

ondansetron (Zofran) Drug that inhibits 5-HT_3 receptors. It is used to treat the nausea and vomiting side effects of cancer chemotherapy. [6]

one-chamber social interaction test Test used to measure the level of anxiety in rodents by recording the time spent investigating other animals. [4]

open State of a receptor channel in which the channel pore is open, thereby permitting ion flow across the cell membrane. [7]

open field test Technique used to measure locomotor activity and exploratory behavior by placing the animal on a grid and recording the number of squares traversed in a unit of time. [4]

operant analgesia testing Technique used to test analgesic drugs. Once an animal is trained to lever press to terminate foot shock, the researchers gradually increase shock stimulation from very low levels until the animal responds by lever pressing, to indicate threshold. Analgesic drugs would be expected to raise that threshold. [4]

operant conditioning Type of learning in which animals learn to respond to obtain rewards and avoid punishment. It explains drug tolerance when an animal learns to engage in behaviors when under the influence of a drug. [1]

opponent-process model Model of addiction in which the initial positive response to a drug is followed by an opposing withdrawal response as the drug wears off. [9]

optogenetics New neurobiological technique based on the ability of certain light-sensitive proteins, when expressed in a specific subset of neurons, to either excite or inhibit the cells when exposed to light of the appropriate wavelength. [4]

oral administration (PO) Method that involves administering a drug through the mouth. [1]

orexin-A Peptide neurotransmitter, also known as hypocretin 1, which is found in the hypothalamic area and which regulates numerous behavioral and physiological functions including feeding behavior, body weight, reward, emotional responses, stress responses, and autonomic nervous system activity. [3]

orexin-B Peptide neurotransmitter, also known as hypocretin 2, which is found in the hypothalamic area and which regulates numerous behavioral and physiological functions including feeding behavior, body weight, reward, emotional responses, stress responses, and autonomic nervous system activity. [3]

organophosphorus compounds General name for organic chemicals containing phosphorus, but sometimes applied more specifically to esters of phosphoric acid. OPs are the basis for many insecticides, herbicides, and nerve gases. [7]

orphenadrine (Norflex) Anticholinergic drug used to treat early symptoms of Parkinson's disease. [7]

osmotic minipump Device placed just under the skin of an animal that allows a drug to be administered continuously over a set period of time. [13]

other hallucinogen use disorder DSM-5 diagnostic category that defines a psychiatric disorder involving use of hallucinogenic drugs other than PCP and related substances. [15]

ovaries Female-specific gonads that secrete the sex hormones estrogen and progesterone. [3]

oxytocin Peptide hormone synthesized by certain hypothalamic neurons and secreted into the bloodstream at the posterior lobe of the pituitary gland. Circulating oxytocin induces uterine contractions during childbirth and milk letdown during lactation. Other oxytocin neurons form synapses within the brain and play an important role in social, including maternal, behaviors in some species. [3]

P

P2X receptors Ionotropic receptors for ATP. [13]

P2Y receptors Metabotropic receptors for ATP. [13]

PAG See *periaqueductal gray*. [2]

palonosetron (Aloxi) Drug that inhibits 5-HT_3 receptors. It is used to treat the nausea and vomiting side effects of cancer chemotherapy. [6]

panic attack Feeling of extreme fear that was not preceded by a threatening stimulus. [17]

panic disorder Disease involving repeated attacks of extreme fear, occurring either without warning or in an environment similar to where previous panic attacks occurred. [17]

pannexins Membrane channels through which ATP is released into the extracellular fluid. [13]

para-chloroamphetamine Drug similar in structure to amphetamine that stimulates 5-HT release. It is also neurotoxic at high doses. [6]

para-chlorophenylalanine (PCPA) Drug that irreversibly inhibits tryptophan hydroxylase, blocking 5-HT synthesis. [6]

parasympathetic Division of the autonomic nervous system responsible for conserving energy, digestion, glucose and nutrient storage, slowing the heart rate, and decreasing respiration. [2]

parasympatholytic agents Drugs that block muscarinic receptors, inhibiting the parasympathetic system. They are deadly at high doses, but at low doses they are used medicinally to dilate pupils, relax airways, counteract cholinergic agonists, and induce drowsiness. [7]

parasympathomimetic agents Drugs that stimulate muscarinic receptors, thereby mimicking the effects of parasympathetic system activation. [7]

parenteral Methods of drug administration that do not use the gastrointestinal system, such as intravenous, inhalation, intramuscular, transdermal, etc. [1]

parietal lobe One of four lobes of the cerebral cortex. It contains the somatosensory cortex and helps integrate information about body senses. [2]

Parkinson's disease (PD) Chronic, progressive, neurodegenerative disorder characterized by tremor, rigidity, difficulty in initiating movement, slowing of movement, and postural instability. [20]

Parkinson's disease dementia (PDD) Condition in which one or more cognitive functions are impaired to the point of interfering with the ability of the individual to navigate everyday life. [20]

Parkinsonian symptoms Undesired response to antipsychotic drugs that resembles Parkinson's disease, including tremors, akinesia, muscle rigidity, akathesia, and lack of facial expression. [19]

Parnate See *tranylcypromine*. [5]

paroxysmal depolarization shift (PDS) Periodic episodes of prolonged neuronal depolarization occurring during the interictal period in the brain of epileptic patients. [8]

partial agonists Drugs that bind to a receptor but have low efficacy, producing weaker biological effects than a full agonist. Hence, they act as agonists at some receptors and antagonists at others, depending on the regional concentration of full agonist. These were previously called mixed agonist-antagonists. [1, 11]

parts per million (ppm) Number of molecules of a gaseous substance per million molecules of air. When applied to an inhaled substance, it is used to describe the amount of exposure to the substance (i.e., dose). [16]

passive avoidance learning Type of learning task in rats and mice in which the animal is trained to avoid a location that it would normally enter (e.g., going into a dark compartment from one that is brightly lit) by administeration of a brief electric footshock when it enters the location. The word "passive" in the name of the task reflects the fact that the animal must withhold its usual response of moving into the dark compartment. [5]

passive diffusion Movement of lipid-soluble materials across a biological barrier without assistance based on its concentration gradient, from higher to lower concentration. [1]

patch clamp electrophysiology Technique used to measure the function of a single ion channel by using a micropipette to isolate the ion channel and obtain an electrical recording. [4]

Pavlovian conditioning See *classical conditioning*. [1]

PCP See *phencyclidine*. [8, 15]

PCPA See *para-chlorophenylalanine*. [6]

PDE See *phosphodiesterase*. [18]

