Nestler, Hyman, and Malenka's Molecular Neuropharmacology

A Foundation for Clinical Neuroscience

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A Foundation for Clinical Neuroscience

FOURTH EDITION

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PREFACE TO THE FOURTH EDITION

Neuropharmacology, the study of drug actions on the nervous system, comprises several areas of critical importance to science and medicine. Neuropharmacology includes the translation of basic neuroscience into the discovery of new therapeutic agents, studies aimed at elucidating the mechanism by which drugs act in disease, and also the use of chemical compounds as tools to investigate the function of cells, synapses, and circuits in the nervous system. Much of what we know about the nervous system has come from such studies. Numerous foundational discoveries in neuroscience, including the identification of many neurotransmitters and their receptors, transporters, and signaling molecules, came from investigation into mechanisms of drug action.

To comprehend the actions of a drug on the nervous system, a great deal more is needed than simply identifying the drug's initial target. Rather, one must understand the entire sequence of events that commences with the binding of a drug to an initial molecular target. The resulting alteration in the functioning of that target, the influence of that occurrence on the complex biochemical networks that exist within neurons and nonneuronal cells, the subsequent changes in the output of the cells, and their consequences for the functioning of neural circuits within which the targeted cells exist are all important for gaining a true understanding of drug action. Only with an awareness of the many steps in the process can we grasp how a drug changes complex nervous system functions such as movement, cognition, pain, or mood.

Neuropharmacology is entering an exciting new era as genetic analysis of many diseases of the nervous system is beginning to identify molecular mechanisms of pathogenesis that suggest new therapeutic targets. Even highly heterogeneous and genetically complex disorders, for instance, many forms of intellectual disability, autism, schizophrenia, epilepsy, and neurodegenerative diseases, among others, are beginning to yield to modern technologies. If these discoveries are ultimately going to yield effective therapeutics, new experimental approaches to neuropharmacology will be much in need.

The organization of this textbook represents an attempt to build an understanding of drug action by adding the different levels of explanation, layer by layer. As a result this book differs significantly from many other pharmacology texts, which are usually organized by drug class or by neurotransmitter. In this book, information on fundamental molecular and cellular building blocks is provided first so that it can serve as the basis for the material associated with neural and glial functions. This permits the reader to relate fundamental neuropharmacology to neural systems and ultimately to clinical neuroscience.

The book is divided into three parts. Part 1 includes a brief discussion of general principles of neuropharmacology (Chapter 1), followed by a detailed presentation of nervous system function (Chapters 2–4), from electrical excitability to signal transduction to gene expression. Drugs that act on these basic components of neuronal function are mentioned in these early chapters.

In Part 2 information about the major neurotransmitter systems in the brain and spinal cord is presented (Chapters 5–8). Highlighted in these chapters are the molecular details of neurotransmitter synthetic and degradative enzymes, receptors, and transporter proteins. These proteins represent the initial targets for the large majority of known psychotropic drugs. Also included in Part 2 is a discussion of several types of atypical neurotransmitters, eg, neurotrophic factors, adenosine, endocannabinoids, and nitric oxide, among others (Chapter 8), which have been shown to profoundly influence the adult nervous system and to be potentially important in therapeutics.

Part 3 uses the basic information contained in Parts 1 and 2 to build a systems–level description of the major domains of complex nervous system function. Chapter 9 focuses on the autonomic nervous system; Chapter 10 on neuroendocrine function, Chapter 11 on pain and analgesia, Chapter 12 on neuroinflammation and autoimmune disorders, Chapter 13 on sleep and arousal, Chapter 14 on cognition and behavioral control, Chapter 15 on emotion and mood, Chapter 16 on reinforcement and addiction, Chapter 17 on schizophrenia and other psychotic disorders (eg, bipolar disorder), Chapter 18 on neurodegenerative diseases, in particular, Alzheimer disease and Parkinson disease, Chapter 19 on seizure disorders, and Chapter 20 on cerebrovascular illnesses such as stroke and migraine. Each chapter

begins with a description of the normal neural and glial mechanisms underlying a particular domain of nervous system functioning, followed by a discussion of the diseases that affect that domain. Drugs are discussed within the context of their influence on the molecules, cells, and circuits involved in both normal function and specific disease states.

The organization of *Molecular Neuropharmacology: A Foundation for Clinical Neuroscience* allows individual drugs to be discussed in several contexts. A drug is mentioned when its initial target is described in Part 1 or 2. The drug is mentioned again in Part 3 in the context of its effect on complex brain and spinal cord functions. Many drugs are discussed in several chapters of Part 3 because they affect more than one domain; for example, first generation antipsychotic drugs not only reduce psychosis (Chapter 17), but also affect motor function (Chapter 18), sleep (Chapter 13), and neuroendocrine function (Chapter 10).

The book's structure also permits the incorporation of a great deal of clinical information, much of it representing the integration of modern molecular genetics and brain imaging with neuropharmacology. New insights on the molecular, cellular, and circuit mechanisms—including mechanisms involving nonneuronal cells—underlying such disorders as Parkinson disease, Huntington disease, depression, schizophrenia, Alzheimer disease, stroke, and epilepsy, to name a few, are provided. Our knowledge of the biologic underpinnings of normal brain function and disease have generally preceded advances in pharmacology. Consequently, the book includes many molecular insights, even though drugs may not yet exist that exploit such molecular knowledge. In this regard the book can be seen as presenting a template for the future, in identifying molecular mechanisms for novel therapeutic approaches. We anticipate that subsequent editions of this book will describe the development of such novel medications and thereby gradually fill in these gaps in pharmacology.

Indeed, there is good reason to be optimistic. After several decades of few advances in the treatment of most nervous system disorders, there is, at long last, significant progress. Since the publication of this book's previous edition in 2015, we have seen the marketing of numerous drugs with fundamentally new mechanisms of action. Suvorexant is a dual OX_1 and OX_2 orexin receptor antagonist approved for the treatment of insomnia (Chapter 13), pitolisant is an H_3 histamine receptor antagonist/inverse agonist approved for the treatment of narcolepsy (Chapter 13), ketamine is a noncompetitive NMDA glutamate receptor antagonist among several other actions approved for the treatment of depression (Chapter 15), brexanolone is a neuroactive steroid approved for the treatment of postpartum depression (Chapter 15), pimavanserin is a $5HT_{2A}$ serotonin antagonist/inverse agonist approved for the treatment of psychosis associated with Parkinson disease (Chapter 18), and several monoclonal antibodies directed against either calcitonin gene–related peptide (CGRP) or its receptor have been approved for the treatment of migraine (Chapter 20). Numerous anti-inflammatory agents—for both parenteral and oral administration—have significantly improved the treatment of multiple sclerosis. Additionally, we are beginning to see highly novel modalities of treatment, such as approved antisense oligonucleotides—delivered intrathecally—for the treatment of spinal muscular atrophy and increased interest in circuit–based treatments (eg, deep brain stimulation) for a range of neurologic and psychiatric disorders. This is an unprecedented degree of progress in neuropharmacology which far exceeds the advances achieved since the book's first edition in 2000.

Despite this progress, there have also been major disappointments. Several amyloid–based treatments for Alzheimer disease failed large clinical trials and we are no closer to nonopioid treatments of opioid or other addictions despite the fact that the health and economic well–being of the world depends in part on devising definitive treatments for these disorders. Nevertheless, the rich science of neuropharmacology—coupled with breakthrough advances in molecular, cellular, and circuit analyses of the brain across species—promises additional clinical achievements in the coming years. The US BRAIN initiative and related programs in several other countries recognize the potential of these groundbreaking new technologies. There is particular excitement for the power of computational approaches for studying neural networks, genomics and several other "omics," brain and cell imaging, and electronic health records, among other applications. We hope and expect that future editions of this book will present clinical progress driven by these and other innovations.

The scientific and clinical explanations in *Molecular Neuropharmacology: A Foundation for Clinical Neuroscience* are written in a style that makes them accessible to a wide audience: undergraduate and graduate students as well as students in the medical and allied health professions. This book is also an excellent resource for residents in psychiatry, neurology, neurosurgery, rehabilitation medicine, anesthesiology, and ophthalmology, and practicing clinicians and scientists in these areas. As a concise treatise of clinical

information that provides descriptions of basic mechanisms and their clinical relevance, this book is suitable for both scientists and clinicians.

We would like to acknowledge the contributing authors who were instrumental in the initial phases of the preparation of this book for the first, second, third, and now fourth editions. We thank Steven Hyman, Robert Malenka, and David Holtzman as past authors of this book, and welcome a new team of coauthors—representing a new generation of neuropharmacologists—to take the reins of the newly named *Nestler, Hyman, and Malenka's Molecular Neuropharmacology: A Foundation for Clinical Neuroscience*. Finally, we would like to thank Andrew Moyer and Kim Davis, and their colleagues at McGraw-Hill, for their crucial role in production of this fourth edition.

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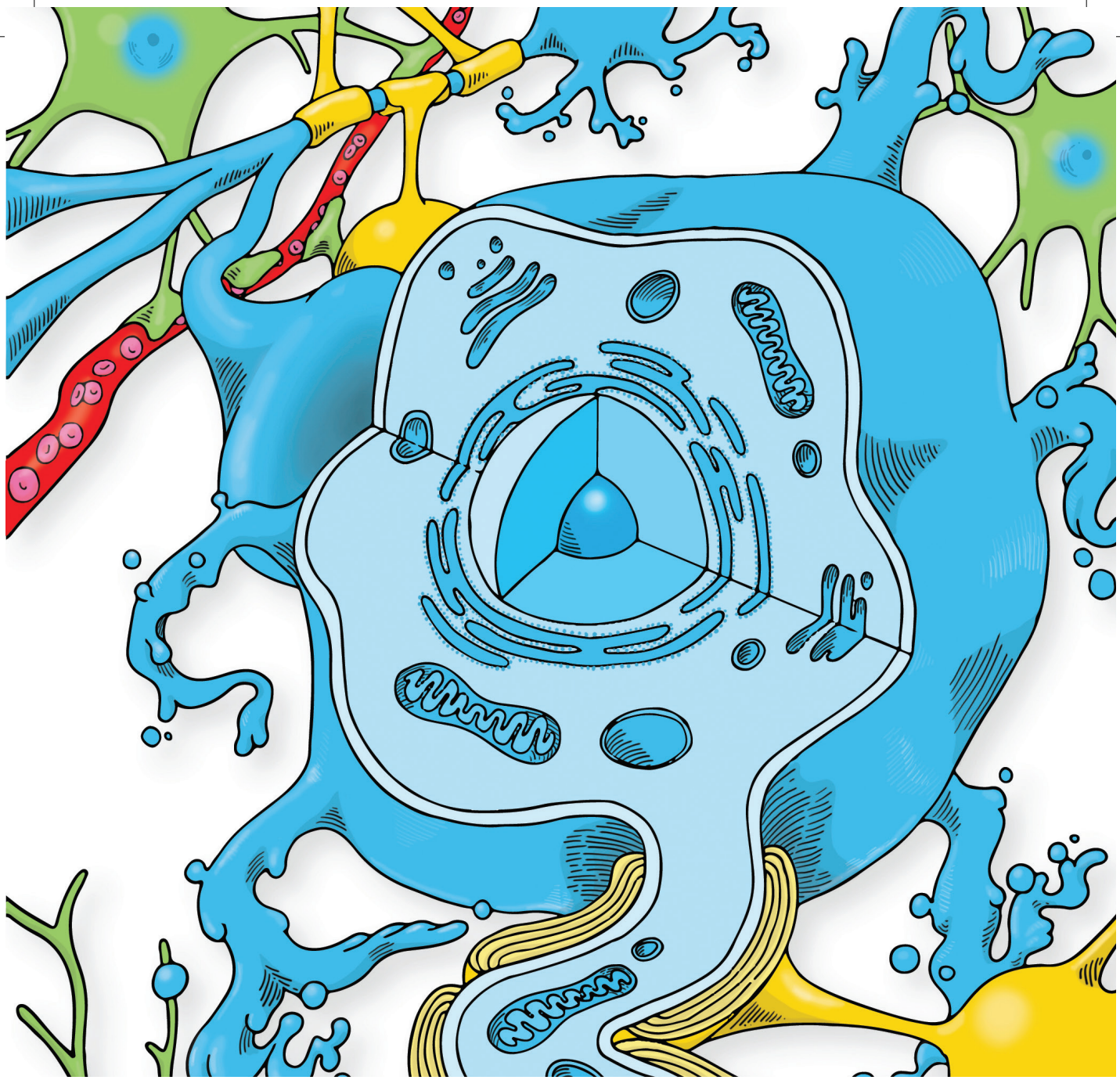

1 Fundamentals of Neuropharmacology

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CHAPTER 1

Basic Principles of Neuropharmacology

KEY CONCEPTS

- An understanding of drug mechanisms in the brain must integrate knowledge of the molecular and cellular actions of a drug with their effects on brain circuitry.
- The clinical actions of a drug in the brain often are due to neural plasticity—the long–term adaptations of neurons or other cell types to the sustained short–term actions of a drug.
- The binding of a drug to its specific target(s) normally is saturable and stereoselective.
- The specific binding of a drug to its target is quantified according to its affinity for the target, expressed as a dissociation constant (K_d), and the total amount of binding (B_{max}).
- Potency of a drug describes the strength of binding between the drug and its target; efficacy describes the maximal biologic effects that the drug exerts by binding to its target.
- Drugs that act at receptors can be classified as agonists, partial agonists, biased agonists, inverse agonists, antagonists, positive allosteric modulators, or negative allosteric modulators.
- Modern neuropharmacology takes advantage of the tools of molecular biology, genetics, and cell biology as well as combinatorial chemistry, which is used to generate novel molecules that may function as new drugs.
- Functional genomics and proteomics will help identify novel drug targets.
- Pharmacogenetics will guide the choice of drug treatments based on an individual's genetic constitution.

Neuropharmacology is the scientific study of the effects of drugs on the nervous system. Its primary focus is the actions of medications for psychiatric and neurologic disorders as well as those of drugs of abuse. Neuropharmacology also uses drugs as tools to form a better understanding of normal nervous system functioning. The goal of neuropharmacology is to apply information about drugs and their mechanisms of action to develop safer, more effective treatments and eventually curative and preventive measures for a host of nervous system abnormalities. The importance of neuropharmacology to medical practice, and to society at large, is difficult to overstate. Drugs that act on the nervous system, including antidepressant, antianxiety, anticonvulsant, and antipsychotic agents, are among the most widely prescribed medications. Moreover, commonly prescribed medications that act on other organ systems often are associated with side effects that involve the nervous system and in turn may limit their clinical utility. In addition, a substantial number of individuals use common substances, such as caffeine, alcohol, and nicotine, that are included in the domain of neuropharmacology because of their effects on the central nervous system (CNS). In a much smaller fraction of the population, these and other drugs are used compulsively, in a manner that constitutes an addiction. Drug abuse and addiction exact an astoundingly high financial and human toll on society through direct adverse effects, such as lung cancer and hepatic cirrhosis, and indirect adverse effects—for example, accidents and AIDS—on health and productivity. Still other common afflictions of the nervous system, such as Alzheimer disease as just one example, are awaiting effective medications, further emphasizing the importance of neuropharmacology.

Neuropsychopharmacology is an all–encompassing term that typically is applied to all types of drug effects that influence nervous system functioning. The term *psychopharmacology* is often used to describe the effect of drugs on psychologic parameters such as emotions and cognition. Drugs that influence behavior are known as *psychotropic* agents. In this book we use the term *neuropharmacology* to describe the study of all drugs that affect the nervous system, whether they affect sensory perception, motor function, seizure activity, mood, higher cognitive function, or other forms of nervous system functioning.

HOW DRUGS WORK

The actions of drugs that affect the nervous system are considerably more complicated than those of drugs that act on other organ systems. To understand how drugs act on the nervous system, it is critical to integrate information about the molecular and cellular actions of a drug with knowledge of how these actions affect brain circuitry—a circuitry that is constantly changing in structure and function in response to both pharmacologic and nonpharmacologic input from the environment. The complexity that underlies such actions can be illustrated by consideration of **fluoxetine**, a widely prescribed antidepressant, and **furosemide**, a widely prescribed diuretic. The chemical actions of these drugs are fairly simple. Both drugs initially bind to their specific protein target: fluoxetine binds to and inhibits serotonin transporters, which normally inactivate the actions of the neurotransmitter serotonin (Chapter 6), and furosemide binds to and inhibits Cl^- channels located in the ascending loop of Henle in nephrons of the kidney. However, the relation between the chemical and clinical actions of these drugs—particularly those of fluoxetine—requires a more elaborate explanation.

The association between furosemide's chemical and clinical activity is relatively straightforward. By inhibiting Cl^- transport in Henle's loop, furosemide causes more Cl^- to remain in the lumen of the nephron tubule, which in turn requires more H_2O to remain in the tubule. Furosemide exerts this same effect on all nephrons in the kidney, and the increase in H_2O in individual nephron tubules combines to cause diuresis at the level of the kidney. Diuresis is achieved as soon as effective concentrations of the drug reach the kidney's extracellular fluid, and is maintained with repeated use of the drug—for example, in the treatment of chronic congestive heart failure.

The relationship between the chemical and clinical actions of fluoxetine is far more complicated and also more speculative. Most drugs that act on the nervous system interact with only the minute subset of the brain's neurons that express the initial protein target of the drug. Fluoxetine directly affects only those neurons that use serotonin as a neurotransmitter—a few 100,000 out of approximately 100 billion neurons in the human brain. By inhibiting serotonin reuptake by these neurons, fluoxetine enhances serotonergic transmission throughout the brain, but it is not known with certainty where in the brain enhanced serotonin function causes an antidepressant effect. Similarly, little is known about which of serotonin's 13 known receptors must be activated to achieve an antidepressant response. Moreover, the mood–elevating effects of fluoxetine are not evident after initial exposure to the drug but require its continued use for several weeks. This delayed effect suggests that it is not the inhibition of serotonin transporters per se, but some adaptation

to sustained increases in serotonin function that mediates the clinical actions of fluoxetine. However, where these adaptations occur in the brain and the nature of the adaptations at the molecular level have yet to be identified definitively (Chapter 15).

