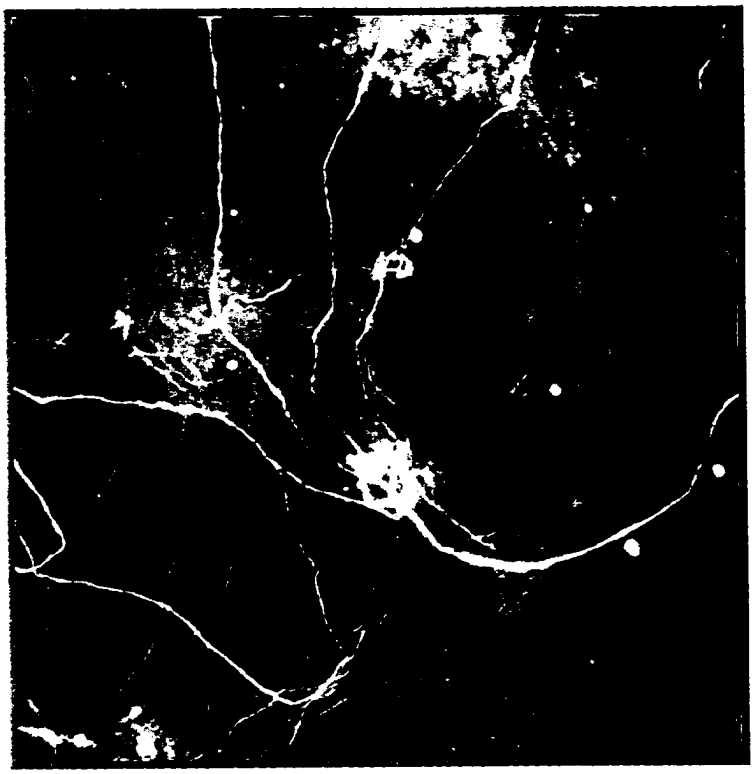
Cells



Cultured rat brain nerve cells with orange/green-fluorescing axons.

The nervous system regulates all aspects of bodily function and is staggering in its complexity. Millions of specialized *neurons* (nerve cells) sense features of both the external and internal environments and transmit this information to other nerve cells for processing and storage. Millions of other neurons regulate the contraction of muscles and the secretion of hormones. The human brain—the control center that stores, computes, integrates, and transmits information—contains about 10^{12} neurons, each forming as many as a thousand connections with other neurons. The nervous system also contains neuroglial (glial) cells that fill the spaces between neurons, nourishing them and modulating their function.

Despite the complexity of the nervous system as a whole, the structure and function of individual nerve cells is understood in great detail, perhaps in more detail than for any other type of cell. The function of a neuron is to communicate information, which it does by two methods: *Electric signals* process and transmit information within a cell. *Chemical signals* transmit information between cells, utilizing processes similar to those employed by other types of cells to signal each other (Chapter 20). Information from the environment creates special problems because of the diverse types of signals that must be sensed —light, touch, pressure, sound, odorants, the stretching of muscles. *Sensory neurons* have specialized receptors that convert these stimuli into electric signals. These electric signals are then converted into chemical signals that are passed on to other cells—called *interneurons*—that convert the information back into electric signals. Ultimately the information is transmitted to muscle-stimulating *motor neurons* or to other neurons that stimulate other types of cells, such as glands. Thus the output of a nervous system is the result of its circuit properties, that is, the wiring, or interconnections, between neurons, and the strength of

these interconnections. At times, the properties of a nervous system must change, for example in the development of new memories. Such changes can be explained as alterations in the number and nature of the interconnections between individual neurons.