PDS See *paroxysmal depoarization shift*. [8]

peak experience Intense psychedelic state experienced during a hallucinogenic drug-assisted therapeutic session that is hypothesized to be an important contributor to the therapeutic benefit. [15]

pedunculopontine tegmental nuclei (PPTg) Structure within the dorsal lateral pons containing cholinergic neurons that project to the substantia nigra (important for stimulating nigral dopamine neurons) and others that project to the brainstem and thalamus (important for behavioral arousal, sensory processing, and inititation of rapid-eye-movement sleep). [7]

penetrance Frequency with which a particular gene produces its main effect. [20]

pentylenetetrazol (Metrazol) Convulsant drug that acts by blocking the function of $GABA_A$ receptors. [8]

penumbra Following an ischemic stroke, this is the outer region of damage (surrounding the core) within which the dying neurons can potentially be rescued. [8]

periaqueductal gray (PAG) Structure of the tegmentum located around the cerebral aqueduct that connects the third and fourth ventricles. It is important for regulating pain; stimulation produces an analgesic effect. [2]

PET See *positron emission tomography*. [4]

peyote button Crown of the peyote cactus, *Lophophora williamsii*, that can be dried and ingested to obtain the hallucinogenic drug mescaline. [15]

peyote cactus Species of cactus, *Lophophora williamsii*, that produces mescaline. [15]

pharmacodynamic tolerance Type of tolerance formed by changes in nerve cell functions in response to the continued presence of a drug. [1]

pharmacodynamic tolerance Type of tolerance formed by changes in nerve cell functions in response to the continued presence of a drug. [10]

pharmacodynamics Study of physiological and biochemical interactions of a drug with the target tissue responsible for the drug's effects. [1]

pharmacogenetics The study of the genetic basis for variability in drug response among individuals (sometimes called pharmacogenomics). [1]

pharmacokinetic Factors that contribute to bioavailability: the administration, absorption, distribution, binding, inactivation, and excretion of a drug. [1]

pharmacological MRI (phMRI) A spin-off of functional MRI (fMRI), is a technique used in drug development to investigate the mechanism of drug action by visualizing changes in brain function following drug administration. [4]

pharmacology Study of the actions of drugs and their effects on living organisms. [1]

pharmacotherapeutic treatment Method of disease treatment that uses drugs to modify a clinical condition. [10]

phasic release Irregularly timed and larger amounts of neurotransmitter release than occurs in the case of tonic release. It is typically associated with burst mode of cell firing and produces surges in extracellular levels of the transmitter. [5]

phencyclidine (PCP) Drug that binds to the PCP site and acts as a noncompetitive antagonist of the NMDA receptor. It is a dissociative anesthetic that was once used medicinally but is now only taken recreationally. [8, 15]

phenelzine (Nardil) MAO inhibitor used to treat clinical depression. [5]

phenethylamine Class of drugs that includes mescaline as well as NE- and amphetamine-related substances. [15]

phenylephrine α_1-receptor agonist that causes behavioral stimulation. [5]

phMRI See *pharmacological MRI*. [4]

phobias Fears of specific objects or situations that are recognized as irrational. [17]

phosphodiesterase (PDE) The enzyme that normally degrades cAMP to 5′-AMP. [18]

phosphoinositide second-messenger system Neurotransmitter signaling mechanism that activates PKC and is controlled by certain receptors for ACh, NE, and 5-HT. [3]

phospholipids Lipid molecules that are major constituents of the cell membrane. They are composed of a polar head and two lipid tails. [1]

phosphorylate Add a phosphate group to a molecule by means of an enzymatic reaction. [3]

physical dependence Developed need for a drug, such as alcohol or opioids, by the body as a result of prolonged drug use. Termination of drug use will lead to withdrawal symptoms (abstinence). [1, 10, 11]

physiological antagonism Drug interaction characterized by two drugs that act in distinct ways and reduce each other's effectiveness in the body. [1]

physostigmine (eserine) Drug that blocks AChE activity. Its symptoms include slurred speech, mental confusion, hallucinations, loss of reflexes, convulsions, coma, and death. It is isolated from Calabar beans. [7]

phytocannabinoids Compounds with a cannabinoid structure that are found in the cannabis plant. [14]

pia mater The innermost of the meninges. The pia mater is a thin tissue immediately surrounding the brain and spinal cord. [2]

picrotoxin Convulsant drug that acts by blocking the function of $GABA_A$ receptors. [8]

pilocarpine Extract of the shrub *Pilocarpus jaborandi* known for its ability to stimulate muscarinic receptors. [7]

pineal gland Specific endocrine gland that is located above the brain stem, covered by the cerebral hemispheres. It secretes melatonin. [3]

pinocytotic vesicles Type of vesicles that envelop and transport large molecules across the capillary wall. [1]

pituitary gland Endocrine gland that is located under the hypothalamus and connects to the brain by a thin stalk. It secretes TSH, ACTH, FSH, LH, GH, PRL, vasopressin, and oxytocin. [3]

PKA See *protein kinase A*. [3]

PKC See *protein kinase C*. [3]

PKG See *protein kinase G*. [3]

place conditioning Pavlovian conditioning procedure used to test the rewarding effects of drugs in rats and mice. [9]

placebo Substance that is pharmacologically inert, yet in many instances produces both therapeutic and side effects. [1]

PO See *oral administration*. [1]

polarized Possessing an electrical charge. [2]

polypharmacy Use of multiple pharmacological agents at the same time. [16]

POMC See *pro-opiomelanocortin*. [11]

positive reinforcers Something (e.g., an abused drug) that, when provided to an organism, increases the strength of the response that was used to obtain the item. In studies of addiction, the positive reinforcing quality of a drug is usually measured by means of a self-administration procedure. [9]

positive symptoms Characteristics of schizophrenia that include delusions, hallucinations, disorganized speech, and bizarre behavior. They are often the more dramatic symptoms. [19]

positron emission tomography (PET) Imaging technique used to determine the distribution of a radioactively labeled substance in the body. It can be used to measure drug binding to neurotransmitter receptors or transporters in the brain as well as measuring changes in metabolic activity reflecting neuron function. [4]

post-traumatic stress disorder (PTSD) Emotional disorder that develops in response to a traumatic event, leaving the individual feeling a sense of fear, helplessness, and terror. Symptoms include sleep disturbances, avoidance of stimuli associated with the trauma, intrusive thoughts reliving the event, and a numbing of general emotional responses. An increase in suicidal thoughts has also been observed. [17]

posterior Located near the back or rear of the nervous system. [2]

posterior pituitary Part of the pituitary gland in which vasopressin and oxytocin are secreted. [3]

postsynaptic cell Neuron at a synapse that receives a signal from the presynaptic cell. [3]

postsynaptic density Protein-rich structure associated with the postsynaptic membrane of many dendrites that contains a high density of neurotransmitter receptors along with other proteins that anchor the receptors to the postsynaptic area near the presynaptic sites of transmitter release. [3]

postural instability Impaired balance and coordination. In Parkinson's disease, manifests as a pronounced forward or backward lean in upright position. [20]

potency Measure of the amount of drug necessary to produce a specific response. It is dependent on the affinity of the drug to the receptor. [1]

potentiation Drug interaction characterized by an increase in effectiveness greater than the collective sum of the individual drugs. [1]