The clinical actions of fluoxetine, like those of many neuropharmacologic agents, therefore reflect drug–induced *neural plasticity*, which is the process by which neurons and other cell types in the brain adapt over time in response to chronic disturbance. Consequently, to understand fully the effects of a neuropharmacologic drug, we must determine not only the initial effects of the drug but also the intracellular signals that control a neuron's or glia cell's adaptations over time, the intercellular signals through which neurons and glia communicate with one another, the ways in which large groups of neurons operate in circuits, and the higher–order interactions between circuits that produce complex brain functions.

Parts I and II of this book explore the intracellular and intercellular signals that enable communication among neurons and glia, which are fairly well understood. Part III addresses the relationships between circuits of neurons and neighboring glia, and the complex brain functions they mediate, about which much remains to be discovered.

DRUGS AS TOOLS TO PROBE BRAIN FUNCTION

Neuropharmacology has contributed to many important advances in the neurosciences during the past several decades. Drugs have been used as tools to dissect the functions of the brain and of individual nerve and glial cells under normal and pathophysiologic conditions. Historically, neuropharmacology has involved the delineation of diverse molecules that function as neurotransmitters in the nervous system, including monoamines, amino acids, purines, and peptides. The identification of many of these neurotransmitters and the elucidation of their synthesis, degradation, and receptors occurred in conjunction with studies of synthetic and plant substances that were known to exert profound effects on behavior. The neuropharmacology of **ergot** alkaloids, **cocaine**, and **reserpine**, for example, led to the discovery and characterization of monoamine neurotransmitter systems; **opiate** alkaloids such as **morphine** led to endogenous opioid systems; **nicotine**, **muscarine**, and **cholinesterase inhibitors** such as **physostigmine** led to cholinergic systems; and **caffeine** and related substances led to purinergic systems.

Neuropharmacology also played a fundamental role in the delineation of the numerous receptor subtypes through which neurotransmitters elicit biologic responses. The early idea that one neurotransmitter acts on only one receptor was replaced decades ago with the recognition that for each neurotransmitter there are multiple receptors. This discovery led to the development of synthetic drugs with increasing selectivity for individual types of receptors, and the evolution of these neuropharmacologic agents has represented important advances in clinical medicine. These advances include the use of selective **β_1-adrenergic antagonists** for cardiovascular disease, selective **β_2-adrenergic agonists** for asthma, **μ-opioid antagonists** for opioid overdose, and **$5HT_{1D}$ serotonin agonists** for migraine, to name just a few examples.

As well, the identification of multiple receptor subtypes for neurotransmitters contributed to the recognition of complex postreceptor signal transduction cascades through which receptors ultimately produce their biologic responses. From G proteins to second messengers to protein phosphorylation pathways to regulation of gene expression, studies of the effects of drugs on the nervous system have provided crucial windows onto the functioning of intracellular signaling. For instance, investigation of the mechanisms by which **organic nitrates** cause vasodilation in the treatment of cardiac angina led to the discovery of nitric oxide as a critical signaling molecule, and studies of **aspirin** and related nonsteroidal anti-inflammatory drugs (**NSAIDs**) led to the discovery of a host of signaling molecules derived from arachidonic acid, including prostaglandins and leukotrienes.

Drugs serve as prototypical external or environmental factors in determining how the brain adapts or maladapts over time in response to repeated perturbations. Many adaptations that occur in response to repeated drug exposure are models for adaptations to other external exposures, including stress and life's experiences.

PRINCIPLES OF GENERAL PHARMACOLOGY

The ability of a drug to produce an effect on an organism is dependent on many of its properties, from its absorption and penetration into target tissues, to its stability and its elimination. To briefly summarize these processes, the first factor to be considered is the *route of administration*, which can determine how rapidly a drug reaches its target organ and which organs it affects. *Oral* administration typically results in a

relatively slow onset of action. *Parenteral* describes all other routes of administration, including *subcutaneous* (under the skin), *intraperitoneal* (into the peritoneal–abdominal cavity), *intravenous* (into the venous system), *intracerebroventricular* (into the cerebral ventricular system), *intrathecal* (into spinal fluid), and *intracerebral* (into the brain parenchyma) delivery. The *bioavailability* of a drug generally refers to the amount that enters the general circulation, which in turn determines how much of the drug is available to reach its target. Bioavailability can be influenced by *absorption* of the drug from the gut if administered orally. It also can be affected by *binding* of the drug to plasma proteins, which makes the drug unavailable to bind to its target. It can be influenced as well by a drug's ability to penetrate the *blood–brain barrier* if the drug acts on the brain (Chapter 2), or its ability to permeate cell membranes if the drug acts on intracellular proteins.

Drug action also depends on the *stability* of the drug once it is absorbed, that is, how rapidly it is metabolized to inactive congeners or eliminated from the body through urine, bile, or exhaled air. Some drugs (*prodrugs*) must be converted into active metabolites before they can exert their biologic effects.

Each of these factors, which can be categorized as *pharmacokinetic* considerations, is a critical determinant of drug action and influences both the clinical use of drugs and the process of developing new agents. However, these pharmacokinetic properties are not discussed in detail in this book because they are not, strictly speaking, related to the underlying mechanisms of drug action—the *pharmacodynamic* features that are the primary concern of these chapters. As an introduction to this topic, a brief description of the process by which a drug interacts with its initial protein target follows. *Pharmacogenetics*, which describes the influence of an individual's genes in determining the response to a given drug, is also critical, but still in the earliest phases of understanding (see below).

Drug Binding

Neuropharmacology is changing rapidly in response to the molecular revolution. In previous decades, neuropharmacology focused on the synapse and, more particularly, on the effects of drugs on neurotransmitters or neurotransmitter receptors. The action of drugs on synaptic targets remains an important field of investigation. The initial target of a drug generally determines the particular cells and neural circuits on which the drug acts and at the same time the potential efficacy and side effects of the pharmacologic agent. However, the molecular revolution has made it clear that the initial binding of a drug to its target—for example, the binding of a drug to a neurotransmitter receptor—is only the beginning of a signaling cascade that affects the behavior of cells and ultimately complex circuits.

When a drug binds to a protein, it affects the functioning of that protein. A drug can conceivably bind to any site on a protein. A simple site may involve just a few contiguous amino acid residues in a protein's primary structure, while a relatively complex site may involve discontinuous residues from the protein's primary structure that are brought near each other by the protein's secondary and tertiary structures. Ultimately, the three–dimensional shape, or conformation, of a binding site and the electrostatic charges distributed across the site must complement the shape and charge of the drug. The interaction of a drug with its binding site can influence the intrinsic activity of the target protein, for example, the catalytic activity of an enzyme or the conductance of an ion channel, or it can influence the ability of the protein to interact with some other molecule, such as the ability of a receptor to bind to its neurotransmitter.

In classic studies of drug mechanisms of action, a mechanism is defined by a drug's ability to bind to an unknown receptor in tissue homogenates or on tissue sections. In these studies the drug, termed the *ligand*, is radiolabeled and incubated with a tissue preparation, which is washed extensively to remove loosely bound drug. A radioactive atom must be added to the drug without altering its ligand binding properties, a process that can be exceedingly difficult. The resulting ligand binding should be *specific*; that is, the ligand must bind to its specific target protein, which must be distinguished from binding to other proteins or even to the wall of a plastic test tube. In many cases, binding is *stereoselective*, or specific for only one stereoisomer of a drug. Binding also should be *saturable*. A limited amount of ligand binding occurs in the preparation because the amount of the specific target is limited. (A tissue preparation contains a finite amount of an individual receptor protein compared with a test tube wall, which is theoretically infinite.) Additionally, binding should attain a *steady state*. Time, temperature, and other conditions of incubation should enable the ligand binding to achieve a state of equilibrium.

The extent to which a ligand binds to a tissue preparation is a function of the concentration of the ligand (Figure 1-1). The total binding comprises two components: (1) specific binding, which is saturable, and (2) nonspecific binding, which is not saturable. In the ideal situation, in which binding to a specific receptor site is competitive and fully reversible in the steady

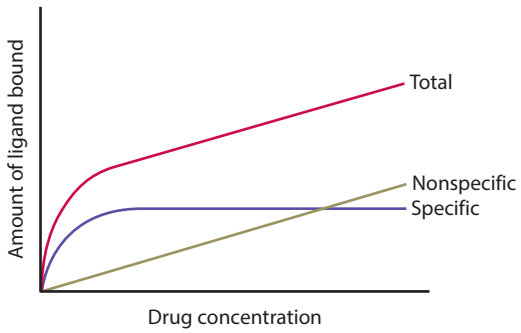

Figure 1-1 Radioligand binding assay. In this theoretical representation the amount of radioligand bound to a tissue preparation (eg, homogenate, brain slice) is a function of the concentration of the radioligand. Total binding is the total amount of binding observed. Nonspecific binding represents the nonsaturable portion of binding that is presumably not associated with the specific binding site under investigation; it is often calculated as the binding of radioligand that persists in the presence of a large excess of nonradiolabeled ligand. Specific binding is calculated as the difference between total and nonspecific binding and reflects the amount of radioligand bound to the specific binding site.

state, specific binding can be defined as the fraction of total binding that can be displaced by incubating the radiolabeled ligand–tissue mixture with a large excess of unlabeled ligand. Conversely, the nondisplaceable radioactive portion of the preparation is considered nonspecific binding.

There are several discrepancies, however, between ideal and actual conditions. Not all binding to target proteins is truly reversible; the affinity of some ligand–receptor interactions is so high that resulting complexes are not readily dissociable. Moreover, artifactual sites may be present and may show striking apparent specificity. While the ideal situation assumes that the tissue preparation contains just one specific target, in actuality many drugs can bind specifically to many related subtypes of a protein target; for example, serotonin binds to numerous subtypes of serotonin receptors. Consequently, the resulting binding curves can be quite complicated and difficult to interpret.

The specific binding of a ligand to a tissue preparation is quantified according to two properties: the *affinity* of the binding, which is expressed as a dissociation constant (K_d), and the *total amount* of the binding (B_{max}) (Figure 1-2). These terms are analogous to those used in studies of enzyme kinetics—for

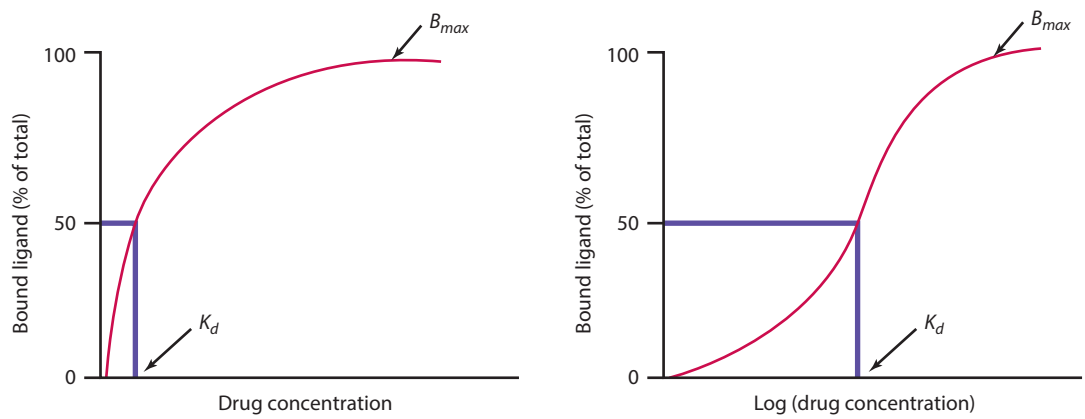

Figure 1-2 Determination of K_d and B_{max} from radioligand binding assays. The amount of specific radioligand binding to a specific site in a tissue preparation (determined in Figure 1-1) is plotted as a function of radioligand concentration, using a normal **A** or semilogarithmic **B** plot. The K_d is calculated as the concentration of radioligand that results in 50% of maximal binding (B_{max}). The semilogarithmic plot, which better illustrates the effects of low radioligand concentrations, places the K_d near the middle of the graph.

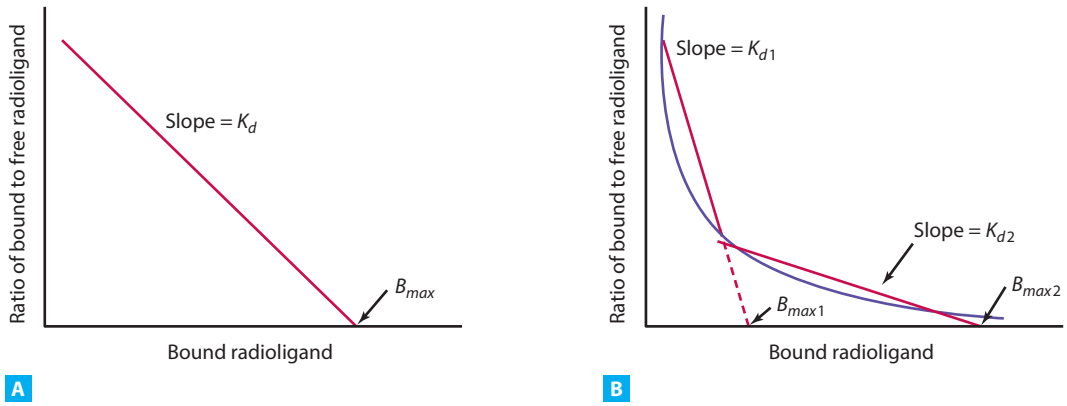

Figure 1-3 **The Scatchard plot.** Specific binding data are mathematically transformed to plot the ratio of bound to free radioligand as a function of bound radioligand. **A.** When one binding site is involved, the data follow a straight line. The slope of the line is the K_d and the *x*-intercept is the B_{max}. **B.** When more than one binding site is involved, the data follow convex curves, which can be converted into multiple straight lines. The slope of each line and its *x*-intercept represent the K_d and B_{max}, respectively, of each binding site.

example, the Michaelis–Menten equation—in which K_a is the activation constant for an enzyme and its cofactor and V_{max} is the maximum catalytic activity of the enzyme. The K_d is defined as the concentration of ligand at which half of the specific binding sites are occupied; larger K_d values (eg, 100 nM vs 1 nM) reflect lower affinities of the drug. When ligand binding is plotted as a function of the log of drug concentration, a sigmoidal curve is obtained (Figure 1-2 B). Ligand binding data are often transformed mathematically to yield a *Scatchard plot*, in which the ratio of bound ligand to free ligand is plotted as a function of bound ligand (Figure 1-3). Because it is difficult to measure the amount of free (unbound) ligand, total ligand minus bound ligand is used. The shape of *Scatchard plots* provides an indication of the number of binding sites in a tissue preparation, as well as the K_d and B_{max} values for each site.

Another method for studying ligand–target interactions makes use of *competition curves*. These curves describe the ability of a drug to compete with a radioligand in binding to a tissue preparation (Figure 1-4). The drug concentration at which half of the radioligand binding is displaced (K_i) is a measure of the affinity of the drug for a binding site in the context of a specific radioligand. Historically, such competition studies have played an important role in defining many subtypes of neurotransmitter receptors. In general, such pharmacologic distinctions of receptors accurately predicted broad categories of receptor proteins, which subsequently were identified with greater precision by means of molecular cloning techniques, as discussed later in this chapter. In ideal situations, the competing drug and the radioligand bind to the same site of the target protein; such binding is termed *competitive*. In more complicated situations, the drug and radioligand bind to different sites on the same protein; in such cases, the binding

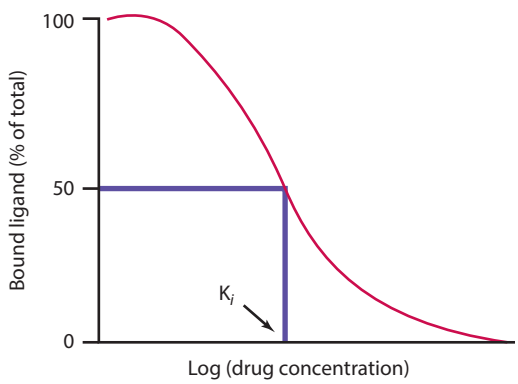

Figure 1-4 **Radioligand competition curve.** The ability of compounds to bind to a particular site in a tissue preparation can be compared by studying the ability of each to compete with a radioligand for a particular binding site. When the binding data are plotted on a semilogarithmic graph, a sigmoidal curve results. The K_i represents the concentration of drug that results in a reduction of radioligand binding to 50% of maximal values.

is *noncompetitive* and results in far more complicated competition curves. These assessments of ligand binding, which have traditionally been performed on tissue homogenates or membrane fractions, can also be performed on brain sections, a process termed *receptor autoradiography*.

As with any technique, the limitations of ligand binding assays must be appreciated. One of the most critical limitations is that binding assays, and the determination of K_d, K_i, and B_{max} values, are highly dependent on experimental conditions and therefore must be interpreted with considerable caution. The specific radioligand, the temperature of incubation, the salt and ionic content of the buffer, and the presence of different guanine nucleotides (Chapter 4) are among the many factors that can exert dramatic effects on ligand binding. The cloning of receptors and other target proteins, and the ability to express them on cells without endogenous expression of the target, has made at least some aspects of characterizing the binding properties of drugs more straightforward.

Drug Efficacy

Binding studies describe the physical relationship between a drug and its target but do not directly assess the biologic consequences of this association. Although drug binding and biologic effect are intricately related, they help define two distinct aspects of drug action: *potency* and *efficacy*. Potency (affinity, or K_d) describes the strength of the binding between a drug and its target. Efficacy describes the biologic effect exerted on the target by virtue of the drug binding. These properties can be understood by considering the effect of a drug on a neurotransmitter receptor. As previously explained, the drug must physically bind to the receptor, which requires a physical attraction between the two. Subsequently, that binding must elicit a change in the receptor that leads to a biologic response. For a G protein–coupled receptor, drug binding must trigger a conformational change in the receptor that alters its interactions with its G protein α subunit (Chapter 3). For a ligand–gated channel (receptor ionophore), drug binding must trigger a conformational change that opens or closes the pore that is intrinsic to the receptor.