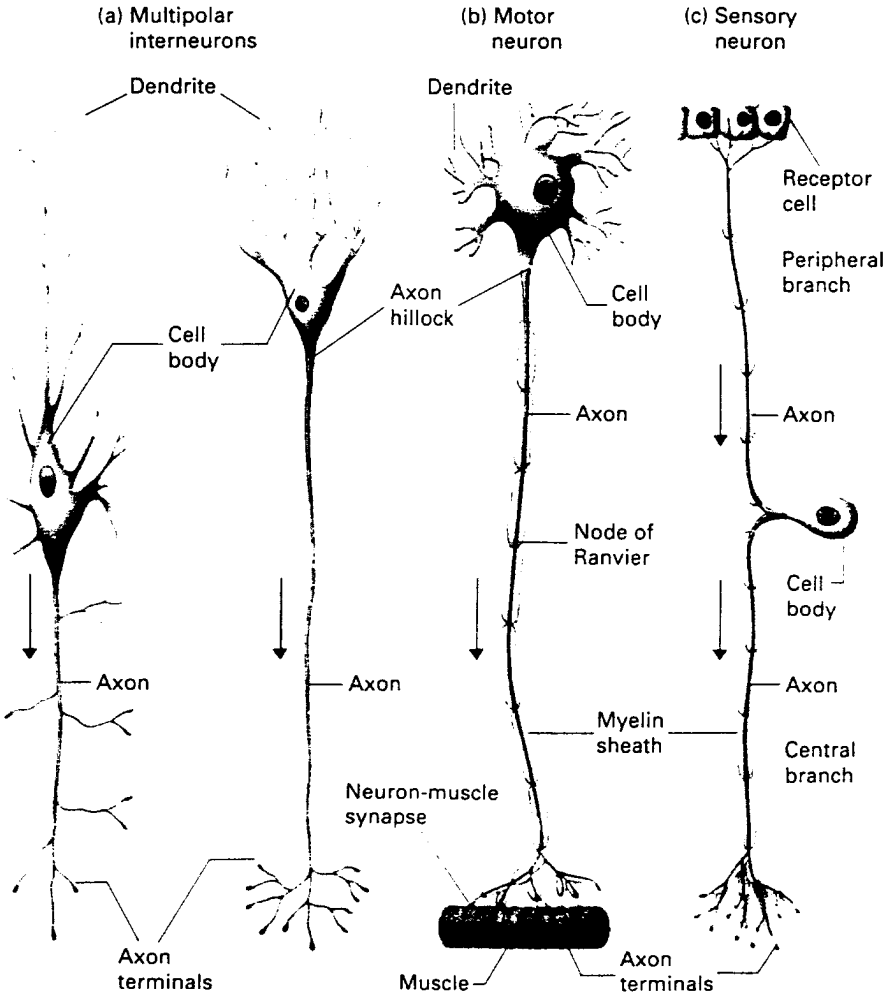
In this chapter we focus on how individual nerve cells function and how small groups of cells function together. A great deal of information has been gleaned from simple nervous systems. Squids, sea slugs, and nematodes have large neurons that are relatively easy to identify and manipulate experimentally. Moreover, in these species, only a few identifiable neurons may be involved in a specific task; thus their function can be studied in some detail. Because the principles studied are basic, the findings are applicable to complex nervous systems including that of humans. Indeed, current techniques make it possible to study the role of individual cell types and even of specific proteins in complex functions such as learning in the mammalian brain.

➤ *Neurons, Synapses, and Nerve Circuits*

Since the neuron is the fundamental unit of all nervous systems, we introduce in this section the structural features that are unique to these cells, and the types of electrical signals that they use to process and transmit information. We then introduce *synapses*, the specialized sites where neurons send and receive information from other cells and some of the circuits that allow groups of neurons to coordinate complex processes. Each of these topics will be covered in more detail in later sections of this chapter.

Specialized Regions of Neurons Carry Out Different Functions

Most neurons contain four distinct regions with differing functions: the *cell body*, the *dendrites*, the *axon*, and the *axon terminals* (Figure 21-1).