PPI See *prepulse inhibition of startle*. [4, 19]

PPTg See *pedunculopontine tegmental nucleus*. [7]

prazosin (Minipress) α_1-receptor antagonist that causes dilation of blood vessels and is useful for treating hypertension. [5]

Precedex See *dexmedetomidine*. [5]

precipitated withdrawal Method used to test dependence and withdrawal by administering an antagonist to block drug effects rapidly. [14]

precursor Chemical that is used to make the product formed in a biochemical pathway (e.g., tyrosine is the precursor of DOPA in the pathway for catecholamine synthesis). [3]

predictive validity A measure of how closely the results from animal tests predict clinically useful effects in humans. [4]

prenatal inflammation After administration of pro-inflammatory agents to pregnant rodents, neurodevelopmental and behavioral outcomes are evaluated in the offspring. [19]

prepulse inhibition of startle (PPI) Method to study the ability of an individual to filter out sensory stimuli by applying a weak "prepulse" stimulus shortly before the startle-inducing stimulus. Well validated model of information-processing deficits in schizophrenia. [4, 19]

presenilin-1 (PS-1) Protein involved in the processing of APP. [20]

presenilin-2 (PS-2) Protein involved in the processing of APP. [20]

presurgical anesthesics Drugs used to decrease preoperative anxiety to make administration of the surgical anesthesia less distressing and quicker. [17]

presynaptic cell Neuron at a synapse that transmits a signal to the postsynaptic cell. [3]

presynaptic facilitation Signaling by the presynaptic cell to increase neurotransmitter release by the axon terminal of the postsynaptic cell. [3]

presynaptic inhibition Signaling by the presynaptic cell to reduce neurotransmitter release by the axon terminal of the postsynaptic cell. [3]

primary cortex The part of each lobe of the cortex that provides conscious awareness of sensory experience and the initial cortical processing of sensory qualities. [2]

primary hypogonadism Type of hypogonadism caused by lack of responsiveness of the testes to LH and FSH. [16]

PRL See *prolactin*. [3]

pro-opiomelanocortin (POMC) One of the four large opioid propeptide precursors that are broken down by proteases to form smaller active opioids (endorphins) in the brain. [11]

prodynorphin One of the four large opioid propeptide precursors that are broken down by proteases to form smaller active opioids (dynorphins) in the brain. [11]

proenkephalin One of the four large opioid propeptide precursors that are broken down by proteases to form smaller active opioids (enkephalins) in the brain. [11]

progesterone Female sex hormone secreted by the ovaries that is present at high levels during pregnancy. [3]

progestins Group of female sex hormones that are important for the maintenance of pregnancy. The principal naturally occurring progestin is progesterone. [3]

programmed cell death Cell death resulting from a programmed series of biochemical events in the cell designed to eliminate unnecessary cells. Also called *apoptosis*. [8]

programmed necrosis Type of programmed cell death provoked by excitotoxic treatment of adult animals in which the appearance of the dying neurons is different from the appearance of neurons undergoing apoptosis. Also called *necroptosis*. [8]

progressive-ratio procedure Method used to measure the relative power of drug reinforcement by steadily increasing the response to reward ratio. [9]

prolactin (PRL) Hormone secreted by the anterior pituitary that promotes milk production by the mammary glands. [3]

promoter region Section of a gene, adjacent to the coding region, that controls the rate of transcription as directed by the binding of transcription factors. [2]

pronociceptin/orphanin FQ One of the four large opioid propeptide precursors, that is broken down by proteases to form smaller active opioids (nociceptin, orphanin FQ) in the brain. [11]

propofol (Diprivan) Positive allosteric modulator of the $GABA_A$ receptor that is used as a surgical anaesthetic agent. [8]

propranolol (Inderal) β-receptor antagonist. It is useful for treating hypertension due to its ability to block β-receptors in the heart, thereby limiting contraction of the heart muscles. [5]

Prostigmin See *neostigmine*. [7]

protein kinase A (PKA) Enzyme that is stimulated by cAMP and that phosphorylates specific proteins as part of a neurotransmitter signaling pathway. [3]

protein kinase C (PKC) Enzyme that is stimulated by diacylglycerol and Ca^{2+} and that phosphorylates specific proteins as part of a neurotransmitter signaling pathway. [3]

protein kinase G (PKG) Enzyme that is stimulated by cGMP and that phosphorylates specific proteins, including proteins involved in cell growth and differentiation. [3]

protein kinases Enzymes that catalyze the phosphorylation of other proteins. [3]

Provigil See *modafinil*. [12]

Prozac See *fluoxetine*. [6]

prucalopride (Resolor) Drug that stimulates $5\text{-}HT_4$ receptors. It is used to treat the diarrhea-predominant form of irritable bowel syndrome in several countries outside of the United States. [6]

pseudobulbar affect Rare neurological disorder seen in a small percentage of patients with brain injury or disease that is characterized by frequent, uncontrollable episodes of laughing or crying that are incongruent with the person's emotional state. [15]

psilocin Metabolite of psilocybin. Psilocin is the actual psychoactive agent. [15]

psilocybin Hallucinogenic drug found in several mushroom species. [15]

psychedelic Substance that alters perceptions, state of mind or awareness. [15]

psychedelic therapy Therapeutic method that involved giving patients a single high dose of LSD to help them understand their problems by reaching a drug-induced spiritual state. [15]

psychoactive drugs Those drugs that have an effect on thinking, mood, or behavior. [1]

psycholytic therapy Therapeutic method that employed LSD in low doses, gradually increasing the dose, in attempts to recover repressed memories or increase communication with the therapist. [15]

psychomotor stimulants Class of drugs that produce strong sensorimotor activation characterized by increased alertness, heightened arousal, and behavioral excitation. Also called *psychostimulants*. [12]

psychopharmacology Area of pharmacology specializing in drug-induced changes in mood, thinking, and behavior. [1]

psychosocial rehabilitation Counseling programs that involve educating the user, promoting behavioral change and alleviating problems caused by drug use. [10]

psychosocial treatment programs Counseling programs that involve educating the user, promoting behavioral change and alleviating problems caused by drug use. [12]

psychotomimetic Substance that mimics psychosis in a subject, such as by inducing hallucinations or delusions. [15]