Drugs differ dramatically with respect to their potency and efficacy. Traditionally, two categories of drug have been described: *agonist* and *antagonist*. The site at which an endogenous neurotransmitter binds to a receptor to produce the conformational changes required to activate the receptor is called the *orthosteric* site. An agonist binds to the orthosteric site on a receptor to mimic the actions of the endogenous neurotransmitter. Antagonists are inherently inert and exert a biologic effect only by interfering with an endogenous ligand. When an antagonist binds, it can do so at the orthosteric site or at other sites on the receptor. An antagonist that binds at the orthosteric site will not elicit the conformational changes required to engage downstream signaling processes. However, the antagonist will compete with the endogenous ligand for the same site and thereby reduce the actions of the ligand. Such antagonists are therefore called *competitive antagonists*, as noted previously. Antagonists that bind at other sites on the receptor are called noncompetitive antagonists. Such antagonists do not block the endogenous ligand from binding to its orthosteric site, but instead prevent the receptor from entering the active conformation despite ligand binding. For opioid receptors, which are receptors for the endogenous opioid peptides such as the enkephalins (Chapter 7), **morphine** and **naloxone** are classic examples of an agonist and a competitive antagonist, respectively. For NMDA glutamate receptors, which are a subtype of receptors for the endogenous neurotransmitter glutamate, **ketamine** is an example of a noncompetitive antagonist because it binds to a site on the receptor different from the site that binds glutamate and thereby prevents glutamate from opening the ion channel intrinsic to the receptor (Chapter 5). The differences in efficacy associated with agonists and competitive antagonists are independent of the affinity with which each binds to the orthosteric site on its receptor; both can exhibit high or low affinities. How can two molecules that bind to the same receptor site exert such different effects on the receptor? A possible explanation is that an antagonist may share one moiety with an agonist that is required for binding to the receptor, but may lack another moiety required for efficacy.

In addition to the actions of classic agonists and antagonists, an intermediate category of drug efficacy is exemplified by *partial agonists*. When a drug binds to the orthosteric site of a receptor and elicits only a partial biologic response, the drug presumably lacks a portion of the molecule required for full biologic effect or binds to the orthosteric site in a slightly different manner (Figure 1-5). An interesting situation arises when partial agonists possess high potency. At low drug doses, a mild agonist effect is obtained. At high doses, a similarly mild agonist effect is obtained because of limits in the intrinsic efficacy of the molecule. However, at high doses, the drug can antagonize the ability of a full agonist, including the endogenous neurotransmitter, to activate the receptor because its

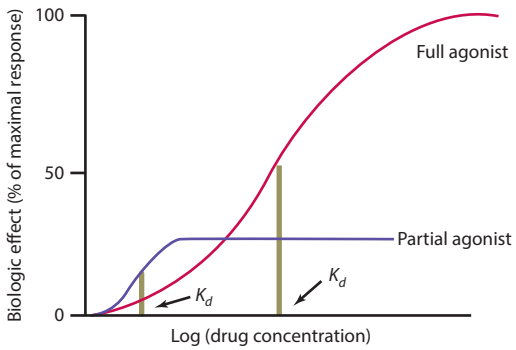

Figure 1-5 Drug efficacy versus drug potency. The biologic responses elicited by two drugs that bind to the same site are presented in this representation as a function of drug concentration. Efficacy refers to the maximal biologic response elicited by each drug. In this theoretical representation the partial agonist elicits a smaller maximal response than the full agonist. However, efficacy is independent of the potency (K_d) of the drug; the partial agonist shown is in fact more potent (possesses a higher affinity for the binding site) than the full agonist.

affinity is greater than that of the full agonist. For this reason, partial agonists are sometimes referred to as *mixed agonists–antagonists*. Partial agonists can be quite useful clinically; for example, **buprenorphine** is a partial agonist at opioid receptors and is used in the treatment of chronic pain and opioid addiction (Chapters 11 and 16). At low doses, buprenorphine elicits a mild analgesic and rewarding effect. Higher doses not only fail to yield a stronger effect, which limits the abuse liability of this drug, but also antagonize the action of full opioid agonists and thereby discourage abuse of other opioids such as **morphine**.

Inverse agonists achieve efficacy in still another way. When an inverse agonist binds to the orthosteric site on a receptor, it elicits the biologic response that is the opposite of that associated with an agonist. If an agonist opens an ion channel, an inverse agonist closes the channel. If an agonist facilitates receptor-to-G protein coupling, an inverse agonist attenuates such coupling. The action of an inverse agonist requires some basal activity on the part of the receptor, which means that the receptor is not quiescent in the absence of ligand but instead possesses some level of intrinsic biologic activity, such as channel conductance or G protein coupling. Indeed, most receptors do exhibit such baseline activity.

In addition to the orthosteric site, receptors contain *allosteric* sites, the binding at which can influence the function of the receptor. *Positive allosteric modulators* (*PAMs*) are ligands that bind to allosteric sites to facilitate agonist–induced activation of the receptor. In the absence of an orthosteric agonist, PAMs do not influence receptor function. Conversely, *negative allosteric modulators* (*NAMs*) are ligands that bind to allosteric sites to attenuate agonist induced activation of the receptor. By definition, NAMs do not influence receptor function in the absence of an orthosteric agonist. Therefore, all NAMs can be viewed as noncompetitive antagonists of a receptor, but not all noncompetitive antagonists are NAMs because some of the former might antagonize a receptor in the absence of an orthosteric agonist.

Very few drugs can be placed in discrete categories—eg, agonist, antagonist, and inverse agonist. Many drugs that are classically described as agonists, such as **morphine**, are not full agonists but strong partial agonists. Conversely, many drugs that are classically categorized as antagonists—for example, **naloxone**—are not completely inert and thus can be very weak partial agonists. Moreover, some neurotransmitters show less efficacy than synthetic drugs, which indicates that they also are partial agonists! Consequently, drugs should be thought of as existing on a continuum ranging from full agonist to inert antagonist to full inverse agonist (Figure 1-6).

The complex nature of the interactions between drugs and their target proteins can be illustrated by a discussion of the γ-aminobutyric acid receptor ($GABA_A$)—an important receptor for the neurotransmitter GABA (Chapter 5). This receptor is a heteropentamer that has two main types of subunits, α and β. GABA binds to a site on the β subunit and triggers the opening of a Cl^- channel that is intrinsic to the receptor complex. **Muscimol** (an agonist) and **bicuculline** (an antagonist) also bind at this orthosteric site and thus compete with GABA. The α subunit of the $GABA_A$ receptor contains a binding site for a class of synthetic molecules known as **benzodiazepines**. Drugs that bind at this allosteric site, such as **diazepam**, are antianxiety agents that, when bound to the site, allosterically facilitate the ability of GABA to bind to and activate the $GABA_A$ receptor (Chapters 5 and 15). Antagonists at this site, such as **flumazenil**, bind to the allosteric site but do not affect receptor function, and are sometimes referred to as *silent allosteric modulators* (*SAMs*). Because this site lacks endogenous ligands, flumazenil is clinically inactive when bound to the $GABA_A$ receptor; however, it can be used to treat diazepam overdose because it displaces diazepam from the binding site. Drugs such as **β-carboline**, which intensify anxiety, bind very near the benzodiazepine agonist site and allosterically inhibit the ability of

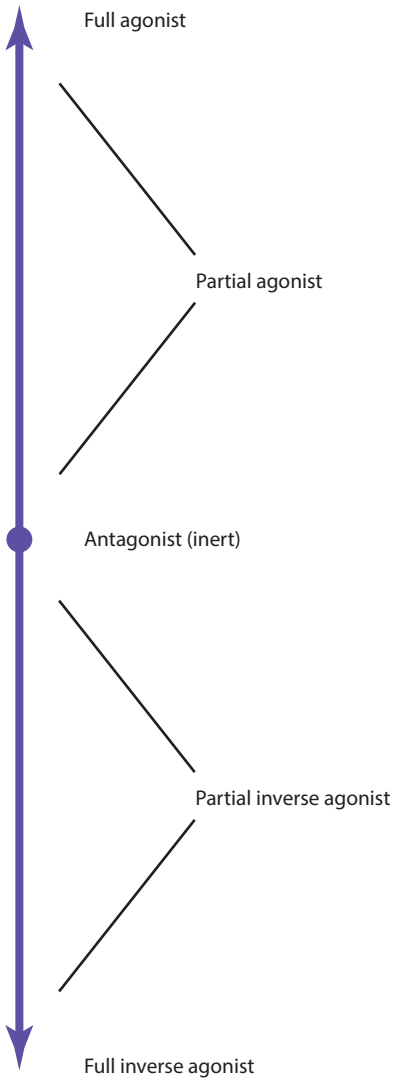

Figure 1-6 Drug efficacy as a continuum. Ligands for a receptor can be described as agonists (agents that activate the receptor), antagonists (agents that have no intrinsic effect on the receptor but can block the ability of agonists and inverse agonists to regulate the receptor), or inverse agonists (agents that regulate the receptor but produce effects opposite to those produced by agonists). However, ligands rarely can be placed into these discrete categories; instead they are distributed across a continuum. In strict pharmacologic terms, there are very few true antagonists, most being very weak partial agonists or inverse agonists, and very few full agonists or inverse agonists, most being strong partial agonists or inverse agonists.

GABA to bind to and activate the receptor. Diazepam and β-carboline therefore represent a PAM and NAM, respectively, of the $GABA_A$ receptor. Although this type of complex receptor pharmacology was first described for the $GABA_A$ receptor, a similar level of complexity can characterize drug interaction at virtually any type of receptor.

In more recent years, more complex actions of receptors have contributed to still additional modes of drug action. Specific types of receptors can form complexes with other receptors, with a resulting generation of novel agonist activity for the receptor heteromer (Figure 1-7A). For example, heterodimers of μ and δ opioid receptors can be activated or inhibited by drugs that show lower affinities to either receptor type alone. As well, different agonists at a given receptor can direct the receptor to signal via distinct intracellular pathways, a process referred to as *ligand–directed* or *biased signaling* (Figure 1-7B). Similar ligand–directed differences can be seen with inverse agonists. The discovery of ligand–directed signaling greatly complicates the consideration of the intrinsic efficacy of a ligand, since ligands might vary qualitatively in addition to quantitatively with respect to their downstream actions. Biased signaling has ushered in a new era of drug discovery in that it raises the possibility of generating drugs that retain certain effects related to clinical efficacy but avoid other actions related to side effects. The possibility, for instance, of generating novel opioid drugs that serve as biased agonists at the μ opioid receptor—and thereby produce potent analgesia without eliciting tolerance, dependence, or addiction with repeated use—has become an active area of research (Chapter 7).

Finally, it must be emphasized that binding sites with a high affinity for drugs do not necessarily have an endogenous ligand. No evidence, for example, supports the existence of an endogenous ligand for the benzodiazepine binding site on the $GABA_A$ receptor. Rather, the discovery of this class of drugs and their binding site is testimony to the power and promise of medicinal chemistry to target distinctive features of proteins that are not exploited by nature. Indeed, our growing knowledge of the many complexities of receptor function and drug action discussed above is contributing to the development of a host of agents with novel pharmacologic and hence clinical activity.

Dose–Dependent Drug Response

That the effect of a drug on a target protein is dependent on the concentration of a drug is implicit in the discussions of drug binding and efficacy presented in the preceding

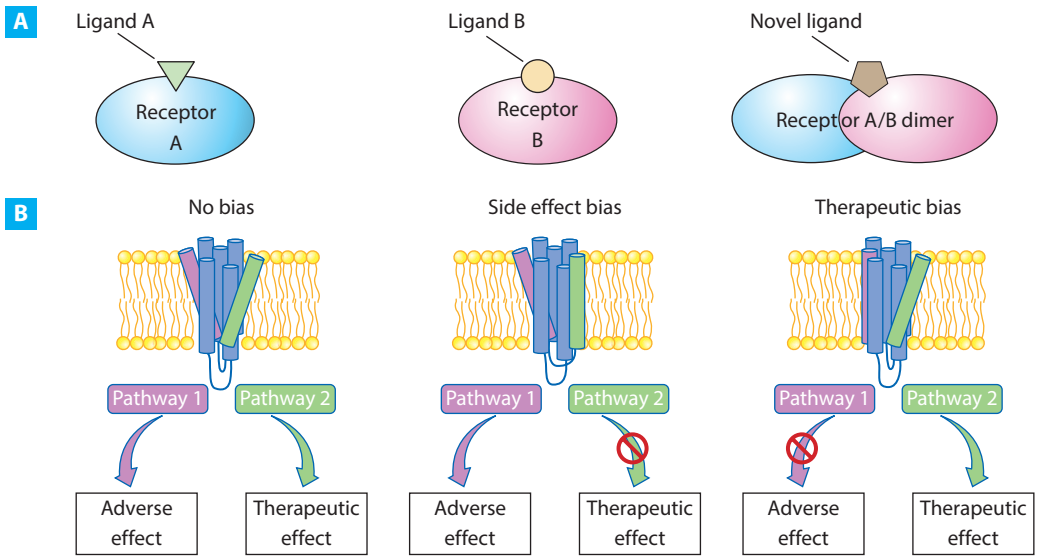

Figure 1-7 More complex considerations of receptor signaling. A. Several types of receptors have been shown to form dimers, with the receptor dimer responding to different ligands (agonists, antagonists, or inverse agonists) compared with the receptor monomers. **B.** Many receptors exhibit ligand–directed or biased signaling. In the scheme shown, one class of agonist (unbiased) at a receptor activates two signaling pathways, one that mediates the agonist's therapeutic actions and another that mediates the side effects elicited by the agonist. Other, "biased" agonists activate only one of these postreceptor signaling pathways to produce only therapeutic actions or only side effects. A specific example of such ligand–directed signaling is provided for the μ opioid receptor in Chapter 7.

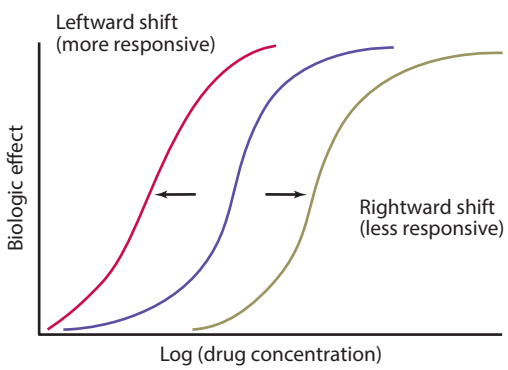

Figure 1-8 Rightward and leftward shifts in dose–response curves. A rightward, or downward, shift indicates a reduction in drug sensitivity: more drug is needed at all concentrations to elicit the same level of biologic response. A leftward, or upward, shift indicates an increase in drug sensitivity: less drug is needed at all concentrations to elicit the same level of biologic response.

sections. This dose dependency of drug action is one of the principal tenets of neuropharmacology and illustrates the importance of studying the effects of a wide range of drug doses.

One application of dose–response curves is in determining whether a form of treatment—for example, chronic exposure to an antidepressant—increases or decreases the responsiveness of a particular receptor system. Hypothetical cases are illustrated in (Figure 1-8), which shows that a reduction in receptor sensitivity in response to treatment is characterized by a rightward or downward shift in the dose–response curve, whereas an increase in receptor sensitivity is characterized by a leftward or upward shift in the dose–response curve.

Dose–response curves also can reveal that the biologic effects of a specific drug may not be a simple (monotonic) function of drug dose. When the effects of a drug are more complex, nonmonotonic, for example, they are represented by an inverted U-shaped curve (Figure 1-9). Such drugs elicit a progressively greater biologic response with greater drug dose up to

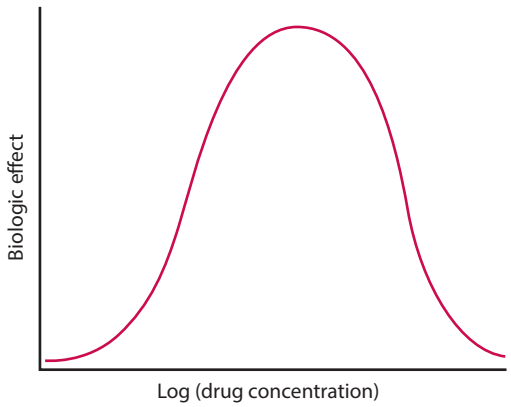

Figure 1-9 Inverted U-shaped dose–response curve. Dose–response curves that are placed on a semilogarithmic plot often are not sigmoidal, such as those in previous figures, but instead form an inverted U shape. Such curves contain an ascending limb at lower drug doses and a descending limb at higher drug doses. These curves indicate that the biologic response elicited by a drug progressively increases as the drug dose increases and subsequently peaks at a moderate dose; higher doses elicit progressively smaller responses.

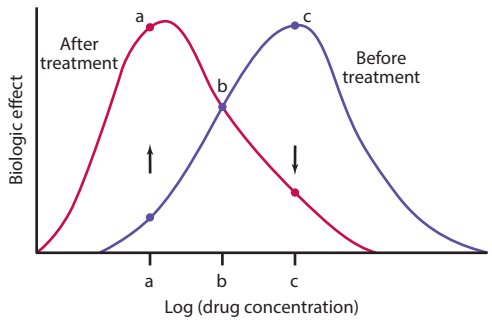

Figure 1-10 Analysis of a full dose–response curve. Because the biologic effects of many drugs are described by an inverted U-shaped dose–response curve, the effects of a drug should be analyzed over a wide range of doses. The graph shows a leftward shift in an inverted U-shaped dose–response curve occurring after an experimental treatment. With analysis of a single drug dose, it might be determined that the treatment causes (1) an increase in drug sensitivity (dose **a**); (2) no change in drug sensitivity (dose **b**); or (3) a decrease in drug sensitivity (dose **c**). The leftward shift in the dose–response curve becomes apparent only after a wide range of doses are analyzed.

a point, after which higher drug doses begin to produce smaller effects. This shift in effect most likely occurs because the drug begins to act on additional target proteins at higher drug doses, and action on these other targets opposes the effects of the first. Alternatively, high doses may cause receptor desensitization.