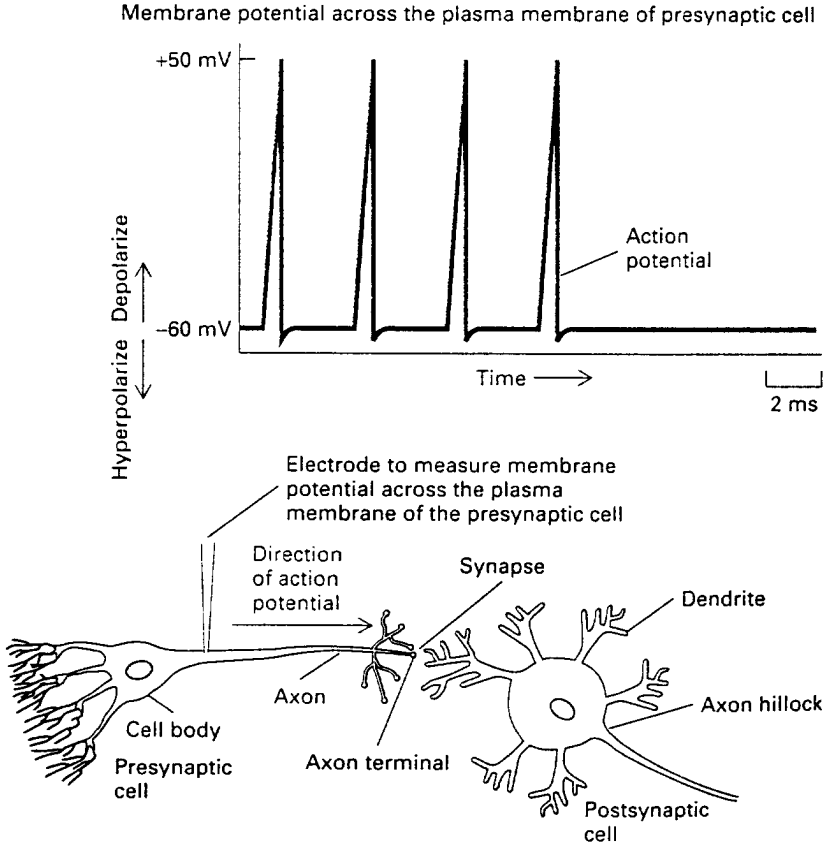


◄ FIGURE 21-1 Structure of typical mammalian neurons. Arrows indicate the direction of conduction of action potentials in axons (red). (a) Multipolar interneurons. Each has profusely branched dendrites (which receive signals at synapses with several hundred other neurons) and a single long axon that branches laterally and at its terminus. (b) A motor neuron that innervates a muscle cell. Typically, motor neurons have a single long axon extending from the cell body to the effector cell. In mammalian motor neurons an insulating sheath of myelin usually covers all parts of the axon except at the nodes of Ranvier and the axon terminals. (c) A sensory neuron in which the axon branches just after it leaves the cell body. The peripheral branch carries the nerve impulse from the receptor cell to the cell body, which is located in the dorsal root ganglion near the spinal cord; the central branch carries the impulse from the cell body to the spinal cord or brain. Both branches are structurally and functionally axons, except at their terminal portions, even though the peripheral branch conducts impulses toward rather than away from, the cell body.

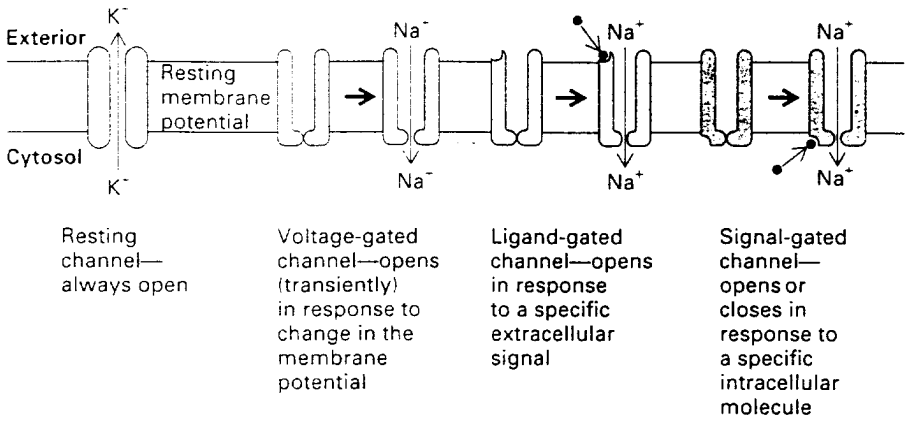
The cell body contains the nucleus and lysosomes, and is where virtually all neuronal proteins and membranes are synthesized. Some proteins are synthesized in dendrites, but axons and axon terminals do not contain ribosomes and no proteins are synthesized there. Proteins and membranes that are required for renewal of the axon and nerve termini are synthesized in the cell body and assembled there into membranous vesicles or multiprotein particles. These are transported along microtubules down the length of the axon—the process called *orthograde axoplasmic transport* (Chapter 23)—to the terminals, where they are inserted into the plasma membrane or other organelles. Axonal microtubules also are the tracks along which damaged membranes and organelles move up the axon toward the cell body, where they are degraded in lysosomes; this process is called *retrograde transport.*