PTSD See *post-traumatic stress disorder*. [17]

pure antagonists Drug that produces no pharmacological activity (i.e., no efficacy) and that can prevent or reverse the effects of a drug agonist by occupying the receptor site. [11]

pyramidal neuron Neuron with a roughly pyramidal shape that serves as the principal type of output neuron in several brain areas, notably the cerebral cortex, hippocampus, and amygdala. [3]

pyramiding Pattern of steroid use characterized by gradually increasing the drug dose until the middle of the cycle, then gradually decreasing the drug dose until the cycle is complete. [16]

pyridostigmine (Mestinon) Synthetic analog of the drug physostigmine that cannot cross the blood–brain barrier. It is used to treat myasthenia gravis due to its ability to block AChE activity in muscle tissue. [7]

pyrolysis Process of chemical decomposition caused by heating. [13]

Q

quantitative EEG (qEEG) Computer-assisted evaluation of EEG data, used to monitor brain function and cognitive processing. [4]

quinpirole Agonist at D_2 and D_3 dopamine receptors. [5]

R

radial arm maze Maze type composed of multiple arms leading from a central choice point. Radial arm mazes are used to test spatial learning. [4]

radioimmunoassay (RIA) A very sensitive method that uses antibodies to measure molecules in body fluids or tissue extracts. The essay depends on competitive binding of an antibody to the antigen of interest. [4]

radioligand binding Technique used to measure the affinity and relative density of receptors in a particular brain area by using a radioactively labeled ligand for the receptor. [4]

raphe nuclei Network of cell clusters in the CNS that contain the cell bodies of serotonergic neurons. They are found almost exclusively along the midline of the brain stem. [6]

rasagiline (Azilect) Selective MAO-B inhibitor used clinically to elevate brain DA levels in Parkinson's disease. [5]

rate-limiting enzyme Enzyme that catalyzes the slowest step in a biochemical pathway. It determines the overall rate of product formation. [5]

reactive depression State of sadness that is appropriate and of a reasonable level in response to a given aversive situation. Not usually considered a clinical condition. [18]

reboxetine (Edronax) Antidepressant that selectively blocks the NE transporter, thereby increasing NE concentration in the synaptic cleft. [5]

receptor agonists Neurochemicals or drugs that can bind to a particular receptor protein and alter the shape of the receptor to initiate a cellular response. [1]

receptor antagonists Molecules that interact with a receptor protein and produce no cellular effect after binding, and also prevent an "active" ligand from binding. [1]

receptor binding studies Technique used to measure the affinity and relative density of receptors in a particular brain area by using a radioactively labeled ligand for the receptor. Also called *radioligand binding*. [18]

receptor cloning Process used to produce large amounts of identical receptor proteins in a cell line. [11]

receptor subtypes Group of receptors that respond to the same neurotransmitter but that differ from each other to varying degrees with respect to their structure, signaling mechanisms, and pharmacology. [1, 3]

receptor trafficking Normal process in which the receptors for a particular neurotransmitter are shuttled into and out of the cell membrane to regulate sensitivity of the cell to that transmitter. [8]

receptor up-regulation Increase in the number of receptors produced and maintained in a target cell. [5]

receptors Proteins located on the surface of or within cells that bind to specific ligands to initiate biological changes within the cell. [1, 2, 3]

rectal administration Drug delivery method requiring placement of a drug-filled suppository into the rectum. [1]

reinstatement of drug-seeking behavior Restoration of a behavior (e.g., an operant response) previously used to obtain a drug after that behavior had been extinguished. It is an animal model for relapse to drug use after a period of abstinence in chronic drug users. [9]

relapse prevention therapy Treatment program for drug abusers that teaches an individual how to avoid and cope with high-risk situations. [12]

relapses Recurrences of drug use following a period of abstinence. [9]

relative refractory period Short hyperpolarizing phase after an action potential during which a more intense excitatory stimulus is necessary to obtain an action potential. [2]

reliability Term used to indicate how dependable test results are and how likely the same test results will be found in subsequent trials. [4]

remissions Periods in which an addict is drug free. [9]

resensitization Receptor state characterized by the return of receptor function and a normal response to agonist stimulation. [7]

reserpine Drug extracted from *Rauwolfia serpentina* (snake root) roots. It inhibits vesicular monoamine uptake by VMAT, thereby reducing monoamine levels in the central and peripheral nervous system. [5]

resident–intruder test Test of aggressive behavior involving attack by a resident adult male rat or mouse against a strange intruder male. [6]

resting membrane potential The difference in the electrical charge inside a neuron at rest compared to the outside. The inside of the cell is more negative, and that potential is –70 mV. [2]

resting tremor Tremor that is present when the limb is relaxed. Can be present in the hand, foot, jaw or face. Generally disappears with intentional movement. [20]

resting-state fMRI (rs-fMRI) This technique is a variation of fMRI that visualizes brain activity and connectivity in individuals when they are not actively engaged in a task that requires attention or task performance. [4]

reticular formation Collection of nuclei within the core of the pons forming a network that extends into the midbrain and medulla. These nuclei are important for arousal, attention, sleep, muscle tone, and some cardiac and respiratory reflexes. [2]

retrograde messengers Chemicals synthesized and released by a postsynaptic cell that diffuse into the nerve terminal of the presynaptic cell, often for the purpose of altering neurotransmitter release by the terminal. [3]

retrograde signaling Signaling mechanism in which the endocannabinoid activates CB_1 receptors on nearby nerve terminals. [14]

retropulsion The need to take a step backward when starting to walk. [20]

reuptake Process that involves transport of neurotransmitters out of the synaptic cleft by the same cell that released them. [3]

reverse tolerance Enhanced response to a particular drug after repeated drug exposure. Also called *sensitization*. [12]

reward circuit Circuit of neurons that, when activated, mediates the rewarding effects of both natural rewards (e.g., food, water, sex) and drugs of abuse. [9]