The analysis of full dose–response curves is necessary to determine reliably whether a particular treatment causes an increase or decrease in drug responsiveness. Figure 1-10 shows a leftward shift in the dose–response curve for a drug whose biologic effects are an inverted U-shaped function of drug concentration. Without an analysis of the full dose–response curve, an investigator may incorrectly interpret effects of the drug; for example, depending on the concentration of drug used to activate the receptor, a shift in the curve may indicate a reduction, an increase, or a lack of change in drug response.

The analysis of full dose–response curves is also required to identify PAMs or NAMs, and determine how they interact with a receptor to enhance or suppress its function, respectively. Figure 1-11 shows an inverted U-shaped dose response to a drug. Increasing concentrations of a PAM can shift the dose–response curve to the left and/or cause an upward shift in the response. Conversely, increasing concentrations of a NAM can shift the dose–response curve to the right and/or can cause an downward shift.

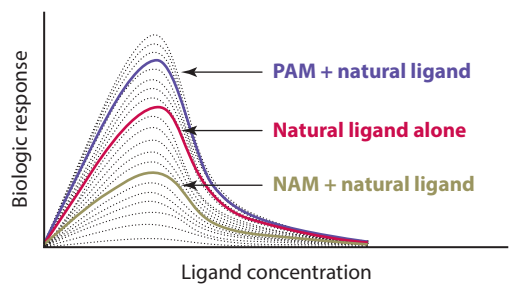

Figure 1-11 Actions of positive and negative allosteric modulators. The dose–response curve to a natural ligand is shown. A positive allosteric modulator (PAM), administered at a given dose, causes an upward (shown) or leftward (not shown) shift in that dose–response curve. Conversely, a negative allosteric modulator (NAM), administered at a given dose, causes a downward (shown) or rightward (not shown) shift in that dose–response curve. The dotted gray lines show the effects of higher and lower doses of the PAM and NAM.

Drug Interaction with Nonreceptor Proteins

Although most principles of drug action have been ascertained from studies of neurotransmitter and hormone receptors, the same general principles apply to interactions between drugs and nonreceptor proteins. A drug binds to a specific site on a protein, which can be determined by means of ligand binding assays. Drug binding influences the function of a protein by either facilitating or inhibiting that protein's normal functioning, including its interactions with other macromolecules. Some drugs create a new function for the protein to which they bind; examples include **FK506** and related drugs that bind immunophilins (Chapter 4). When such drugs bind to immunophilin proteins, the proteins become potent inhibitors of calcineurin, a protein phosphatase.

The conditions under which two proteins interact are conceptually similar to those for drug–target interactions. Protein–protein interactions have emerged as a central theme of cell regulation (Chapter 4). The binding of proteins such as transcription factors to specific sequences of DNA, which is a key mechanism of gene regulation in development and in neural plasticity throughout life, also operates according to principles like those of drug–target interactions.

NEUROPHARMACOLOGY IN THE MOLECULAR ERA

Neuropharmacology originally was a phenomenologic science. An investigator administered a drug to an animal or a cell preparation and examined the response. There were two major drawbacks to this black box approach (Figure 1-12). First, it did not elucidate the mechanisms of drug action and thus did not enable investigators to relate the initial action of a drug on its protein target to the clinical effects of the drug. Earlier in this chapter, it was pointed out that the actions of fluoxetine extend beyond inhibiting serotonin transporters or increasing serotonin function. To understand fully how fluoxetine works it is necessary to determine its action on the overall workings of the brain, from its effects on molecules and cells to its effects on neural circuits and ultimately on behavior.

Second, traditional neuropharmacology depended on ligand binding studies to identify the protein targets of a drug and to understand its actions on brain function. Such protein targets were typically defined by potency series, which compared the ability of ligands to interact with different binding sites. However, many of the neurotransmitter receptors that were originally identified by ligand binding studies proved to be misleading once it became possible to identify individual proteins by molecular means with great precision.

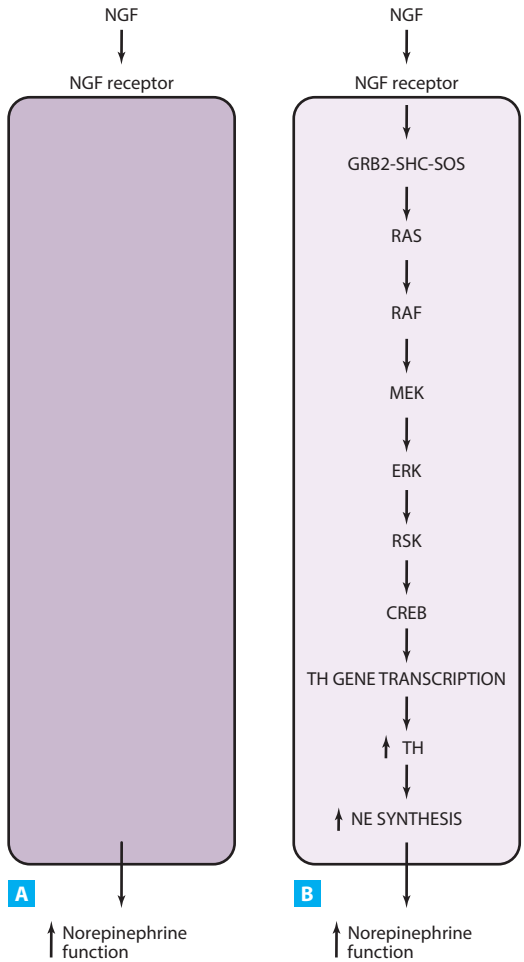

Figure 1-12 A comparison of black box and mechanistic approaches to neuropharmacology. The increased norepinephrine function in a sympathetic neuron caused by nerve growth factor (NGF) can be described in different ways. **A.** In classic studies, the effect of NGF was described in narrow, superficial terms—for example, NGF activates an NGF receptor—and insufficient attention was given to the detailed mechanisms by which drug–receptor interactions lead to a biologic response. **B.** In contrast, the tools of molecular and cell biology enable a detailed mechanistic description of NGF action, encompassing the delineation of the precise molecular steps by which activation of the NGF receptor leads to increased transcription of tyrosine hydroxylase (TH), the rate–limiting enzyme in norepinephrine synthesis. (See Chapters 4 and 8 for definitions of the various proteins shown in this figure.)

Whereas pharmacologic studies identified 3 major subtypes of serotonin receptors, we now know that 14 subtypes exist, with some subtypes misclassified by ligand binding experiments (Chapter 6).

Neuropharmacologists are currently using the penetrating tools of molecular biology and cellular physiology to extend their experimental repertoire. New research is aimed at defining the action of a drug on its cloned protein target in precise molecular terms and analyzing the protein target in various functional states, for example, in phosphorylated versus dephosphorylated states. Ultimately, investigators delineate the crystal structure of these proteins before and after they are bound to a drug to better understand the ways in which a drug alters the shape and surface charges of a protein.

Neuropharmacologists are using these new approaches to identify and validate novel targets for drug development, so that novel drugs can be synthesized to interact with those targets. *Combinatorial chemistry* is enabling the development of a large number of drugs with unique and diverse chemical structures. It incorporates knowledge of drug–target interactions at the molecular level, or *structure–activity relationships*, to determine what types of chemical moieties can be added to a drug to alter its actions on a protein target (Figure 1-13). Much of this initial characterization is carried out virtually, by exploring theoretical interactions between chemical structures of small molecules and three–dimensional structures of protein targets. *High–throughput screening* then allows the testing of a large number of chemical agents, which are promising by such chemi-informatic analyses, to discover their actual abilities to influence cloned proteins of interest. Finally, exploratory research is under way to use higher throughput behavioral analyses to facilitate the discovery of novel classes of compound that exert interesting, and potentially clinically useful, behavioral effects in laboratory animals. Other forms of *phenotypic screening* might search for molecules that exert desired

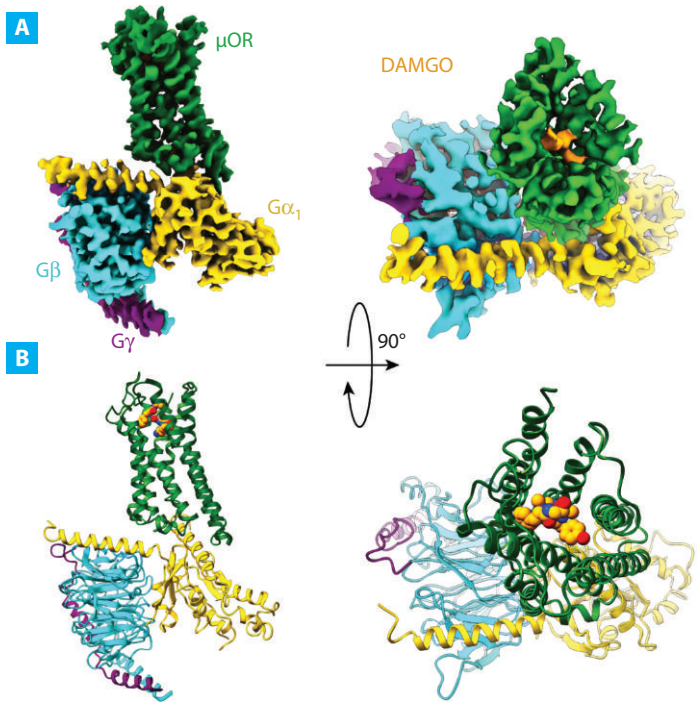

Figure 1-13 Example of using protein structure to inform medicinal chemistry. Cryo-EM structure of the μ opioid receptor–G_i complex is shown. **A.** Orthogonal views of the cryo-EM density map of the receptor–G_i heterotrimer complex colored by subunit. Green, μ opioid receptor; orange, DAMGO ([D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin), a synthetic peptide agonist of the receptor; gold, $G_{\alpha i}$ RAS-like domain (see Chapter 4); cyan, G_β; purple, G_γ. **B.** Model of the μ opioid receptor–G_i complex in the same views and color scheme as shown in **A.** (Reproduced with permission from Koehl A, Hu H, Maeda et al. Structure of the μ-opioid receptor–G_i protein complex. *Nature*. 2018; 558:547–552.)

effects on neuronal or glial structure or function in vitro. Together, these tools are enabling researchers to develop any desired types of drugs—for example, agonists, antagonists, weak partial agonists, or inverse agonists—for any targeted proteins or behavioral or cellular end points.

An interesting question in the field is whether a specific drug is more desirable than a less specific one. The initial era of modern neuropharmacology focused on the search for ever more specific medicinal agents, for instance, moving from **antipsychotic drugs** that antagonize numerous receptors to more specific agents that antagonize just one receptor type (Chapter 17). The expectation was that such "cleaner" drugs would show similar or even greater efficacy without the unwanted side effects of the "dirty" drugs. To date, however, such specific agents have been disappointing clinically, which has led to the realization that drugs with multiple targets may offer some advantages for complex brain diseases. The focus on specific agents is also somewhat semantic: fluoxetine is quite specific in that to a first approximation it antagonizes serotonin transporters only, but in doing so it promotes serotonin action at 14 receptors that are expressed by a large fraction of all of the brain's 100 billion nerve cells.

Molecular Diversity of the Brain

Traditional neuropharmacology focused on a very narrow subset of cellular proteins, which included neurotransmitter receptors and transporters and proteins involved in the synthesis or degradation of neurotransmitters. Such proteins account for only a few hundred of perhaps hundreds of thousands of distinct gene products that are expressed in the brain once alternative splicing and protein processing are taken into account (Chapter 4).

The concentration on neurotransmitter–based drugs excessively narrowed the scope of neuropharmacology and interfered with the development of drugs based on new mechanisms of action. Focusing on the 100,000 or more proteins expressed in the brain but not based on neurotransmitters is likely to lead to the identification of fundamentally novel classes of neuropharmacologic agents, and thereby mirror the tremendous successes in drug discovery achieved recently for cancer, immunologic conditions, and heart disease. Over the next decade, *functional genomics* and *proteomics*—the processes of sequencing, identifying, and characterizing individual gene products—will provide a template for exploring this vast array of proteins. Large numbers of proteins that regulate receptor sensitivity already have been identified, as have large numbers of modulatory proteins that govern these regulatory proteins. Indeed, it is an exciting prospect that the completion of the various genome projects is allowing us to know the full set of receptors and regulatory proteins expressed by nerve and glia cells. Genomic and proteomic methods are also making it possible to better understand the complex effects of a drug or disease state on target neurons. For example, advanced bioinformatic analysis of global changes in gene expression induced in a given cell type in a given brain region by a drug is making it possible to deduce key regulatory proteins that drive important aspects of drug action. Such *gene networks* are then used to drive the search for novel drug targets. As well, advances in proteomics are making it possible to screen large collections of compounds for specific molecular or physiologic actions, and to then leverage proteomics to identify the targets through which those compounds act. This type of phenotypic screening is likely to reveal entirely new and unexpected classes of drug targets.

Unconventional Therapeutic Approaches

This chapter has thus far focused solely on drugs that interact with target proteins to produce their functional effects. Increasingly, the molecular era has created opportunities for targeting other biologic molecules from RNAs to the genome itself. There are several ways to target RNAs. Small molecules are being developed to affect the alternative splicing or protein translation machinery in cells to alter the expression levels of a targeted RNA. Alternative approaches include *antisense oligonucleotides*, which are short (~20 nucleotides) double–stranded DNAs, and *microRNAs*, which are short (~20 nucleotide) single–stranded RNAs. These molecules can bind to specific RNAs of complementary sequence and thereby inhibit protein translation or in some cases influence alternative splicing (see Box 18-4 in Chapter 18). **Nusinersen** is the first antisense oligonucleotide approved by the US Food and Drug Administration (FDA) for a CNS disorder; it is administered intrathecally and works by controlling levels of expression of the *SMN2* gene and thereby eases the symptoms of **spinomuscular atrophy**. Other antisense oligonucleotides, also delivered intrathecally, are now in advanced clinical trials for the treatment of several neurodegenerative diseases (eg, **Huntington disease**, **amyotropic lateral sclerosis**), which involve the expression of toxic mutant proteins (Chapter 18).

Viral–mediated gene therapy has been in clinical trials for several neurologic conditions, including

neurodegenerative disorders. The greatest promise is seen for **retinitis pigmentosa**, which involves degeneration of the retina including its photoreceptor cells. It is a genetic disorder that leads to blindness. Numerous genetic mutations have been identified as the cause of retinitis pigmentosa. This knowledge, coupled with the relative ease of viral delivery directly into the eye (*intravitreal* or *subretinal injection)*, is driving several late–stage clinical studies to correct the genetic lesion locally, eg, to overexpress normal copies of *MYO7A, RPGR,* or *PDE6B* in patients harboring loss of function mutations in these genes. A related approach is to virally overexpress *channelrhodopsin* (a light–sensitive bacterial ion channel; Chapter 2) in surviving retinal cells to render them light–sensitive. Most of these approaches use different subtypes of *adeno–associated viruses* (*AAVs*), which mediate long–lived transgene expression without serious side effects. Clinical trials involving the direct injection of AAVs into the brain are ongoing for several CNS disorders, mostly notably, **Parkinson disease** and intractable **epilepsy**. Systemic delivery of AAVs is under investigation for **spinal muscular atrophy** (Chapter 18).

The advent of *CRISPR* and other *gene editing* methods is raising the possibility of repairing a genetic mutation and thereby restoring normal functioning or preventing disease in the first place. CRISPR describes a system—which mediates a form of induced immunity in bacteria—where an enzyme termed *Cas9* is tethered to a single guide RNA (sgRNA) that targets the Cas9–sgRNA complex to a single region of the genome of complementary sequence to the sgRNA. Cas9 is a nuclease and thereby cleaves and disrupts the targeted genomic region. Enzymatically dead forms of Cas9 (dCas9) can be fused to any functional moiety—eg, a DNA or histone methylating enzyme, a transcription factor—to induce more subtle effects on the epigenetic state of the targeted gene and its expression levels within the targeted cells. Today such genome or epigenome editing tools would have to be delivered into the brain by viral vectors, although efforts are under way to develop methods to deliver such agents systemically.

It is also conceivable that cell engineering will be introduced for the treatment of psychiatric and neurologic diseases. There is intense interest in the use of neuron– or glia–like cells derived from a patient's skin biopsy or other peripheral tissue to study the pathophysiology and treatment of that individual's illness (Box 1-1). Might cells derived from a patient but corrected for an underlying disease–causing genetic mutation be transplanted into the brain as a treatment? This scenario sounds farfetched but such approaches are being used today to treat certain cancers, as exemplified by *chimeric antigen receptor T cell* (*CAR T cell*) therapies. In this approach, T cells are isolated from a patient and engineered by CRISPR or other methods to increase the ability of the cells to recognize and neutralize cancerous cells more effectively and infused back into the patient as a therapy. CAR T cell therapies are showing encouraging results in the clinic.

This discussion highlights the fact that neuropharmacology in the postgenomics era will involve the development of small molecule drugs and many other types of treatment modalities aimed at a new set of proteins as well as novel RNA and genomic targets to regulate brain function and behavior. This potential transformation of current treatment of psychiatric and neurologic disorders holds great promise for the future.

Pharmacogenetics

Pharmacogenetics represents the ultimate application of the molecular era to neuropharmacology. It holds the promise of *personalized or precision medicine*, where an individual's particular genetic makeup, and perhaps other "omic" characterizations (transcriptomics, metabolomics, lipidomics, etc.), predict his or her response to a given medication. For instance, it should be possible one day to identify distinct etiologic subtypes of heterogeneous disorders, such as autism, schizophrenia, and epilepsy, to name just a few examples, based on genetic and other omic testing. These findings could be combined with brain imaging or other technologies to define pathophysiologic subtypes of the illnesses and to thereby permit treatment with drugs or other approaches aimed specifically at the underlying biologic abnormalities.