Most neurons have a single axon, whose diameter varies from a micrometer in certain nerves of the human brain to a millimeter in the giant fiber of the squid. Axons are specialized for the conduction of a particular type of electric impulse, called an *action potential,* away from the cell body. An action potential is a series of sudden changes in the electric potential across the plasma membrane (Figure 21-2). In the resting (or nonstimulated) state the potential is ≈ -60 mV (the inside is negative relative to the outside), which is similar to the potential in most nonneuronal cells (see Figure 15-9). At the peak of an action potential the membrane potential can be as much as +50 mV (inside positive), a net change of ≈ 110 mV. This *depolarization* of the membrane is followed by a rapid *repolarization,* or return to the resting potential. These characteristics distinguish an action potential from other types of changes in electric potential across the plasma membrane and allow an action potential to move along an axon without diminution. These and other changes in membrane potential are generated and propagated by the opening and closing of specific ion channel proteins in the neuron plasma membrane, many of which have been cloned and characterized in great detail (Figure 21-3).

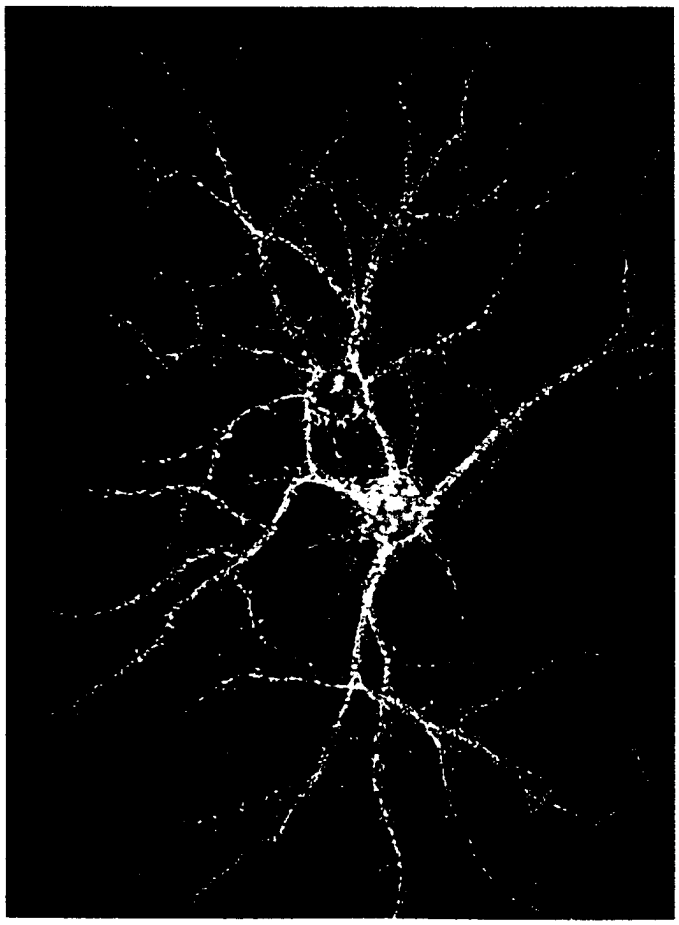
Action potentials move rapidly, at speeds up to 100 meters per second. In humans, axons may be more than a meter long, yet it takes only a few milliseconds for an action potential to move along their length. An action potential originates at the *axon hillock,* the junction of the axon and cell body, and is actively conducted down the axon into the axon terminals, small branches of the axon that form the synapses, or connections with other cells. At synapses signals are passed to other neurons, to a muscle cell at a neuromuscular junction, or to any of various other types of cells. A single axon in the central nervous system can synapse with many neurons and induce responses in all of them simultaneously.



➤ FIGURE 21-2 Action potentials and synaptic transmission in neurons. The membrane potential across the plasma membrane of the presynaptic neuron is measured by a small electrode inserted into it. In this example the neuron is "firing" about every 4 milliseconds, or generating about 250 action potentials a second; the action potentials move down the axon at speeds up to 100 meters per second. Arrival of an action potential at a synapse causes release of neurotransmitters that bind to receptors in the postsynaptic cell, generally inducing an action potential in it. Typical neurons have many axon termini; only one is shown here.