RIA See *radioimmunoassay*. [4]

ribosomes Organelles in the cytoplasm that decode the nucleotide sequence provided by mRNA and link the appropriate amino acids together to form a protein. [2]

rigidity Stiffness and inflexibility in the joints. Two types are present in Parkinson's disease: "lead-pipe" rigidity, which is characterized by maintenance of inflexibility of the joint through the entire range of movement, and "cog-wheel" rigidity, which is characterized by a ratchet-like interruption in movement. [20]

riluzole Drug that inhibits glutamate release and that is used to treat ALS. [8]

rimonabant Antagonist selective for the CB_1 receptor. It is also called SR 141716. [14]

risperidone (Risperdal) Drug that inhibits 5-HT_{2A} and D_2 dopamine receptors. It is used to treat schizophrenia. [6]

Ritalin See *methylphenidate.* [12]

ritanserin Drug that inhibits 5-HT_{2A} receptors. [6]

rivastigmine (Exelon) Drug that blocks AChE activity. It is used in the treatment of Alzheimer's disease. [7]

rostral Located near the front or head end of the nervous system. [2]

rotarod An animal test using a horizontally-oriented cylinder that is mechanically rotated at set speeds. Researchers time latency to fall (e.g., how long mice remain balanced on the rod). [4]

S

Sabril See *vigabatrin*. [8]

saclofen Chemical analog of baclofen that is a competitive antagonist at the GABAB receptor. [8]

sagittal Section that is taken parallel to the plane bisecting the nervous system into right and left halves. [2]

saltatory conduction Mode of action potential conduction along a myelinated neuron characterized by jumps from one node of Ranvier to the next. [2]

salvinorin A Active compound in the hallucinogenic plant *Salvia divinorum*; acts as a κ-opioid receptor agonist. [15]

sarin Toxin that causes irreversible inhibition of AChE. It is used as a nerve agent for chemical warfare. [7]

SC See *subcutaneous*. [1]

SCH 23390 D_1 receptor antagonist that may induce catalepsy when administered in high doses. [5]

Schedule of Controlled Substances System established by the Controlled Substances Act in 1970 that classifies most substances with abuse potential into one of five schedules. Schedules I and II have the strictest guidelines. [9]

schedule of reinforcement Predetermined schedule used to determine when an animal will be rewarded for performing a specific behavior. A fixed ratio (FR) schedule refers to rewards given after a set number of responses; a fixed interval (FI) schedule refers to rewards given to the first response that occurs after a set amount of time has elapsed. [4]

Schwann cells Glial cells that myelinate peripheral nerve axons. [2, 20]

scopolamine Drug that blocks muscarinic receptors. It is found in nightshade, *Atropa belladonna*, and in henbane, *Hyoscyamus niger*. [7]

second messenger Substance that, when activated by signaling molecules bound to receptors in the cell membrane, will initiate biochemical processes within the cell. [3]

second-messenger systems Biochemical pathways that use second messengers to mediate intercellular signaling. [3]

secondary cortex Section of the cerebral cortex containing the neuronal circuits responsible for analyzing and recognizing information from the primary cortex, and for memory storage. [2]

secondary hypogonadism Type of hypogonadism caused by a decline in LH and FSH secretion. [16]

section Tissue slice showing structures of the body or nervous system. [2]

sedative–hypnotics Class of drugs that depresses nervous system activity. They are used to produce relaxation, reduce anxiety, and induce sleep. [17]

selective D_2 receptor antagonists Drugs that selectively block D_2 receptors, including sulpiride, raclopride, and remoxipride. [19]

selective serotonin reuptake inhibitors (SSRIs) Antidepressants used to treat major depression, panic and anxiety disorders, obsessive-compulsive disorder, obesity, and alcoholism by blocking the presynaptic membrane transporter for 5-HT. [6, 18, 20]

selegiline (Eldepryl) Selective MAO-B inhibitor used clinically to elevate brain DA levels in Parkinson's disease. [5]

self-administration method Test used to measure the abuse potential of a drug by allowing an animal to give itself the drug doses. [4]

self-medication hypothesis Theory that addiction is based on an effort by the individual to treat oneself for mood or other ill feelings. [9]

sensitization Enhanced response to a particular drug after repeated drug exposure. Also called *reverse tolerance*. [1, 11, 12]

sensory afferents Neurons carrying sensory information from the body surface or internal organs into the CNS. [2]

sensory neurons Nerve cells that are sensitive to environmental stimuli and convert the physical stimuli into electrical signals that are sent to the CNS. [2]

serenics Class of drugs capable of inducing a state of calmness, thereby reducing aggressive behaviors. [6]

serotonergic neurons Neurons that use serotonin as their transmitter. [6]

serotonin Neurotransmitter found in the central and peripheral nervous system and synthesized by serotonergic neurons. [6]

serotonin deficiency hypothesis of aggression Hypothesis that low serotonergic activity in the CNS is associated with hyperaggressiveness. [6]

serotonin reuptake transporter (SERT) Protein in the membrane that is responsible for serotonin (5-HT) reuptake from the synaptic cleft. [18]

serotonin syndrome Effects associated with an overdose of SSRIs or serotonergic agonists, including severe agitation, disorientation, confusion, ataxia, muscle spasms, fever, shivering, chills, diarrhea, elevated blood pressure, and increased heart rate. [6, 18]

SERT See *5-HT transporter* and *serotonin reuptake transporter*. [6, 18]

shared etiology Situation in which multiple disorders are caused by the same set of factors. [9]

side effects Undesired physical or behavioral changes associated with a particular drug. [1]

silent receptors See *drug depots.* [1]

single-nucleotide polymorphisms (SNPs) Allelic variations in genes consisting of a changes in single nucleotides in one of the two copies of a gene. They are distinguished from mutations by having a prevalence of at least 1% in the population. [9]

single-photon emission computerized tomography (SPECT) Imaging technique used to view changes in regional blood flow or drug binding by using radioactively labeled compounds injected or inhaled into the body. [4]

single-spiking mode Mode of neuronal cell firing characterized by the production of single action potentials at intervals that may be regular or irregular, depending on the cell type. For midbrain DA neurons, the intervals are irregular. [5]

sinsemilla The potent marijuana produced by preventing pollination and seed production in the female cannabis plants. [14]

SKF 38393 Selective dopamine D_1 receptor agonist. [5]

sleep deprivation Lack of proper sleep, either unintentional (e.g., jet lag), or intentional (such as all-night studying). [18]

sleep paralysis Loss of muscle tone leading to a feeling of paralysis that occurs during the daytime in some patients with narcolepsy. [3]