Pharmacogenetics is in the earliest stages of development, particularly for diseases of the nervous system, but a few examples are currently in clinical practice. *Cytochrome P450 enzymes*, which are encoded by the *CYP* gene superfamily, are involved in the metabolism of most drugs. We now know that individuals with particular *CYP* gene variants metabolize certain drugs much more or less effectively than most people. Such genotypes account for some of the differences in drug responsiveness observed clinically, and have been used to approximate the unusually high or low drug doses required in these individuals.

To date, pharmacogenetics is being applied most widely to the treatment of cancer. **Monoclonal antibodies** directed against a protein involved in a particular type of cancer, or even specific for an individual's cancer, are in clinical use. The use of **tamoxifen**, an estrogen receptor antagonist or partial agonist

Box 1-1 Induced Neurons and Glia from Patients

Recent technologic advances have made it possible to generate neuron– and glia–like cells from a peripheral tissue, typically a person's skin biopsy. This involves culturing fibroblasts from the biopsy and dedifferentiating them into *induced pluripotent stem cells* (*iPSCs*), that is, stem cells that are capable of giving rise to virtually any cell type. The key discovery that enabled this new approach was the 2006 finding that overexpressing a combination of four transcription factors—OCT3/4, SOX2, c-MYC, and KLF4—in fibroblasts yields iPSCs. Those iPSCs can then be redifferentiated into a cell type of choice, including a neuron or glia cell. There has since been intense research into refining the methods to generate iPSCs and to generate different types of neurons (eg, excitatory, inhibitory, dopaminergic) and glia (ie, astrocytes, oligodendrocytes, microglia) from a patient's peripheral biopsy. Some methods even skip the iPSC intermediate and induce neuron– and glia–like cells directly from fibroblasts.

Much work is needed to further improve these methods. The cells that can be generated today from iPSCs or related approaches are not true fully differentiated neurons or glia: their gene expression patterns more closely resemble cells from early embryonic development. As just one example, induced "dopamine neurons" are not true dopamine neurons: they express tyrosine hydroxylase, the rate–limiting enzyme in dopamine synthesis (Chapter 6), but differ from adult dopamine neurons in many other ways. Nevertheless, induced neurons and glia offer great promise for the future. Under the right conditions and provided with three–dimensional scaffolds, iPSCs in vitro can form "*organoids*," which contain mixtures of different neuronal and glial cell types and even form cell layers reminiscent of the developing brain. Such cells and organoids are now being used to study disease pathogenesis for many illnesses, for example, **Alzheimer disease** and **schizophrenia**, with highly complex genetic underpinnings and yielding encouraging findings. The cells and organoids are also being used in high–throughput assays to screen for pharmacologic agents that reverse functional abnormalities observed in the cells. For illnesses where animal models are inherently limiting, as is the case for many neuropsychiatric disorders, such approaches might lead to important advances in the field. A longer–term prospect is the possibility that engineered cells derived from a patient's biopsy might one day be used as a treatment.

depending on the tissue, in the treatment of breast cancer is reserved for those individuals whose tumors express the estrogen receptor. Likewise, the use of epidermal growth factor (EGF) receptor antagonists, such as **gefitinib** or **erlotinib**, for treatment of several types of cancers is increasingly being targeted to individuals whose tumors express mutant forms of the receptor or its signaling proteins. This has recently been applied to **glioblastomas**, a particularly severe form of brain cancer: only 20% of glioblastomas respond to EGF receptor antagonists and recent work has shown that it is possible to predict those individuals who respond based on EGF receptor function in the tumors.

As large–scale genetic studies of nervous system diseases progress, and specific causative or vulnerability genes are discovered, it should be possible to increasingly match a particular treatment with a particular patient. While this is many years on the horizon, it will truly usher in the molecular era of pharmacotherapeutics.

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CHAPTER 2

Cellular Basis of Communication

KEY CONCEPTS

- Neurons are the principal cells in the brain that process information. There is a great diversity of neuronal cell types based on morphology, molecular constituents, location, and connections.
- The nucleus and major cytoplasmic organelles in the cell body of neurons synthesize and process proteins, which are subsequently transported within the soma or along axons and dendrites to their appropriate locations.
- The axon conducts action potentials to presynaptic terminals to initiate communication with other neurons, which occurs at synapses.
- Dendrites, multiple fine processes that extend from the neuronal cell body, together with the cell body, serve as the primary structure for the reception of synaptic contacts from other neurons.
- The cytoskeleton—the inner scaffold of a neuron formed by a system of interconnected protein filaments called microtubules, intermediate filaments, and actin filaments—plays a key role in the structure of neurons and in the transport of various proteins and organelles from the cell body to axonal and dendritic processes.
- Three major classes of glia—astrocytes, microglia, and oligodendrocytes—play important roles in brain function.
- Astrocytes have diverse functions, including maintenance of the extracellular milieu, metabolism of certain neurotransmitters, formation of the blood–brain barrier, and response to brain injury.
- Microglia are essential components of the brain's immune system, but function more broadly by sculpting synaptic connections between neurons.
- Oligodendrocytes are the source of myelin sheaths that insulate many axons in the brain, a requirement for rapid transduction of electrical impulses.
- The blood–brain barrier—formed by capillary endothelial cells, connections between which are sealed by tight junctions and then wrapped by astrocyte end–feet and pericytes—allows only small lipophilic substances to enter the brain from the general circulation.
- In their resting state, neurons maintain a negative electrical potential in relation to the extracellular environment. This results primarily from differences between the intracellular and extracellular concentrations of K^+, Na^+, and Cl^- and the relative permeability of the cell membrane to these and other ions. The energy–consuming Na^+–K^+ pump helps to maintain appropriate ionic gradients across the membrane.
- The generation of all-or-none action potentials relies on the activities of voltage–dependent ion channels, highly specialized proteins that allow the flow of a specific ion (K^+, Na^+, or Ca^{2+}) across neuronal membranes in response to changes in neuronal membrane potential.
- Sodium channels are the targets of many important drugs including local anesthetics and some antiseizure medications.

- Potassium channels are a large and diverse family of proteins that regulate neuronal excitability and control the shape of the action potential.
- Entry of Ca^{2+} into neurons through voltage–dependent Ca^{2+} channels, of which there are three major classes—Ca_V1 (L-type), Ca_V2 (P/Q-, N-, and R-type), and Ca_V3 (T-type)—is important for neurotransmitter release and activation of intracellular signaling cascades.
- Blockers of Ca_V1 Ca^{2+} channels are used to treat ischemic heart disease and hypertension.
- Mutations in ion channels are the cause of several neurologic disorders, including certain inherited neuromuscular disorders, epilepsy, and migraine syndromes.

It has been estimated that there are ~100 billion neurons in the human brain, although this number in itself reveals little of the brain's complexity. Unlike other organs, the brain contains an enormous diversity of cell types. Depending on the definition of a neuronal cell type, there may be thousands in the brain—each with their own structural, molecular, and functional properties. Yet, the complexity of the brain as an information–processing organ extends beyond its cellular diversity. Neurons in the brain form diverse circuits that can range in scale from small local neuronal groups to long–distance projections. Many neurons function in more than one circuit and can communicate with thousands of other neurons.

Neuronal communication depends on both electrical and chemical carriers of information. The electrical mechanisms rely on the ability of each neuron to control the flow of ions across its membrane and thus to process and store information. Chemical signals are the means by which the vast majority of neurons communicate with each other. The vast majority of neurons are not physically connected but form contact points, called a *synapse* (Figure 2-1), at which their plasma membranes are separated by a minute space, as small as 20 nm. The simplest synaptic arrangement involves an electrical impulse in one neuron, the presynaptic neuron, that triggers the release of a chemical substance, or *neurotransmitter*, which diffuses across the synaptic cleft and binds to specific receptors on another, the postsynaptic neuron. The binding of a neurotransmitter to its appropriate receptors precipitates changes in the electrical activity of the postsynaptic neuron, which in turn leads to the release of its neurotransmitter and further interneuronal communication. In a small fraction of cases, neurons physically connect with one another via *gap junctions* so that molecules and ions can pass between the two neurons, allowing for direct electrical transmission.

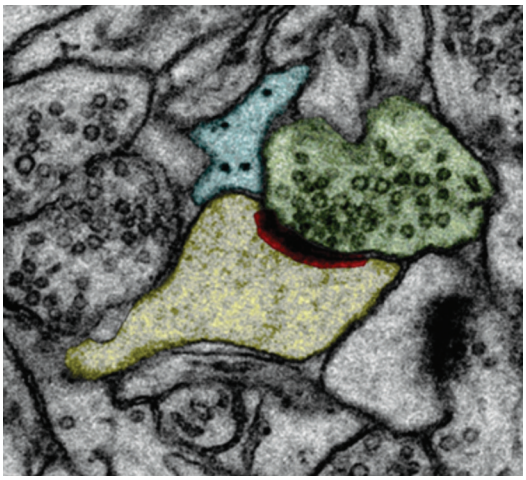


Figure 2-1 Electron micrograph of excitatory synapses. Each excitatory synapse forms an asymmetric junction, which exhibits a prominent postsynaptic thickening called the postsynaptic density (red). Axon terminals opposite the postsynaptic density contain small, spherical vesicles. One terminal (green) can be seen making contact with a dendritic spine (yellow). Most excitatory synapses are surrounded by astroglial processes (blue). (Reproduced with permission from Witcher MR, Kirov SA, Harris KM. Plasticity of perisynaptic astroglia during synaptogenesis in the mature rat hippocampus. *Glia*. 2007;55(1):13–23.)

Neurons in the brain can form thousands of synapses with other neurons; extreme examples are a Purkinje cell in the cerebellum or a monoamine–containing cell in the brainstem that may form more than 100,000 synapses. Overall, in a single human brain, there are likely to be on the order of 100 trillion synapses. Complex processes of brain development that remain incompletely understood result in connections among neurons—some local, some over long distances—that are both highly specific and highly plastic.

The overall patterns of neural connectivity in the mammalian central nervous system (CNS) are dictated by a complicated set of genetically programmed interactions. Nevertheless, both spontaneous neural activity and neural activity that occurs in response to stimulation during gestation and throughout life have profound influences on the fine–tuning of an individual's pattern of synaptic connections. Although there are finite critical periods of early postnatal development during which the pattern of neural connectivity in some circuits is markedly influenced by experience, the adult brain is far more plastic than previously thought and synaptic connectivity is modified throughout life. Thus, unlike computers, which are sometimes represented as artificial brains, the neural circuitry of the mammalian brain is not hardwired but instead constantly reacts and adapts to an ever–changing environment. The advent of *optogenetics*, *chemogenetics*, and related tools has vastly expanded the ability to study the function of precise neural circuits in the brain (Box 2-1).

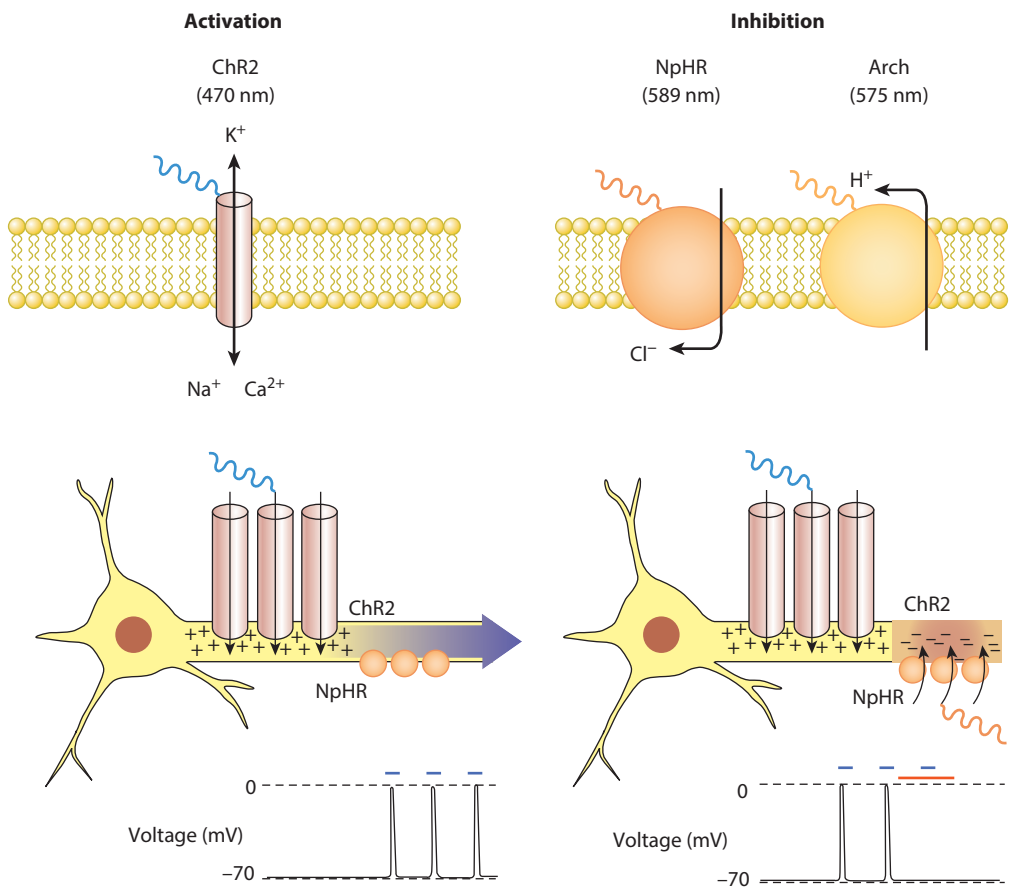
Although the neuron is the critical cell type for communication in neural networks, essential roles are played by glial cells. The brain contains three major classes of glia: *astrocytes*, *oligodendrocytes*, and *microglia*. Astrocytes have diverse functions, which include maintenance of the extracellular milieu (the composition, including ion concentrations, of extracellular fluid

Box 2-1 **Optogenetics and Chemogenetics: Revolutionizing the Study of Brain Circuits**

Major scientific advances are often driven by the development of new methodologies. Two such advances over the last decade—optogenetics and chemogenetics—have dramatically enhanced the study of the neural circuit basis of brain function.

Optogenetics refers to the targeting of light–sensitive recombinant proteins to certain cells in the brain followed by optical stimulation to achieve precise spatial and temporal control over the activity of those cells. By probing the genomes of primitive organisms, scientists discovered light–sensitive proteins, termed *opsins*. These opsins function normally when expressed in neurons and include light–activated ion channels and ion pumps that are targeted to membranes. One of the most important ion channel opsins is called *channelrhodopsin* (ChR2). When activated by blue light, it functions as a nonspecific cation channel and strongly depolarizes cells. In the absence of blue light, which normally never enters the brain, it is inert. By expressing ChR2 in neurons and exposing the neurons to very short pulses of blue light, it is possible to generate action potentials and to mimic the neurons' natural firing patterns. By targeting ChR2 (or related opsins) to a specific cell type in the brain, by use of viral gene transfer or transgenic approaches, investigators can precisely activate these cells and determine the behavioral consequences in awake behaving animals. It is also possible to activate the presynaptic terminals of ChR2–expressing neurons and thereby determine the behavioral consequences of activating different afferent inputs to a given brain region.

Of equal importance is the existence of opsins that inhibit the activity of neurons. Two examples are an inward chloride pump termed halorhodopsin (NpHR) and an outward proton pump termed Arch. NpHR and Arch are activated by yellow light and can hyperpolarize cells for prolonged periods



(*Continued*)

Box 2–1 Optogenetics and Chemogenetics: Revolutionizing the Study of Brain Circuits (*Continued*)

of time. Thus, by targeting these inhibitory opsins to specific cell types, scientists can determine the behavioral consequences of inhibiting the activity of specific sets of neurons in awake behaving animals.

Chemogenetics describes a related approach that targets the expression of designer receptors exclusively activated by designer drugs (*DREADDs*) to specific brain cells followed by activation or inhibition of those cells upon administration of a small molecule that activates the DREADDs. DREADDs are modified G protein–coupled receptors that signal through a particular G protein (eg, G_q, G_i) (Chapter 4). The receptors are modified to respond to a synthetic ligand (eg, clozapine N-oxide) that has minimal effects on endogenous receptors. In this manner, it is possible to experimentally activate or inhibit a particular signaling pathway in affected neurons. While this approach lacks the temporal resolution of optogenetics, it offers other advantages.

Optogenetics and chemogenetics have become standard, and ever improving, experimental tools to study the function of specified circuits in the brain. Optogenetics is also being employed to gain light control over other types of proteins in the brain, for example, intracellular signaling proteins. It is even conceivable that optogenetics and chemogenetics might eventually be applied to the human nervous system as a therapeutic modality to repair malfunctioning circuits that cause debilitating symptoms.

in the brain) for healthy neuronal function, metabolism of certain neurotransmitters, and formation of the blood–brain barrier. Astrocytes also are critical in the CNS response to injury. Oligodendrocytes produce myelin sheaths that encase axons and facilitate the conduction of action potentials. Microglia, together with lymphocytes and macrophages that migrate to the CNS from the periphery, are the cellular components of the brain's immune system and are important for removing cellular debris from the brain and shaping synaptic contacts between neurons. All types of glia elaborate soluble factors, including neurotrophic factors and cytokines, which are involved in the maintenance of the nervous system and in its adaptation to changes in the environment (Chapter 8). Indeed, astrocytes and microglia are important in modulating the process of synaptic transmission. Glia also are key components in guiding the migration of growing neurons during development.

This chapter focuses on the basic features of neurons and glia and on the electrical excitability of neurons. The molecular and cellular basis of synaptic transmission and signal transduction are covered in subsequent chapters.