▲ FIGURE 21-3 The types of ion channels in neuron plasma membranes. Each type of channel protein allows movement only of certain ions. Resting channels are open in unstimulated cells and are responsible for generating the resting membrane potential across the membrane. Voltage-gated channels open, generally only for a fraction of a second, in response to a change in membrane potential; they are responsible for propagation of the electric signals called action potentials along the membrane. Two types of channels that open (or close) in response to chemical signals are responsible for generating electric signals. Ligand-gated channels open in response to a specific extracellular signaling molecule. Some signal-gated channels open (or close) in response to changes in the concentration of specific intracellular "second messenger" molecules, such as Ca^{2+} or cyclic GMP; others open or close in response to a G_α or $G_{\beta\gamma}$ subunit of a transducing G protein that is activated by a cell-surface receptor.



Most neurons have multiple dendrites, which extend outward from the cell body and are specialized to receive chemical signals from the axon termini of other neurons. Dendrites convert these signals into small electric impulses and transmit them to the cell body. Neuronal cell bodies can also form synapses and thus receive signals, as can be seen in the cell depicted in Figure 21-4. Particularly in the central nervous system, neurons have extremely long dendrites with complex branches. This allows them to form synapses with and receive signals from a large number of other neurons, perhaps up to a thousand. Electric disturbances generated in the dendrites or cell body spread to the axon hillock. If the electric disturbance there is great enough, an action potential will originate and will be actively conducted down the axon.

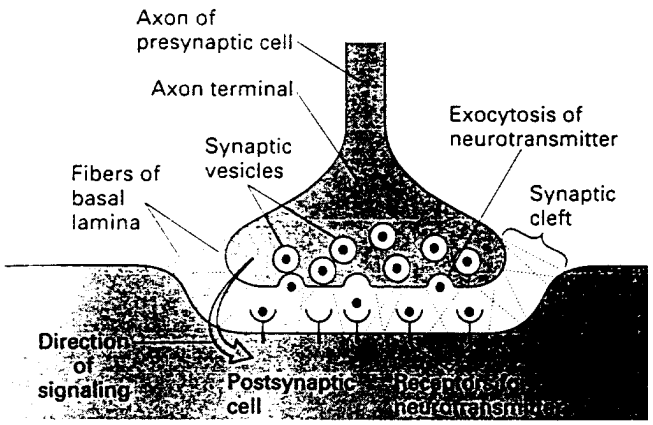
FIGURE 21-4 A typical interneuron from the hippocampal region of the brain makes about a thousand synapses. The dendrites and cell body of this cultured neuron fluoresce green because the cell was stained with a fluorescent antibody specific for the microtubule-associated protein MAP2 (see Figure 23-17 and Table 23-1) found only in dendrites and cell bodies. The synapses are stained orange-red by a second fluorescent antibody specific for synaptotagmin, a protein found in presynaptic axon terminals (see Figure 21-37). Thus the orange-red dots indicate presynaptic axon terminals from neurons that are not visible in this field. [Photograph courtesy of O. Mundigl and P. deCamilli.]

Synapses Are Specialized Sites Where Neurons Communicate with Other Cells

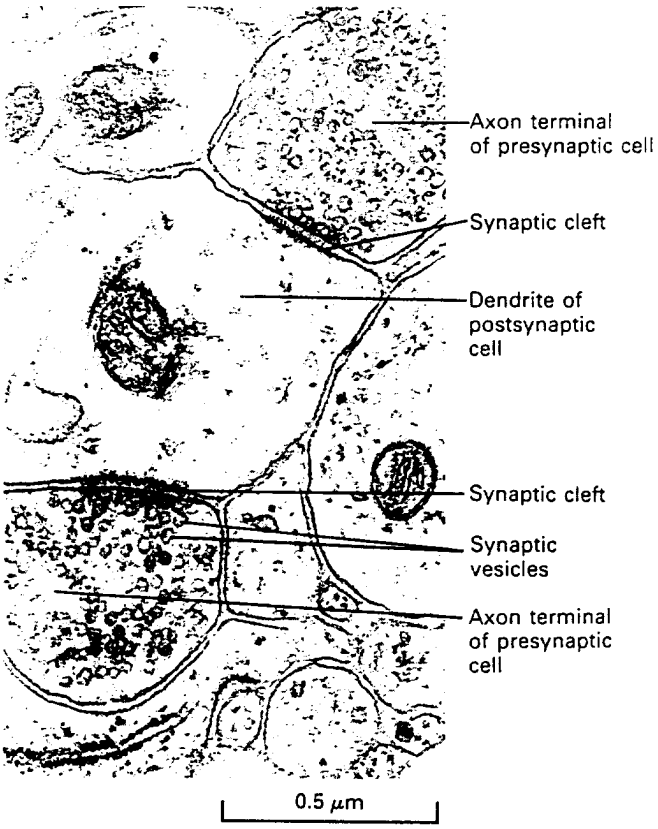
Synapses generally conduct signals in only one direction. An axon terminal from the presynaptic cell sends signals that are picked up by the postsynaptic cell. There are two types of synapses, *electric* and *chemical*, which differ in both structure and function. Chemical synapses are much more common than electric synapses.