SNPs See *single-nucleotide polymorphisms.* [9]

sodium oxybate (Xyrem) The sodium salt of γ-hydroxybutyrate (GHB). It is used as a treatment for narcolepsy. [16]

soma Cell body of a neuron, containing all of the organelles needed to maintain the cell. [2]

soman Toxin that causes irreversible inhibition of AChE. It is used as a nerve agent for chemical warfare. [7]

somatodendritic autoreceptors Autoreceptors located on the dendrites or cell body that slow the rate of cell firing when activated. [3]

spasticity Constant unwanted contraction of one or more muscle groups. [20]

specific drug effects Physical or behavioral changes associated with biochemical interactions of a drug with the target site. [1]

specific neurotoxins Chemical that damages a specific neural pathway leaving others intact. [4]

SPECT See *single-photon emission computerized tomography*. [4]

spinal interneurons Nerve cells with short axons within the spinal cord. [11]

SR 141716A See *rimonabant*. [14]

SSRIs See *selective serotonin reuptake inhibitors*. [6, 18, 20]

stacking Pattern of anabolic steroid use characterized by the simultaneous use of multiple steroids, such as a short- and a long-acting steroid. [16]

state-dependent learning Condition characterized by better performance of a particular task that was learned in a drugged state in the same drugged state, rather than in a nondrugged state. Tasks learned in a nondrugged state are likewise performed better in a nondrugged state. [1]

status epilepticus Dangerous condition characterized either by continuous epileptic seizures or a sufficiently short period between seizures so that the patient has insufficient time to recover. [8]

steady state plasma level The desired blood concentration of drug achieved when the absorption/distribution phase is equal to the metabolism/excretion phase. [1]

stereotyped behaviors Repeated, relatively invariant behaviors associated with a particular situation or drug treatment. They often occur following a high dose of a psychostimulant such as cocaine or amphetamine. [5]

steroids Class of hormones that are derived from cholesterol and regulate a variety of biochemical pathways. [3]

stop-signal task Test used to evaluate impulsivity (e.g., lack of behavior control). It requires the subject (human or otherwise) to rapidly press one button or lever when a square is displayed, and the other button or lever when any other shape appears. Periodically, a tone, which is the "stop" signal, is sounded following the visual presentation. The tone indicates that the subject should withhold responding. [4]

Strattera See *atomoxetine*. [5]

subcutaneous (SC) Method that involves injection of a drug just below the skin. [1]

sublingual administration Method of drug administration that requires placing the drug under the tongue in contact with the mucous membrane, which has a rich capillary network for rapid absorption into the blood. [1]

substance use disorders New *DSM-5* designations for psychiatric disorders with features typically associated

with addiction. This designation replaces both substance abuse and substance dependence categories in *DSM-IV*. [9]

substance-induced disorders New *DSM-5* designations referring to reversible substance-specific syndromes caused by recent ingestion of a substance. [9]

substance-related disorders New *DSM-5* category that encompasses both substance use disorders and substance-induced disorders. [9]

substantia nigra Collection of dopaminergic cell bodies within the tegmentum of the mesencephalon that innervate the striatum by way of the nigrostriatal tract. Damage to cells in this region leads to Parkinson's disease. [2, 5, 20]

subunits Individual protein components that must join in the cell membrane to form a complete receptor. [3, 7]

succinylcholine Chemical similar to ACh that is resistant to metabolism by AChE. It is used as a muscle relaxant during some surgical procedures. [7]

sucrose preference test A test dependent on rodents' natural preference for sweet solutions. Failure to prefer a sucrose solution over water is an indication of anhedonia, a symptom of clinical depressive disorder in humans. [4]

sudden sniffing death syndrome Fatal cardiac arrhythmia associated with inhalant use. [16]

sulci (sing. sulcus) Small grooves of the cerebral cortex. [2]

sumatriptan (Imitrex) Drug that stimulates 5-$HT_{1B/1D}$ receptors, thereby causing constriction of cerebral blood vessels. It is used to treat migraine headaches. [6]

superior Located near the top of the brain in humans. [2]

suppressor T cell Type of T cell that reduces or suppresses the immune response of other T cells (or B cells) to an antigen. [20]

supraspinal Located above the spinal cord or spine. [11]

suvorexant (Belsomra) Antagonist at both OX1R and OX2R receptors that is approved for the treatment of chronic insomnia. [3]

suxamethonium Drug that causes a short-term depolarization block of skeletal muscles. It is used as an adjunct to general anesthesia to produce muscular relaxation during surgery. [7]

swimming performance A rodent test of coordination used in the study of motor deficit diseases. [4]

sympathetic Division of the autonomic nervous system responsible for providing energy expenditure to deal with a challenge by triggering the "fight-or-flight" response: increasing heart rate, increasing blood pressure, stimulating adrenaline secretion, and increasing blood flow to skeletal muscles. [2]

sympathomimetic Substance that produces symptoms of sympathetic nervous system activation. [12]

synapse Structural unit of information transmission between two nerve cells. It consists of the presynaptic nerve terminal, the synaptic cleft, and a small area of the postsynaptic cell (typically associated with a dendrite or region of the cell body) that receives the incoming signal. [2, 3]

synaptic cleft Small gap, about 20 nm wide, between the presynaptic and postsynaptic cells. [3]

synaptic plasticity Ability of synapses to change structurally (i.e., growth of new synapses or loss of existing ones) and functionally (i.e., increased or decreased strength of existing synapses). In the adult nervous system, synaptic plasticity is particularly important for learning and memory and for the development of addiction following repeated exposure to abused drugs. [3]

synaptic vesicles Sac-like structures located in the axon terminal that are filled with molecules of neurotransmitter. [2, 3]

synaptobrevin Small protein located in synaptic vesicle membranes that plays a critical role in exocytotic fusion of vesicles with the axon terminal membrane. [3]

synesthesia Mixing of sensations such that one kind of sensory stimulus creates a different kind of sensation, such as a color producing the sensation of sound. [15]

T

T cells A type of white blood cell. [20]

T-maze Maze type that involves an alley ending in a "T" shape, giving the animal two path choices to reach food in goal box. [4]

T3 See *triiodothyronine*. [3]

T4 See *thyroxine*. [3]

tail suspension test Used in the study of animal models for affective disorders; mice are suspended by the tail from a lever, and the duration of movements (a period of agitation followed by immobility) is recorded. Antidepressant drugs prolong the active struggling. [4]

tail-flick test Technique used to measure pain sensitivity in an animal by placing a beam of light on the animal's tail and recording the time it takes for the animal to remove its tail from the beam. [4]

tar Mixture of hydrocarbons created by the vaporization of nicotine in tobacco. Tar is a major component of cigarette smoke. [13]

tardive dyskinesia (TD) Undesired response to antipsychotic drugs characterized by involuntary muscle movements, particularly of the face, head, and neck, that may be irreversible in some patients. [19]