THE NEURON

Neurons are highly asymmetric (polarized) cells that have three major components: a cell body (also known as a *soma* or perikaryon), a single long process called an *axon*, and a varying number of branching processes known as *dendrites* (Figure 2-2). Aggregations of neuronal cell bodies form the gray matter of the brain (named for its appearance on freshly cut sections). Axons make up the white matter, whose appearance results from the myelin sheaths that insulate many axons. Although neurons share a common set of features, they have a variety of sizes and shapes (Figure 2-3) and serve very different functions within the networks in which they operate.

Overview of the Neuron

The cell body contains the nucleus and major cytoplasmic organelles such as the rough and smooth endoplasmic reticulum (ER) and Golgi apparatus (Figure 2-4). It is primarily responsible for synthesizing and processing proteins, which are subsequently transported to their appropriate locations within the neuron. The nucleus contains genomic DNA that is transcribed into RNA; mRNAs are exported from the nucleus to the cytoplasm where they are translated into proteins on ribosomes (Chapter 4). Although most mRNAs remains in the cell body, some are transported to dendrites and axon terminals. Polyribosomes, which are multiple ribosomes arrayed on an mRNA, and ER are found in dendrites, often right beneath synapses, where they presumably permit localized protein synthesis. Local control of protein synthesis permits alterations to be made to specific synapses within a neuron, a mechanism that may underlie very precise changes in neural circuit function.

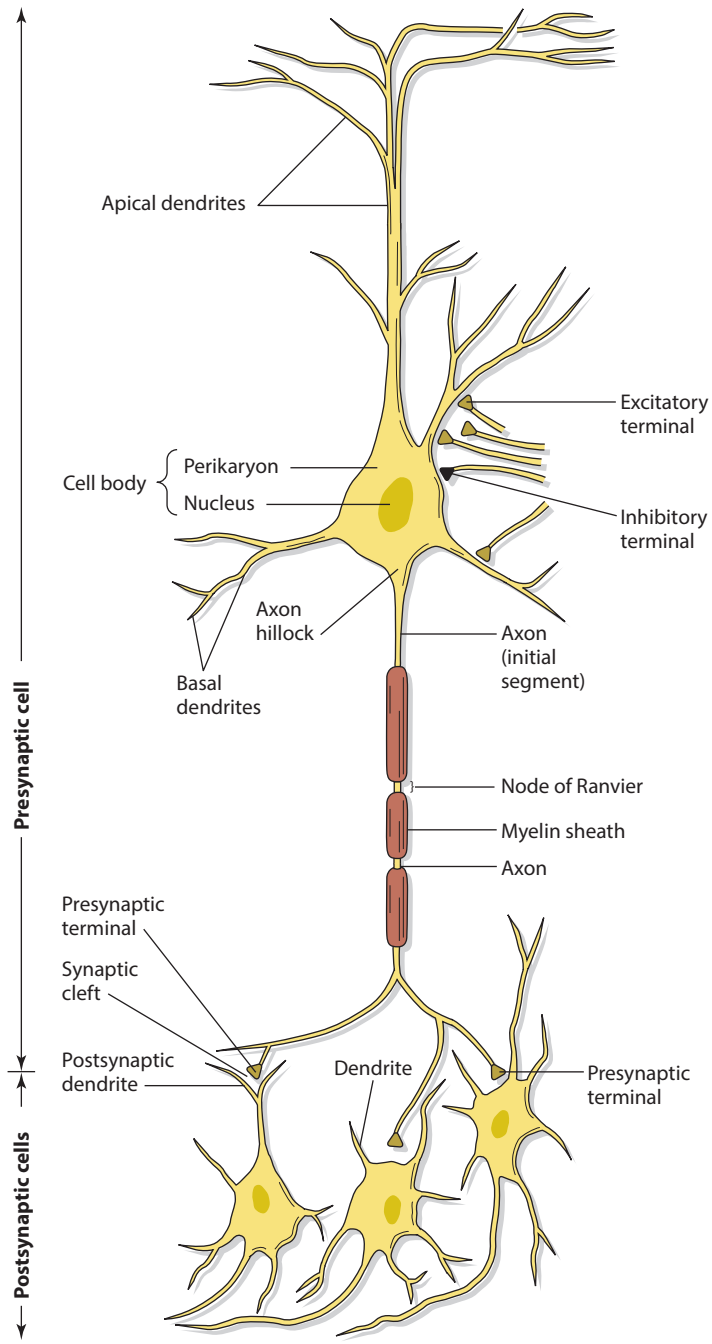


Figure 2-2 **Principal features of a typical vertebrate neuron.** Dendrites, which are the primary postsynaptic sites of synaptic contact, receive most of the incoming synaptic communication. The cell body contains the nucleus and is the site of gene transcription. The axon transmits information and often has multiple branches, the terminals of which form synapses with the dendrites of other neurons. (Reproduced with permission from Kandel ER, Schwartz JH, Jessell TM. *Principles of Neural Science*, 4th ed. New York: McGraw-Hill; 2000.)

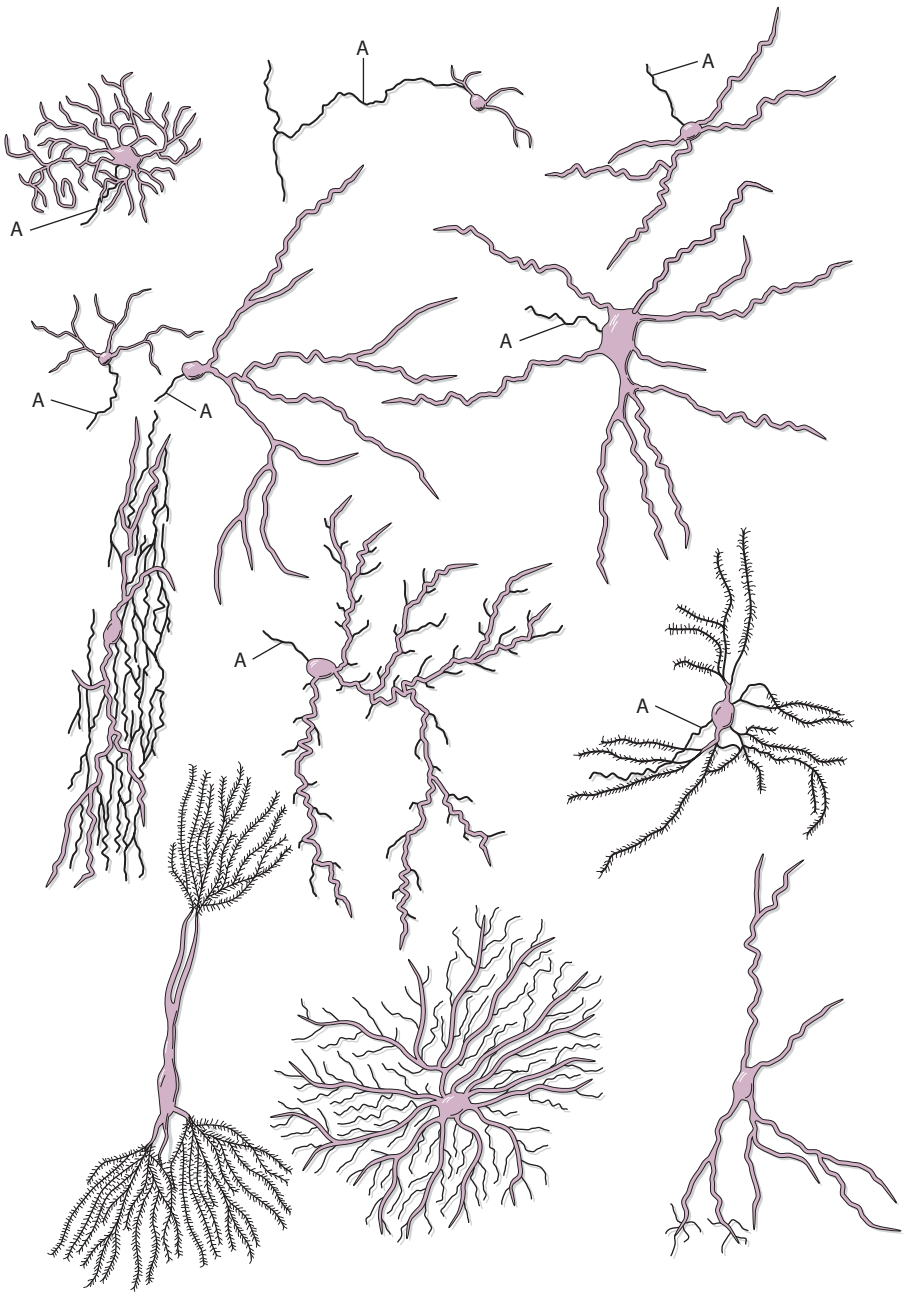


Figure 2-3 **Drawings of typical neurons in the CNS.** "A" marks the axons of some of these neurons.

The cell body is the smallest part of the neuron; the bulk of cytoplasmic volume is distributed throughout the axon and the dendritic arbor. Yet, because it must produce components that sustain the rest of the neuron, the metabolic and synthetic demands on the neuronal cell body are immense. Thus, the cell body contains large numbers of mitochondria, which are the sites of oxidative phosphorylation and provide the main form of energy (adenosine triphosphatase [ATP]) used by all eukaryotic cells.

The axon is a fine tubular process that extends from the neuronal cell body; it conducts electrical impulses from the cell body to the axon terminals (presynaptic boutons) that form the presynaptic component of a

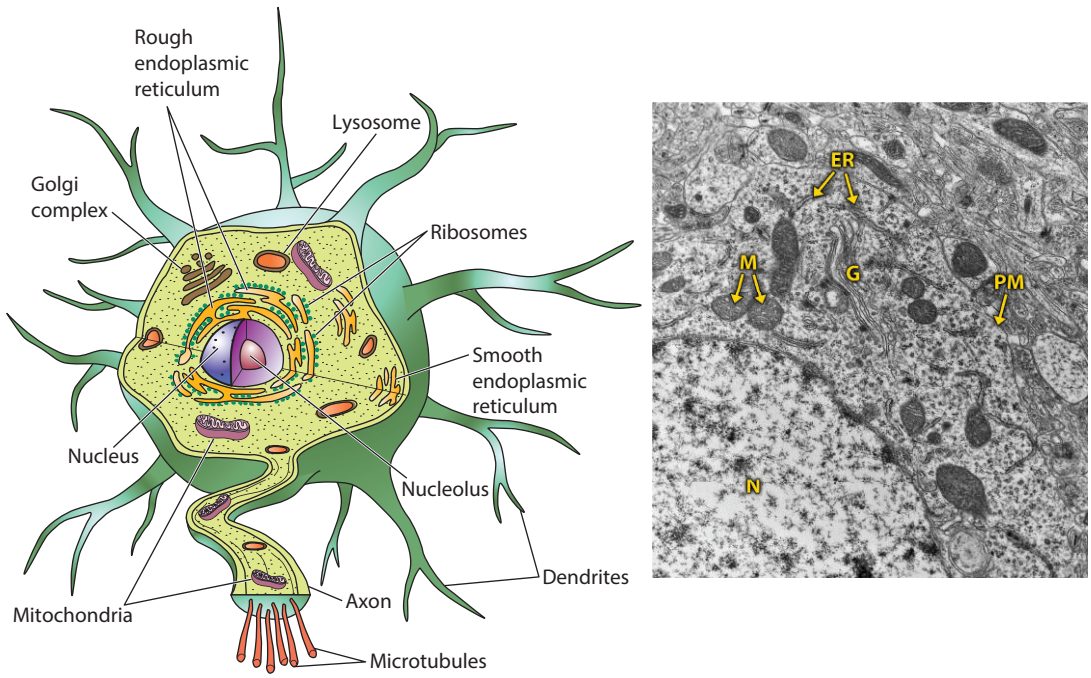


Figure 2-4 **Diagram and electron micrograph of intracellular organelles.** The nucleus (N) is the site of transcription of DNA to RNA. Proteins are synthesized in the endoplasmic reticulum (ER) and subsequently transported to the Golgi apparatus (G) where they undergo further modifications before they are transported to their final destination. Also shown are mitochondria (M), the energy powerhouses for the cell, and the plasma membrane (PM). (Used with permission from William Janssen, Icahn School of Medicine at Mount Sinai.)

synapse. *Projection neurons* are those that send axons to another region of the CNS or to the periphery, as opposed to *interneurons* whose axons remain within the CNS region of origin. Neurons generally have a single axon, the length of which varies from less than 1 mm for interneurons to more than 1 m for motor neurons that innervate the extremities. The axons of long projection neurons typically are myelinated; those of local circuit neurons usually do not have myelin sheaths. The axon normally emerges from a region of the cell body called the axon hillock (Figure 2-2), the region of the neuron from which an action potential is most often generated. As it approaches its terminal field of innervation, an axon may branch to varying degrees, depending on the number of neurons with which it makes synaptic contact. Axons also may give rise to recurrent collaterals that innervate other neurons in their vicinity and thereby serve feedback regulatory functions.

Dendrites are multiple fine processes that extend from the neuronal cell body and, together with the cell body, serve as the primary structure for the reception of synaptic contacts from other neurons. The geometry of dendritic arbors can be very complex and indeed beautiful (see Figure 2-3). The precise location and extent of a dendritic arbor determine the role of a neuron in a network. Many types of neurons have discrete spines protruding from their dendrites (Figure 2-5). Such spines typically receive the major excitatory inputs directed to their respective cells. Moreover, the spines are thought to structurally and biochemically isolate synapses so that each synapse serves as a small, individual unit of information processing (Chapter 3). Dendritic spines are not fixed structures; neurons regulate the number and morphology of spines, in some cases over minutes and hours, in response to neural activity and environmental signals.

The main functions of the dendritic tree include the reception, processing, and integration of incoming synaptic communications. Dendrites are both electrically and biochemically quite complex. Dendrites, like axons, contain voltage–dependent ion channels and thus can fire action potentials and actively propagate information to the soma. They also contain a wide

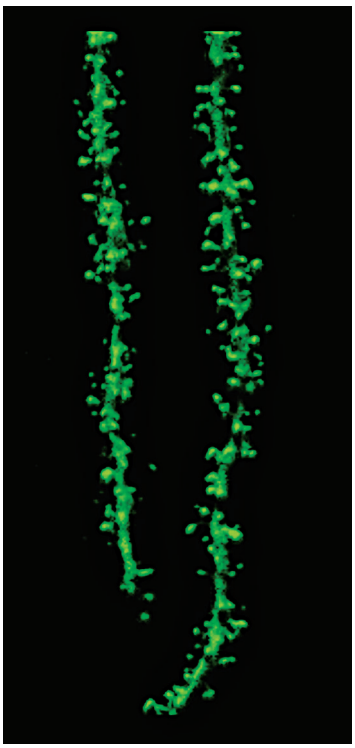


Figure 2-5 3-Dimensional image of dendritic branches of a hippocampal pyramidal neuron reveals a high density of dendritic spines. This cell was injected with Lucifer yellow dye and imaged using laser confocal fluorescence microscopy. (Used with permission from William Janssen and Ana Pereira, Icahn School of Medicine at Mount Sinai.)

variety of intracellular signaling molecules (Chapter 4) that are activated during synaptic communication and alter neuronal function.

The Cytoskeleton and the Transport of Proteins

The *cytoskeleton*, which represents the inner structure, or scaffold, of a neuron, is formed by a system of interconnected molecular filaments termed *microtubules, intermediate filaments*, and *actin filaments*. Microtubules are made of polymers of tubulin, a globular protein that forms a heterodimer between α and β tubulin. Microtubules copurify with several microtubule–associated proteins (MAPs) such as *tau*, which have significant roles in the assembly of microtubules, in cross–linking them to other filaments, and in transport functions. Intermediate filaments of neurons, called *neurofilaments*, are formed by three polypeptide subunits of low, middle, and high molecular masses. Actin filaments (also called *microfilaments*) are made of actin, a globular protein that self–assembles into a linear polymer. Microtubules and intermediate filaments are cross–linked to form a longitudinal scaffold for axons and dendrites. Actin microfilaments form a network underneath the entire surface membrane of the neuron; in dendrites and axons they are connected to microtubules and intermediate filaments. Actin microfilaments are heavily concentrated in dendritic spines and growth cones, both of which are dynamic structures that can respond to extracellular signals by changing shape and size. Many and perhaps all neurons in the CNS also possess *cilia,* small somatic processes. Cilia are specialized biochemical signaling compartments that are important for the migration of neurons during development but may also continue to serve important functions in the adult brain.

The cytoskeleton not only has important structural functions but also controls the transport of proteins between the cell body and its axonal and dendritic processes. Both fast anterograde and retrograde transport of proteins (100–400 mm per day) and slow anterograde transport (0.1–3.0 mm per day) occur in axons. Fast anterograde axonal transport involves the movement of transport vesicles, derived from the Golgi, along axonal microtubules. These vesicles contain many of the proteins necessary for the functioning of presynaptic terminals.

The power to move vesicles along microtubules by fast axonal transport is derived from two force–generating proteins, *kinesin* and *dynein*. These proteins are ATPases that, by binding to microtubules, are stimulated to transport vesicles. Because microtubules have an intrinsic polarity, with plus ends pointing toward presynaptic terminals and minus ends pointing toward the cell body, they can serve as a compass to direct vesicle traffic. Kinesin and dynein also have an intrinsic polarity; kinesin moves vesicles only toward the plus end of microtubules and therefore is the motor protein for anterograde fast transport, while dynein moves them only toward the minus end and thus is the motor protein for retrograde fast transport. Retrograde axonal transport functions to return various membrane molecules to the soma for elimination and is also important for communicating information from nerve terminals to the soma.