In a chemical synapse (Figure 21-5a), the axon terminal of the presynaptic cell contains vesicles filled with a particular neurotransmitter substance (Figure 21-5b), such as epinephrine or acetylcholine. The postsynaptic cell can be a dendrite or cell body of another neuron, a muscle or gland cell, or, rarely, even another axon. When the postsynaptic cell is a muscle cell, the synapse is called a *neuromuscular junction* or *motor end plate*. When an action potential in the presynaptic cell reaches an axon terminal,

(a) Chemical synapse

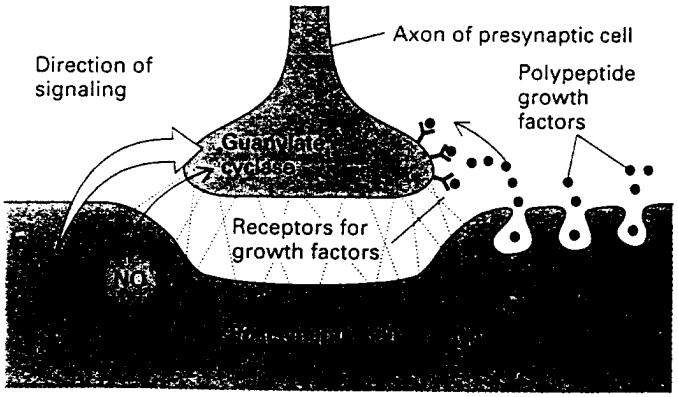


(b) Ultrastructure of chemical synapse



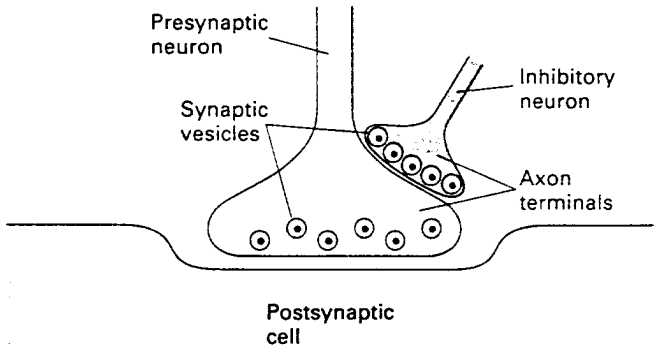
◀ FIGURE 21-5 Chemical synapses (a) A narrow region—the synaptic cleft—separates the plasma membranes of the presynaptic and postsynaptic cells. Transmission of electric impulses requires release of a neurotransmitter by the presynaptic cell, its diffusion across the synaptic cleft, and its binding by specific receptors on the plasma membrane of the postsynaptic cell. (b) Electron micrograph showing a cross section of a dendrite synapsing with two axon terminals filled with synaptic vesicles. In the synaptic region, the plasma membrane of the presynaptic cell is specialized for vesicle exocytosis; synaptic vesicles, which contain a neurotransmitter, are clustered in these regions. The opposing membrane of the postsynaptic cell (in this case, a neuron) contains receptors for the neurotransmitter. (c) In retrograde signaling, a signal is sent from the postsynaptic to the presynaptic cell. The signal can be a gas, such as nitric oxide or carbon monoxide, or a polypeptide growth factor. Retrograde signals can alter the ability of the presynaptic cell to release neurotransmitter, and are thought to be important in learning. [Part (b) from C. Raine, G. J. Siegel et al., eds., 1981, *Basic Neurochemistry*, 3d ed., Little, Brown, p. 32.]