Tasmar See *tolcapone*. [5]

tau Protein associated with NFTs. [20]

TCA See *tricyclic antidepressant*. [18]

tegmentum Division of the midbrain. The tegmentum is composed of several important structures including the PAG, substantia nigra, and the VTA. [2]

Tegretol See *carbamazepine*. [18]

temporal lobe One of four lobes of the cerebral cortex. It contains the auditory cortex and helps integrate auditory information. [2]

teratogen Any agent including a virus, drug, or radiation that induces abnormal fetal development, causing birth defects. [1]

terminal autoreceptors Autoreceptors that are located on axon terminals and that inhibit neurotransmitter release. [3]

terminal buttons Small enlargements at the axon terminal, in close proximity to the dendrites of the postsynaptic cell, containing synaptic vesicles. Also known as boutons. [2]

tertiary association areas Section of the cerebral cortex where the three sensory lobes can interact, providing a higher order of perception and memory. [2]

testes Male specific gonads that secrete androgens. [3]

testosterone The principal androgen (male sex steroid) secreted by the testes. [3]

tetanic stimulus Electrical stimuli delivered repeatedly, in a brief train of electrical bursts. Also referred to as tetanus. [8]

tetanus A train of electrical stimuli that is used experimentally to induce LTP. Also referred to as a tetanic stimulus. [8]

TH See *tyrosine hydroxylase*. [5]

thalamus Structure of the diencephalon that is responsible for processing and distributing sensory and motor signals to the appropriate section of the cerebral cortex. [2]

THC See *Δ^9-tetrahydrocannabinol*. [14]

theophylline Stimulant drug similar to caffeine that is found naturally in tea. [13]

therapeutic drug monitoring Taking multiple blood samples to directly measure plasma levels of a drug after administration, to identify the optimum dosage for maximum therapeutic potential and minimal side effects. [1]

therapeutic effects Desired physical or behavioral changes associated with a particular drug. [1]

therapeutic index The relationship between the drug dose that results in a toxic response compared to the dose required for the desired biological response. It is represented by the equation $TI = TD_{50}/ED_{50}$ where TD_{50} is the dose that is toxic for 50% of the population and ED_{50} is the effective dose for 50%. [1]

thiosemicarbazide Drug that blocks GABA synthesis, inducing convulsions. [8]

threshold Membrane potential, typically –50 mV, at which voltage-gated Na^+ channels will open, generating an action potential. [2]

thyroid gland Specific endocrine gland that is located in the throat and secretes T3 and T4. [3]

thyroid-stimulating hormone (TSH) Hormone that stimulates the thyroid gland. It is secreted by the anterior pituitary. [3]

thyrotropin-releasing hormone (TRH) Hormone that stimulates TSH release. It is synthesized by neurons of the hypothalamus. [3]

thyroxine (T4) Hormone that is synthesized from tyrosine and helps control normal energy and metabolism in the body. It is secreted by the thyroid gland. [3]

tiagabine (Gabitril) Drug that is a selective inhibitor of GAT-1. It is used in pharmacological studies and to treat patients with partial seizures who are resistant to standard antiepileptic drugs. [8]

tianeptine Tricyclic antidepressant (TCA) that modulates glutamate function. [18]

tight junctions Connection between cells characterized by a fusing of adjoining cell membranes. In cerebral capillaries, the lack of small gaps prevents the movement of molecules across the capillary wall unless the molecules are lipid soluble. [1]

tolcapone (Tasmar) COMT inhibitor used in conjunction with L-DOPA to treat Parkinson's disease. [5]

tolerance Decreased response to a drug as a direct result of repeated drug exposure. [1, 10, 11, 12]

tonic release Slow, consistent release of neurotransmitter that is typically associated with single-spiking mode of cell firing. It maintains low but relatively constant extracellular levels of the transmitter. [5]

topical Method that involves administration of a drug through a mucous membrane such as the oral cavity, nasal mucosa, or vagina. [1]

TPH See *tryptophan hydroxylase*. [6]

tracts Bundles of nerve axons in the CNS sharing a common origin and target. [2]

transcription Process whereby mRNA produces a complementary copy of an active gene. [2]

transcription factors Nuclear proteins that regulate the rate of gene transcription within a cell. [2, 3]

transdermal Method that involves administration of a drug through the skin (e.g., with a patch). [1]

transfection Process used to introduce genetic material into a cell by injecting it with a DNA sequence coding for the desired protein product. [11]

transgenic mice Mice bred to replace one gene with another (e.g., a normal gene with a mutant version of that gene). They are used to study genetic disorders. [4]

translation Process whereby proteins are produced using the nucleotide sequence carried by mRNA to direct the amino acid sequence. Translation is performed by ribosomes. [2]

translational medicine Process by which basic scientific findings (e.g., using animal models) increase the understanding of important disease processes and aid the development of new medical therapies. [8]

transporters Specific proteins in the cell membrane that transport molecules into and out of the cell (e.g., proteins that remove neurotransmitters from the synaptic cleft following their release). They are sometimes called transporter proteins. [3]

tranylcypromine (Parnate) MAO inhibitor used to treat clinical depression. [5]

TRH See *thyrotropin-releasing hormone*. [3]

tricyclic antidepressants (TCAs) Class of antidepressants characterized by a three-ring structure. They block reuptake of NE and 5-HT, thereby increasing their concentration in the synaptic cleft. [5, 18, 20]

triggers Classically conditioned cues associated with drug taking that cause craving. [11]

trihexyphenidyl (Artane) Anticholinergic drug used to treat early symptoms of Parkinson's disease. [7]

triiodothyronine (T3) Hormone that is synthesized from tyrosine and helps control normal energy and metabolism in the body. It is secreted by the thyroid gland. [3]

trinucleotide repeat A form of mutation characterized by a stretch of three nucleotides (a codon) repeated in multiple times in the DNA sequence. [20]

TRPV1 Nonspecific cation channel receptor that was first discovered in sensory neurons where it plays a key role in the heat and pain sensations produced by capsaicin.