The molecular mechanisms responsible for slow axonal transport and for the transport of proteins to dendrites are not well understood. Slow axonal

transport appears to utilize the proteins that comprise the cytoskeleton itself—a dynamic rather than a static structure that is continually renewed. Proteins that are specifically directed toward dendrites rather than axons must be guided by molecular recognition signals that dictate the direction of transport. The dendritic cytoskeleton is different from that of axons. Unlike axons, in which the polarity of microtubules is fixed, dendrites have microtubules whose polarity varies because equal numbers of microtubules are oriented in each direction. The proteins associated with dendritic microtubules also differ from those of axonal microtubules; MAP2 is found only in dendrites and cell bodies, whereas the protein tau is found almost exclusively in axons. Perhaps these differences help guide the transport of specific proteins to specified sites within neurons.

The Synapse

A synapse is a specialized structure involved in the transmission of information from one neuron to another. Synapses typically are composed of a single presynaptic element—the presynaptic terminal or bouton of an axon—and a postsynaptic element, which for excitatory synapses is often a dendritic spine. Dendritic spines contain a *postsynaptic density*—named for its dark appearance by electron microscopy—which immediately opposes the innervating presynaptic nerve terminal (Figure 2-1; see also Figure 3-2 in Chapter 3). Postsynaptic densities are enriched for many types of signaling proteins including neurotransmitter receptors, proteins that anchor those receptors to the synapse, and a host of proteins that serve intracellular signaling functions. Although the *synaptic cleft* lies between the presynaptic and postsynaptic elements, it is incorrect to think of the cleft as empty space that separates two independent structures. Instead, the presynaptic and postsynaptic elements of a synapse are tightly bound to one another and to the *extracellular matrix* (composed of proteoglycans, other polysaccharides, and specialized proteins) by means of numerous scaffolding proteins, such as cell adhesion molecules (CAMs), and by astrocytes (see Figure 2-2). The synapse should therefore be considered an individual unit whose purpose is to transmit, process, and store information.

Most synapses in the brain utilize chemical transmission. Neurotransmitters (Box 2-2) are typically released from presynaptic terminals, and diffuse across a synaptic cleft and bind to specific receptor proteins on postsynaptic cells (Chapters 3 and 4). The presynaptic terminal contains specialized cellular structures that allow it to remain, to a certain extent, metabolically and functionally independent from the neuronal cell body. It contains large numbers of mitochondria to provide energy, enzymes to synthesize and degrade neurotransmitters, and synaptic vesicles to store substantial concentrations of neurotransmitters while waiting for a signal to be released. The postsynaptic dendritic membrane is markedly enriched with appropriate neurotransmitter receptors and elaborate intracellular signaling machinery as previously noted.

Synapses that involve the innervation of a postsynaptic dendrite by a presynaptic nerve terminal represent just one anatomic arrangement of chemical synapses. Other arrangements, in particular, where substances are released by dendrites that act on nerve terminals, are described in Box 2-3. Overall, chemical synapses are predominant in the CNS but do not represent the only form of synaptic transmission. In a small minority of cases, neurons are connected by means of a *gap junction* rather than separated by an intervening space. Gap junctions, which are formed by a large number of tightly packed proteins (*connexons*), produce so-called electrical synapses that permit electrical currents to flow directly between cells.

THE ELECTRICAL PROPERTIES OF NEURONS

Every heartbeat, every nerve impulse, every movement, and every thought is critically dependent on the tightly controlled and precisely timed flow of ions across cell membranes. A disruption of this flow, for example, by the puffer fish poison tetrodotoxin, can be fatal. Ion channel abnormalities are responsible for many human diseases (now called channelopathies). Mutations of ion channels can result in severe deficits in neuromuscular functioning, including episodic ataxias and paralyses, myotonia, and long QT syndrome of the heart as well as seizure disorders (Table 2-1). Moreover, ion channels are the targets of widely used and efficacious pharmacologic agents. For example, phenytoin and carbamazepine, which are used to treat epilepsy, act by altering Na^+ channel kinetics. Lidocaine and procaine, common local anesthetics, block voltage–gated Na^+ channels and prevent the conduction of nerve impulses that signal the occurrence of tissue damage and therefore pain. An awareness of ion channel structure and function is crucial for understanding neuropharmacology and neuronal disease processes.

Box 2-2 Identification of Neurotransmitters in the Brain

Our understanding of the molecular basis of neuropharmacology is significantly dependent on our ability to identify neurotransmitters in the mammalian brain. In theory, a substance that is released in response to stimulation of a neuron and that is capable of generating a measurable postsynaptic response, electrophysiologic or biochemical, might be classified as a neurotransmitter. However, to help elucidate the extraordinary complexity of neural signaling, more explicit criteria have been used to identify neurotransmitters.

Localization

A putative neurotransmitter must be localized to presynaptic terminals (or in some cases to dendrites or somas) in specific neural pathways (Box 2-3). Techniques for such localization include immunohistochemical staining and biochemical analysis of regional concentrations of the substance under study. The localization of enzymes required for the synthesis, degradation, or uptake of the substance helps to confirm the identification of a neurotransmitter.

Release

Classically, neurotransmitters are released in a Ca^{2+}–dependent manner, which can be established, for example, by depleting Ca^{2+} or blocking Ca^{2+} entry into neurons (Chapter 3). In an intact brain, it can be determined whether stimulation of a pathway causes the release of a candidate neurotransmitter into the extracellular fluid. This can be accomplished by techniques such as *microdialysis* to collect the extracellular fluid and *high performance liquid chromatography* to measure the neurotransmitter, or by direct electrochemical detection (eg, *voltammetry*) of certain neurotransmitters. So-called sniffer cells, which express a given neuropeptide receptor, are a recently developed innovation to measure levels of released neuropeptide neurotransmitters in the brain (Chapter 7).

Synaptic mimicry

The action of a suspected neurotransmitter should be mimicked by exogenous application of the substance. Such mimicry can be accomplished in vitro by application of the substance to reduced brain preparations, or in vivo by means of *microiontophoresis*. The actions of the substance can be evaluated by means of electrophysiologic, biochemical, or behavioral measurements.

Synaptic pharmacology

Neurotransmitters act on receptors for which there may exist pharmacologic antagonists. Thus, if the action of a synaptically released substance is blocked by a selective receptor antagonist, the identity of the neurotransmitter is strongly suggested. Receptor agonists also may be used to demonstrate synaptic mimicry but may provide inaccurate results because several receptor subtypes can be coupled to the same postsynaptic effector mechanisms.

Electrical Potential in Cells

An animal's nervous system receives information from the environment, integrates this information with past experience, and creates a behavioral response that promotes the survival of the organism. Sensory neurons receive information from both the external environment—for example, sounds and sights—and the internal environment, such as the sensation of hunger or the stretch of a muscle, and relay messages to other neurons. Neurons that receive these messages are responsible for directing appropriate signals to various locations that may include muscles, internal organs, glands, or other neurons. The swift communication of these signals typically benefits an organism. It can, for example, enable a prey's rapid response to the appearance of a predator, or produce a reflexive postural correction that is necessary to prevent a fall.

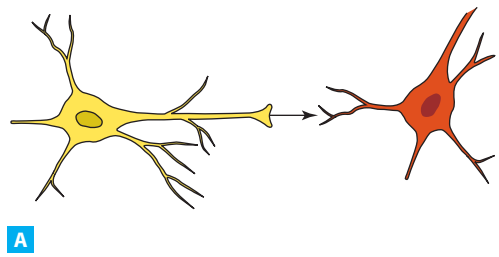
Neurons convey signals rapidly by alternately maintaining and varying their electrical potentials in relation to the extracellular environment. All neurons maintain a negative electrical potential (also known as a *membrane potential*) relative to their extracellular environment. This negative potential provides a driving force for charged particles; in the absence of other forces, positive charges tend to be drawn into the cell, and negative charges tend to be repelled from the cell.

Box 2-3 **Diverse Modes of Synaptic Transmission**

A synapse was initially thought to involve a postsynaptic dendrite or cell body innervated by a presynaptic nerve terminal A. This classic definition described a synapse both structurally and functionally in terms of presynaptic and postsynaptic elements. Although such synapses, which can be termed *axodendritic* or *axosomatic*, are widespread in the CNS and may represent the predominant mode of synaptic transmission involving excitatory and inhibitory amino acids, we have learned that many additional types of synaptic transmission occur in the brain.

Axoaxonic synapses occur when neurotransmitters released from one nerve terminal act on receptors located on other nearby nerve terminals B. Such nerve terminals may be functionally presynaptic in one synapse but functionally postsynaptic in another. Neurotransmitters also can act by means of autoreceptors located on the same terminals that release them C. Neurotransmitters can be released from cell bodies or dendrites (eg, nitric oxide, endogenous cannabinoids, dopamine) and diffuse to act on neighboring nerve terminals, resulting in a *dendroaxonic* synapse D, or on their own cell bodies or dendrites.

It is likely that several types of synaptic relationship coexist in most regions of the brain. Consider a hypothetical situation in which an excitatory amino acid nerve terminal innervates a dendritic spine by means of a classic axodendritic synapse E. In addition to acting on the dendritic spine, the released glutamate can affect further glutamate release by acting on autoreceptors at its own nerve terminals. Glutamate release is further modulated by nearby γ-aminobutyric acid (GABA)-ergic nerve terminals, which function as axoaxonic synapses with the glutamatergic terminals. Released monoamines or endogenous cannabinoids modify glutamate release from glutamatergic nerve terminals by means of actions on presynaptic receptors and modify postsynaptic responses to glutamate through actions on receptors near or on the dendritic spines.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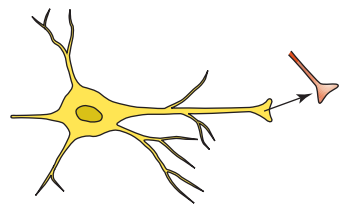
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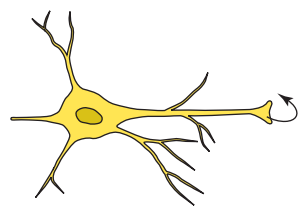
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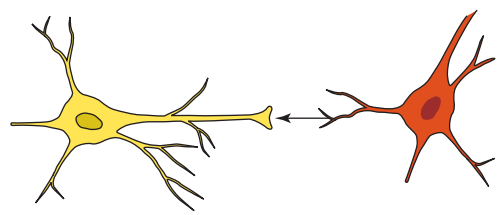
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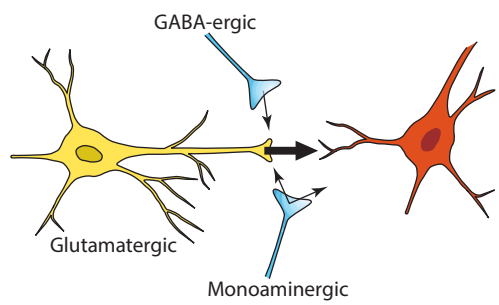


E

Table 2-1 Examples of Human Channelopathies

Family	Disease and Symptoms
K_{ir}, inwardly rectifying K^+ channels	Bartter syndrome (renal salt loss) Congenital hyperinsulinism Neonatal diabetes Dilated cardiomyopathy
K_v, voltage-gated K^+ channels	Episodic ataxia type 1, neuromyotonia Long QT syndromes Atrial fibrillation, short QT syndrome Benign neonatal febrile convulsions Nonsyndromic deafness
TRP, TRP channels	Autosomal dominant polycystic kidney disease Focal segmental glomerulosclerosis, defective Mg^{2+} reabsorption Mucolipidosis IV
CNG, retinal cGMP-gated channels	Retinitis pigmentosa Achromatopsia
K_{Ca}, Ca^{2+}-activated K^+ channels	Generalized epilepsy with paroxysmal dyskinesia
Na_v, voltage-gated Na^+ channels	Generalized epilepsy with febrile seizures type Severe myoclonic epilepsy of infancy Neuropathic pain Benign familial neonatal seizures Paramyotonia congenital Hypokalemic periodic paralysis Long QT syndrome Progressive cardiac conduction defect Familial erythromelalgia
Ca_v, voltage-gated Ca^{2+} channels	Episodic ataxia Familial hemiplegic migraine Spinocerebellar ataxia Congenital stationary night blindness Hypokalemic periodic paralysis Juvenile myoclonic epilepsy Generalized epilepsy and praxis-induced seizures Timothy syndrome
Cl, chloride channels	Cystic fibrosis Vitelliform macular dystrophy (Best disease) Generalized myotonia (Becker disease) Several types of epilepsy

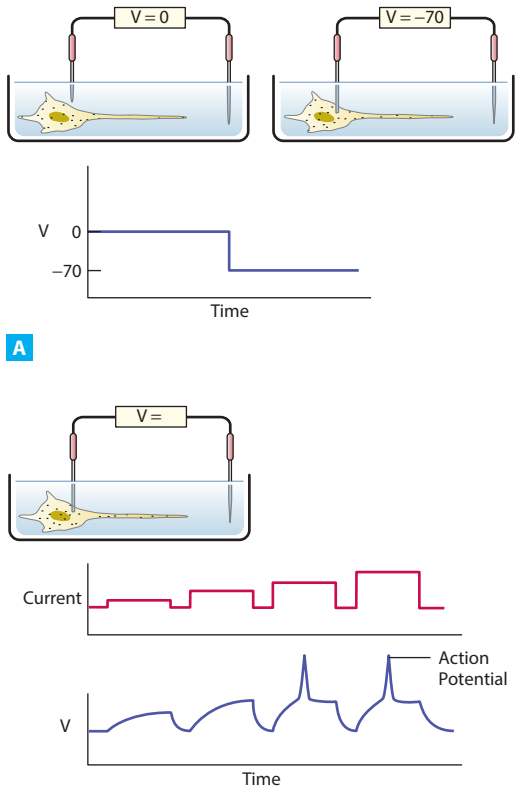


Figure 2-6 Membrane potential. A. As a sharp electrode penetrates a neuron, the difference in potential between the recording electrode and the bath drops from 0 to −70 mV. **B.** When small currents pass through the electrode, the potential of the cell changes in a passive manner. Larger currents elicit an all-or-none action potential.

When a neuron is activated by an external stimulus such as a chemical signal from another neuron, or by an event in the environment, it may *depolarize*; that is, its electrical potential may become less negative relative to the extracellular milieu. A neuron may, for example, depolarize from −70 to −50 mV. If a neuron undergoes significant depolarization, it may generate an *action potential*—a brief, all-or-none depolarization and repolarization of the membrane potential (Figure 2-6). Such substantial, rapid depolarization will often stimulate a neuron to release neurotransmitters and thus convey information to other cells through chemical signals; for example, a signal conveyed to muscle cells may cause the contraction of a muscle, and a signal conveyed to an internal organ may stimulate or attenuate its activity.

How Neurons Maintain Electrical Potential

Two characteristics of nerve cells contribute to their ability to maintain an electrical potential. First, different types of ions are unequally distributed across the neuronal cell membrane. Generally, the neuron's interior contains a higher concentration of K^+ and

lower concentrations of Na^+, Ca^{2+}, and Cl^- than does the extracellular space. These ionic gradients, which occur not only in neurons but also in most cell types,[1] are maintained through ion–specific pumps that require energy. Moreover, the neuron's interior contains many negatively charged, membrane–impermeable proteins that do not exist in the extracellular milieu.

Second, the neuronal cell membrane is differentially permeable to ions. In the resting state, most neurons are highly permeable to K^+, somewhat permeable to Cl^-, and very slightly permeable to Na^+ and Ca^{2+}. Because the cell membrane is a lipid bilayer, it would be completely impermeable to ions if it did not contain specialized proteins for ion transit. Ions strongly prefer interaction with the polar water molecules in intracellular and extracellular spaces to interaction with the hydrophobic lipid groups that comprise the bulk of the cell membrane. The selective permeability of the cell membrane depends on the numbers and states of its various ion channels. These neuronal properties—unequal permeability of ions across the cell membrane and unequal distribution of ions—are theoretically sufficient to maintain an electrical potential in a cell.

A Simple Cell Model

Consider a very simple model of a cell. The interior of the cell (*I*) contains 100 mM K^+A^-, where A^- is an impermeant anion such as a negatively charged protein. Exterior to the cell (*O*), the concentration of K^+A^- is 5 mM (Figure 2-7). To simplify this model, the effects of osmosis are ignored.

If the cell's lipid bilayer is impermeable to both K^+ and A^-, no movement of ions occurs across the bilayer, and the concentration of ions on each side of the bilayer remains constant. However, if the lipid bilayer of this cell became permeable to K^+, and only to K^+—for example, from the opening of K^+–specific ion channels in the lipid bilayer—K^+ ions but not A^- ions would be free to move across the lipid bilayer in both directions. Because *I* contains 20 times more K^+ than *O*, many more K^+ ions would be likely to travel from *I* to *O* than from *O* to *I*. Consequently, a net efflux of K^+ from *I* to *O* would occur; because A^- would remain impermeable, it would not cross the membrane.

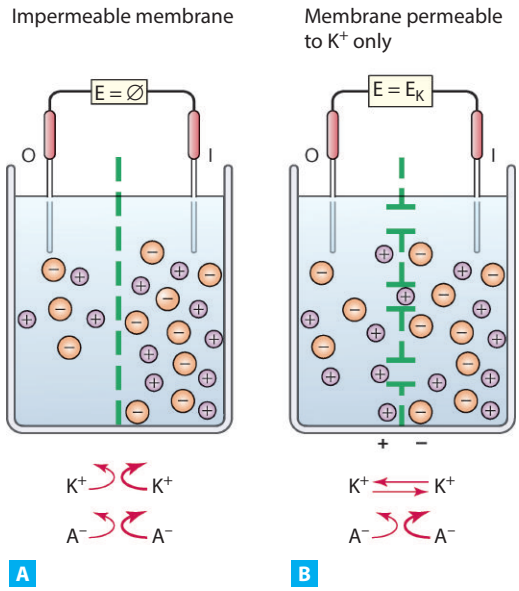


Figure 2-7 A cell model. A. If an impermeable membrane is placed between two compartments that contain different concentrations of the ionic solution K^+A^-, no movement of electrical charge can occur and the difference in potential between the two compartments (E) is zero. **B.** If the membrane becomes permeable to K^+, K^+ initially flows down its concentration gradient from *I* to *O*, creating an electrical potential that opposes the movement of K^+. The net movement of K^+ between *I* and *O* stops when the electrical force repelling K^+ equals the force of the concentration gradient; at this point K^+ has reached its equilibrium potential (E_K), which can be estimated with the Nernst equation.

As soon as K^+ ions begin to move to *O*, however, a net positive charge develops in *O* because K^+ has left the cell without accompanying A^-. This net positive charge *repels* K^+ from *O*. Thus, two tendencies act in opposition to one another: (1) the tendency of K^+ to move out of *I* because more K^+ is present in *I* than in *O* and (2) the tendency of K^+ to be drawn into *I* because of its relative negative potential.

Eventually, these two tendencies reach an equilibrium whereby the net efflux of K^+ from *I* (favored by the concentration gradient) is equal to the net efflux of K^+ from *O* (promoted by the electrical potential). Only a minuscule fraction of K^+ must venture from *I* to *O* to create an electrical potential strong enough to balance the tendency of K^+ to leave *I* along its concentration gradient. As a consequence, a minuscule fraction of the A^- ions in *I* exists without

[1]Evolutionarily, the maintenance of ionic gradients may have developed from a drive to maintain an extracellular environment similar to that of seawater, where it is speculated that life began. Although higher in osmolarity than mammalian extracellular fluid, seawater possesses a similar K^+ to Na^+ to Cl^- ratio.

accompanying K^+ ions, and this tiny separation of charge creates a negative potential of −75 mV in *I* relative to *O*. This negative charge within the cell exerts just enough force on the K^+ ions that the net flow of K^+ ions across the membrane is zero, despite the existing concentration gradient of K^+. The transmembrane electrical potential at which this equilibrium occurs (−75 mV) can be determined by the Nernst equation[2] and is termed the *equilibrium potential* (or the *Nernst potential*) for K^+.

A More Complicated Cell Model

A basic understanding of membrane potential drawn from the previous cell model can assist in understanding a model that more closely resembles a mammalian nerve cell. It involves: (1) a more complete complement of extracellular and intracellular ions and (2) more realistic ionic permeabilities. Table 2-2 describes several features of this cell model. First, the net total charge on each side of the cell is zero, a condition that is necessary for electrical neutrality.[3] Second, the cell membrane is far more permeable to K^+ than to any other ion, which is the case for most neurons at rest. All other permeabilities are described relative to the permeability of K^+.

Table 2-2 Idealized Free Ion Concentrations Inside and Outside a Nerve Cell

Ion	Concentration Inside Cell (mM)	Concentration Outside Cell (mM)	Relative Permeability
K^+	100	5	1
Na^+	10	100	0.01
Cl^-	10	105	0.2
A– (large anions)	100	0	0

What is the electrical potential at which the net flux of charge across the cell membrane equals zero? If the cell were *only* permeable to K^+, the membrane potential would rest at the equilibrium potential of K^+, because all other ions would be trapped on one side of the cell and could not migrate to contribute to the generation of an electrical potential. However, the cell *is* permeable to other ions, although to a much lesser degree than it is to K^+. If the membrane were permeable *only* to Na^+, the resting potential would be at the equilibrium potential of Na^+. In this case, the cell interior would develop a *positive* potential relative to the exterior of the cell. The positive potential in the cell interior would repel Na^+ ions and balance the statistical tendency of these ions to flow down their concentration gradient and into the cell.

The true equilibrium potential for this cell model would be expected to lie somewhere between the equilibrium potentials for the various ions involved. The membrane permeability for each type of ion determines its relative contribution to the equilibrium potential. An equilibrium potential of −68 mV for a hypothetical cell lies between the equilibrium potentials estimated for K^+, Na^+, and Cl^-.[4] Because the membrane's permeability to K^+ is so much greater than its permeability to the other ions, the neuron's resulting membrane potential is much closer to the equilibrium potential of K^+ (−75 mV) than to that of Na^+ (+58 mV) or Cl^- (−59 mV).

Many electrophysiologic concepts and the roles of ion channels and ion channel–targeting drugs in physiologic processes can be understood intuitively by understanding the change in membrane potential caused by changing the permeability of the membrane to different types of ions via the opening and closing of ion channels. When only one type of ion channel opens, it drives the membrane potential of the cell toward the equilibrium potential of that ion. For example, if many of the Na^+ channels in this cell model were suddenly opened, causing Na^+ to be three times more permeable than K^+, the membrane potential would move toward the equilibrium potential for Na^+. If the Na^+ channels were to close suddenly, bringing the permeability ratio of Na^+ to K^+ back to its original value of 0.01, the membrane potential would return to −68 mV in response to the efflux of K^+ through the many open K^+ channels.

[2]The Nernst equation is $E_m = 58 \log(K_o/K_i)$, where E_m is the equilibrium membrane potential, K_o is the concentration of K^+ ions on the outside, and K_i is the concentration of K^+ ions on the inside. The factor of 58 in this equation derives from several chemical values (eg, gas constant, temperature) as well as the valence of each ion and the conversion of natural logarithms to base 10 logarithms.

[3]Note that electrical neutrality cannot be achieved because a separation of charge persists across the cell membrane. It is also important to note that ionic concentrations in Table 2-2 are given in millimolar values, although a resting potential in a normal cell of −75 mV can be produced by a femtomolar net separation of charge. Moreover, both the cytoplasm of the cell and the extracellular milieu are electrically neutral, having an equal number of positive and negative charges; the difference in charge exists across the cell membrane, which acts as a capacitor.

[4] This can be calculated using the Goldman–Hodgkin–Katz equation. For details, see Hille (2001).

It also is important to emphasize that the equilibrium potential for a particular ion depends on the relative concentrations of that ion inside and outside the cell, which can vary considerably among different cell types and tissues. For example, the equilibrium potential for Cl^- can range from −60 to −90 mV.

Maintenance of Membrane Potential by ATP–Dependent Pumps

The equilibrium potential provides for an equal exchange of cations back and forth across the cell membrane: for every excess Na^+ ion that sneaks across the membrane, a K^+ ion moves out, holding the membrane stable at the predicted potential. This exchange occurs slowly enough that the ionic concentrations may be considered constant for short periods of time. However, if a slow exchange of Na^+ for K^+ were allowed to continue for hours or days, the concentration gradients would eventually degenerate and the membrane potential would slowly begin to dissipate. *Na^+–K^+ pumps* maintain the ionic gradients across a cell membrane by extruding Na^+ from and pumping K^+ into the cell against their respective concentration gradients at the cost of energy (Figure 2-8). Each pump is a multimeric integral membrane protein consisting of transmembrane α subunits which possess the catalytic and iontophoretic (ion pore–containing) domains of the pump, accessory transmembrane β subunits, which mediate the trafficking of the catalytic α subunit to the cell membrane, and tissue–specific regulatory subunits.

The catalytic subunit of each pump has extracellular binding sites for K^+ and intracellular binding sites for Na^+ and ATP. ATP transfers its terminal phosphate to the catalytic subunit in a Na^+–dependent manner; because the pump is a protein that cleaves ATP by means of ion–dependent enzymatic activity, it often is referred to as a *Na^+/K^+-ATPase*. At the expense of the energy of hydrolysis of ATP, typically three Na^+ ions are transported out of the cell and two extracellular K^+ ions are transported in. Thus, the pump is *electrogenic*: it exports more positive charge than it imports. The phosphorylated catalytic subunit is subsequently hydrolyzed in the presence of K^+ ions, returning the catalytic subunit to its resting state. The resting potential of a cell with active Na^+–K^+ pumps is usually a few

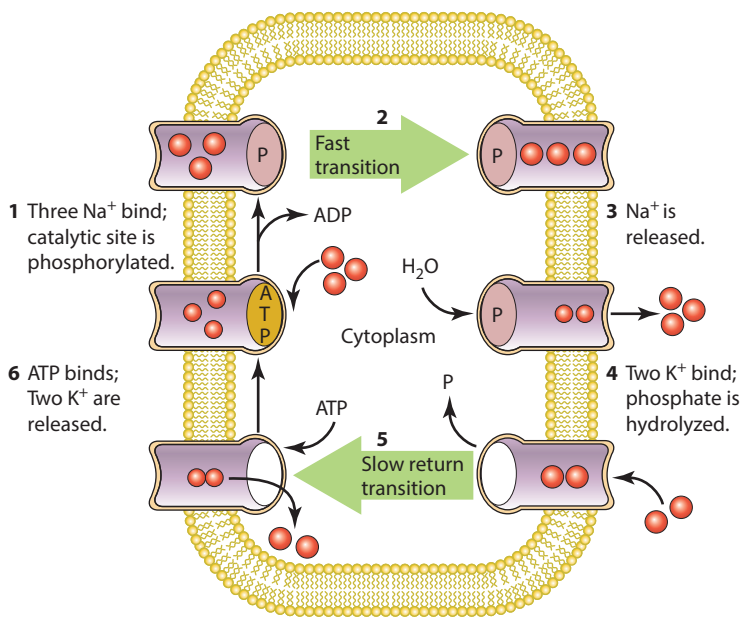
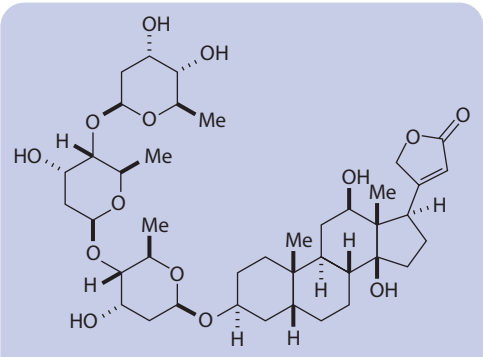


Figure 2-8 **The adenosine triphosphate (ATP)–dependent Na^+–K^+ pump.** The pump removes three Na^+ ions from the cell and introduces two K^+ ions. **1.** Three Na^+ ions bind to the interior face of the catalytic subunit, which subsequently is phosphorylated. **2.** Through a conformational transition Na^+ ions become less tightly bound to the pump and obtain access to the extracellular space. **3.** Na^+ ions dissociate from the pump. **4.** When two K^+ ions bind to the pump, it undergoes dephosphorylation. **5.** The pump changes conformation, providing the two bound K^+ ions with access to the cytoplasm. **6.** After ATP binds to the catalytic subunit, the two K^+ ions are released into the cytoplasm. ADP, adenosine diphosphate; P, phosphorylation.

millivolts more negative than would be predicted on the basis of ion distribution and relative permeabilities alone.

The importance of Na^+–K^+ pumps in the maintenance of cellular membrane potential becomes quite evident when they are inhibited by pharmacologic agents. **Cardiac glycosides** such as **ouabain** and **digoxin**, which increase the contractile force of cardiac muscle and are used in the treatment of **congestive heart failure** and some cardiac **dysrhythmias**, are the best known inhibitors of the Na^+–K^+ pump. Normally, cardiac myocytes maintain low levels of intracellular Ca^{2+}, partly through a Na^+–Ca^{2+} pump that uses energy from the movement of Na^+ down its concentration gradient to transport Ca^{2+} out of the cell. Because cardiac glycosides inhibit Na^+–K^+ pumps and increase intracellular Na^+ concentrations, they make Na^+–Ca^{2+} pumps less effective. Consequently, intracellular Ca^{2+} concentrations increase, which increases the contractile force of cardiac muscle. These drugs also can slowly reduce the resting potential of neurons eventually to zero; in large neurons, this decline in potential occurs after several hours. This action of the drugs in the CNS accounts for common side effects of cardiac glycosides, including disturbed vision, confusion, and delirium.



Digoxin

The brain contains many other types of transporters that control its extracellular contents. An *antiporter* is a type of active cotransport across a cell membrane where one molecule is transported down its electrochemical gradient, whereas the other is transported against its electrochemical gradient. This is in contrast to primary active transport processes, such as that mediated by the Na^+–K^+ pump, where both molecules are transported against their electrochemical gradients, a process powered by ATP. An example of an antiporter is the cystine–glutamate transporter (also known as xCT; SLC7A11), which regulates glutamate function at some excitatory synapses (Chapter 5).

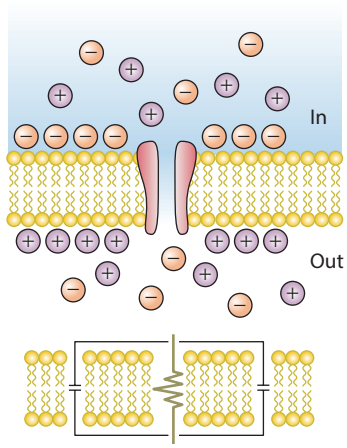
BIOPHYSICAL PROPERTIES OF THE CELL MEMBRANE

In the cell model described previously, it was possible to create a potential across the membrane by making it selectively permeable to the ions distributed unequally across it. It also was possible to alter the potential of the membrane by changing the relative permeabilities of various ions, for example, by increasing the permeability of Na^+ relative to K^+. Likewise, the membrane's original potential could be restored by reinstating the original permeabilities. In addition to these features, real neurons have two biophysical properties that affect the movement of charge and the development of potential across a neuronal membrane.

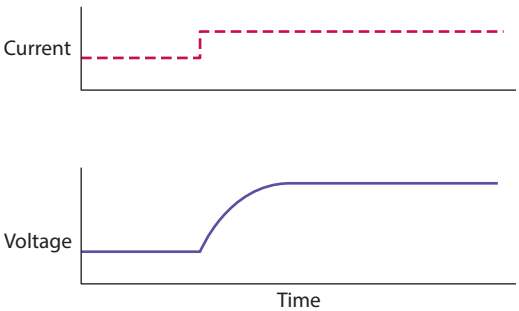
First, unlike the movement of charge in the cell model, charge is not transferred instantaneously from one neuronal compartment to another. Electrically, the membrane can be thought of as a *resistor* and a *capacitor* (Figure 2-9). Resistance describes a membrane's ability to pass ions, and it is determined by the number and properties of the ion channels, and the thickness of the membrane. Membranes with added insulation, such as myelinated axon membranes, are high resistors that are difficult to let ions through. Capacitance describes a membrane's ability to store charge; the larger the membrane's capacitance, the more charge is required to raise the membrane's potential. Several properties such as size and, most significantly, thickness can affect a membrane's capacitance. A very large membrane area requires more stored charge to bring it to a given potential, or, in other words, it has a greater capacitance, than does a small membrane area. Very thick membranes are poor capacitors: because ions separated across a greater distance possess greater potential energy, fewer ions are required to reach a certain membrane potential. Because a myelin sheath increases a membrane's thickness, a myelinated axonal membrane is a high resistor and poor capacitor compared with a nonmyelinated membrane.

Sensory, Synaptic, and Action Potentials

Neurons exploit their ability to rapidly change their transmembrane potentials in order to receive information from the environment and to relay messages. Input from a variety of sources, including other neurons, can cause a neuron's membrane potential to fluctuate. If a neuron depolarizes enough or reaches threshold, it produces an *action potential*: a rapid, all-or-none depolarization that propagates down its axon. The firing of an axon generally leads to the release of neurotransmitter from the axon's terminals, which in



A



B

Figure 2-9 The cell as a resistor–capacitor circuit. **A.** The lipid bilayer of the cell's plasma membrane separates charge and thus can be represented as a simple electrical circuit comprising resistance and capacitance. The number and state of the various ion channels in a cell membrane determine that membrane's *resistance*, or the ease with which ions can cross the membrane. Cell membranes with many open ion channels have low resistance; cell membranes with only a few open ion channels have high resistance. The *capacitance* of a membrane, or its ability to store charge, is determined by factors such as the area and thickness of the membrane. **B.** The opening of ion channels and the subsequent flow of current do not result in an instantaneous change in membrane potential. High–resistance and high–capacitance membranes require more time to develop a charge than low–resistance and low–capacitance membranes.

turn conveys the neuron's signal to muscle cells, to effector organs such as glands, or to other neurons.

Receiving information Some neurons are equipped with specialized systems that enable them to receive information from the environment. Hair cells in the cochlea, for example, are sensitive to vibrational energy: vibrations cause the movement of tiny cilia on the surface of these cells. Such movement activates mechanosensitive ion channels that increase the membrane's permeability to Na^+ that in turn leads to depolarization of the hair cell. Photoreceptor cells in the retina can respond to light because photons activate a series of chemical reactions that cause changes in the ionic permeability of the cell membrane, which in turn leads to changes in the membrane potential of the cell.

Neurons generally receive signals from other neurons through chemical neurotransmitters. They typically release neurotransmitter at synapses where it binds to receptors on an adjacent neuron's cell membrane. The binding of neurotransmitter to a receptor routinely leads to changes in the receiving neuron's ion permeability. This change in permeability may occur directly; many neurotransmitter receptors are ligand–gated ion channels. However, changes in permeability also take place indirectly; many types of neurotransmitter receptors activate second messenger systems within a cell that in turn modify ion channels, causing changes in membrane potential (Chapters 3 and 4).

Integrating information The opening of ion channels in a localized area, such as a synapse, produces a transmembrane current that changes the membrane potential of a cell. However, this change in potential does not occur instantaneously or remain localized to the narrow region of membrane in which the current was generated. The integration of local changes in the transmembrane voltage of a neuron is affected by two basic types of summation: *spatial summation* and *temporal summation* (Figure 2-10).

Neurons are "decision makers"—they must continually decide whether to respond to particular sets of stimuli by firing action potentials and in turn communicate with fellow neurons or with effector organs. A motor neuron in the spinal cord, for example, receives thousands of excitatory and inhibitory inputs from pathways that descend from the brain. These descending pathways deliver enormous amounts of information integrated from many areas of the brain, including motor planning, vestibular, and visual centers. The currents produced by excitatory and inhibitory inputs continually undergo summation to produce membrane potentials that fluctuate in time and space. A neuron reads fluctuating potentials at the base of its axon, the *axon hillock*, where it has a high concentration of voltage–dependent Na^+ channels. If the sum of these potentials produces a sufficient level of depolarization, an action potential is triggered.