Within the brain, TRPV1 receptors can be activated by anandamide. [14]

tryptophan Amino acid characterized by the presence of an indole group. It is a precursor to 5-HT. [6]

tryptophan depletion challenge Research method used to investigate the role of serotonin in depressive disorders, in which subjects consume a tryptophan-deficient amino acid cocktail that transiently reduces 5-HT level in the brain. [18]

tryptophan hydroxylase (TPH) Enzyme that catalyzes the conversion of tryptophan into 5-HTP. [6]

tryptophan loading Administration of pure tryptophan for the purpose of elevating blood tryptophan concentrations. [6]

TSH See *thyroid-stimulating hormone*. [3]

tuberohypophyseal dopamine pathway Pathway that controls the secretion of the hormone prolactin by the pituitary gland. [5]

twin studies Studies used to understand how heredity contributes to a disorder by comparing the concordance rate for the disorder in pairs of monozygotic and dizygotic twins. [18]

type 2 diabetes Disorder characterized by chronically elevated blood glucose levels. One form of the disorder is produced by insulin resistance, which means that ability of insulin to promote glucose uptake has become compromised. [7]

tyrosine Amino acid characterized by a phenol group. It is necessary for the synthesis of the catecholamine neurotransmitters. [5]

tyrosine hydroxylase (TH) Enzyme that catalyzes the first step of catecholamine synthesis in neurons, the conversion of tyrosine to DOPA. [5]

tyrosine kinase receptors Family of receptors that mediate neurotrophic factor signaling. [3]

U

ultrafast endocytosis Mechanism for extremely rapid retrieval of synaptic vesicle membrane components from the membrane of the axon terminal. [3]

ultrasonic vocalizations High-frequency rodent calls typically emitted by juveniles as when separated from their mothers, indicating distress. [4]

up-regulation Increase in the number of receptors, which may be a consequence of denervation or of chronic antagonist treatment. [1]

V

VaChT See *vesicular ACh transporter*. [7]

vacuous chewing movements test A technique used to evaluate the potential motor side effects of antipsychotic drugs. [19]

Valium See *diazepam*. [8]

valproate (Depakote) Simple branched-chain fatty acid that was the first anticonvulsant approved by the U.S. FDA for treatment of acute mania. [18]

vanillymandelic acid (VMA) Metabolite of NE, formed primarily by NE breakdown in the peripheral nervous system. [5]

varenicline (Chantix) Drug that is a partial agonist at high affinity $\alpha4\beta2$ nAChRs. It is used in the treatment of tobacco dependence. [13]

varicosities Repeated swellings of nerve fibers that contain large numbers of synaptic vesicles and that serve as sites of neurotransmitter release. The fibers of dopaminergic and noradrenergic neurons characteristically show varicosities in the brain areas or (in the case of norepinephrine) peripheral organs that they innervate. [5]

vasopressin Peptide hormone secreted by the posterior pituitary that increases water retention by the kidneys. [3]

Ventolin See *albuterol*. [5]

ventral Located toward the underside of the brain or front of the body in humans. [2]

ventral tegmental area (VTA) Region containing dopaminergic cell bodies within the tegmentum of the mesencephalon (midbrain) that form the mesolimbic and mesocortical tracts. [2, 5]

vesamicol Drug that blocks the vesicular ACh transporter. [7]

vesicle recycling Multi-step process consisting of removal of synaptic vesicle membrane components from the membrane of the axon terminal after exocytosis, followed by formation of new vesicles. [3]

vesicular ACh transporter (VAChT) Vesicle membrane protein that transports ACh into synaptic vesicles. [7]

vesicular GABA transporter (VGAT) Vesicle membrane protein that transports both GABA and glycine into synaptic vesicles; also known as VIAAT. [8]

vesicular glutamate transporter (VGLUT) Vesicle membrane protein that transports glutamate into synaptic vesicles. There are three such proteins, designated **VGLUT1** to **VGLUT3**, which differ in their location within the brain. [8]

vesicular inhibitory amino acid transporter (VIAAT) See *vesicular GABA transporter*. [8]

vesicular monoamine transporter (VMAT) Vesicle membrane protein that transports monoamines (i.e., catecholamines and 5-HT) into synaptic vesicles. Monoamine neurons express a particular form of VMAT called VMAT2, whereas the epinephrine- and norepinephrine-secreting chromaffin cells of the adrenal medulla express a different form called VMAT1. [5]

VGLUT See *vesicular glutamate transporter.* [8]

VIAAT See *vesicular GABA transporter*. [8]

Vigabatrin (Sabril) Drug that irreversibly inhibits GABA-T. It is used to treat epilepsy. [8]

vilazodone (Viibryd) Drug that blocks the 5-HT transporter and also acts as a 5-HT_{1A} receptor partial agonist. It is used to treat anxiety disorders and depression. [6]

viral vectors Use of viruses as a delivery system (called a vector) to carry a gene into the nuclei of target cells to alter protein synthesis. [1]

VMA See *vanillymandelic acid*. [5]

VMAT See *vesicular monoamine transporter.* [5]

Vogel test Water-lick suppression test (a conflict procedure) that reliably screens anxiety-reducing drugs in rodents. [4]

volatile solvents Class of inhalants characterized by chemicals, such as adhesives, ink, and paint thinner,

that are liquid at room temperature, but readily give off fumes that can be easily inhaled. [16]

voltage-gated channels Type of ion channels that are regulated by voltage differences across the membrane. [2]

volume transmission Phenomenon characterized by the diffusion of a chemical signal (e.g., a neurotransmitter) through the extracellular fluid to reach target cells at some distance from the point of release. [3]

VTA See *ventral tegmental area*. [2, 5]

W

water-lick suppression test (Vogel test) Technique used to measure anxiety in rodents by recording their propensity to lick a drinking spout that will also deliver a mild electric shock. Also called Vogel test. [4]

WAY 100635 Drug that selectively inhibits 5-HT_{1A} receptors. [6]

Western blot A research method that uses antibodies to quantify a specific protein in a tissue homogenate. [4]

wiring transmission Point-to-point communication between neurons in which the neurotransmitter acts locally within the synapse to affect the target cell. The opposite of wiring transmission is volume transmission. [3]

withdrawal syndrome Rebound physiological state that occurs at drug cessation in an individual following chronic drug use. It is the defining element of physical dependence. [1]

withdrawal See *abstinence syndrome*. [11]

X

Xyrem See *sodium oxybate.* [16]

Y

yohimbine α_2-antagonist that blocks autoreceptors and increases noradrenergic cell firing. It enhances symptoms of opioid withdrawal. [5]

Z

Zecuity Transdermal (patch) form of sumatriptan that is used to treat migraine headaches. [6]

zero maze Elevated donut-shaped platform used to measure anxiety in rodents. [4]

zero-order kinetics Term used to describe a constant rate of drug removal from the body, regardless of drug concentration in the blood. [1]

zolmitriptan (Zomig) Drug that stimulates 5-$HT_{1B/1D}$ receptors, thereby causing constriction of cerebral blood vessels. It is used to treat migraine headaches. [6]

Zyban See *bupropion.* [13]

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