(c) Retrograde signaling



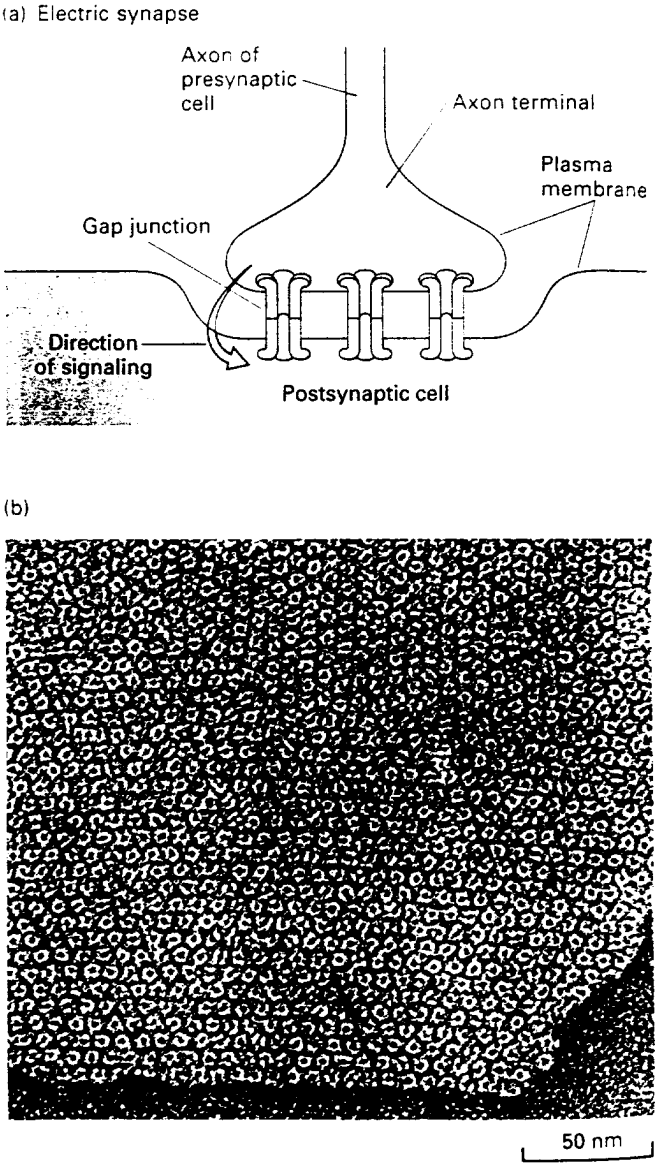
some of the vesicles fuse with the plasma membrane, releasing their contents into the synaptic cleft, the narrow space between the cells. The neurotransmitter diffuses across the synaptic cleft and, after a lag period of about 0.5 millisecond (ms), binds to receptors on the postsynaptic cells. The bound neurotransmitter changes the ion permeability of the postsynaptic plasma membrane, which, in turn, changes the membrane's electric potential at this point. If the postsynaptic cell is a neuron, this electric disturbance may be sufficient to induce an action potential. If the postsynaptic cell is a muscle, the change in membrane potential following binding of the neurotransmitter may induce contraction; if a gland cell, the neurotransmitter may induce hormone secretion. In some cases, enzymes attached to the fibrous network connecting the cells destroy the neurotransmitter after it has functioned; in other cases, the signal is terminated when the neurotransmitter diffuses away or is transported back into the presynaptic cell.

In certain types of synapses, the postsynaptic neuron sends signals to the presynaptic one (Figure 21-5c). Such *retrograde* signals can be gases, such as nitric oxide (NO) and carbon monoxide, or peptide hormones. This type of signaling modifies the ability of the presynaptic cell to signal the postsynaptic one; it is thought to be important in many types of learning. Sometimes an axon terminal of one neuron will synapse with the axon terminal of another neuron (Figure 21-6). Such a synapse may either inhibit or stimulate the ability of an axon terminal to secrete the contents of its synaptic vesicles and signal to a postsynaptic cell. We shall see later how the molecular properties of such synapses permit certain types of learning.

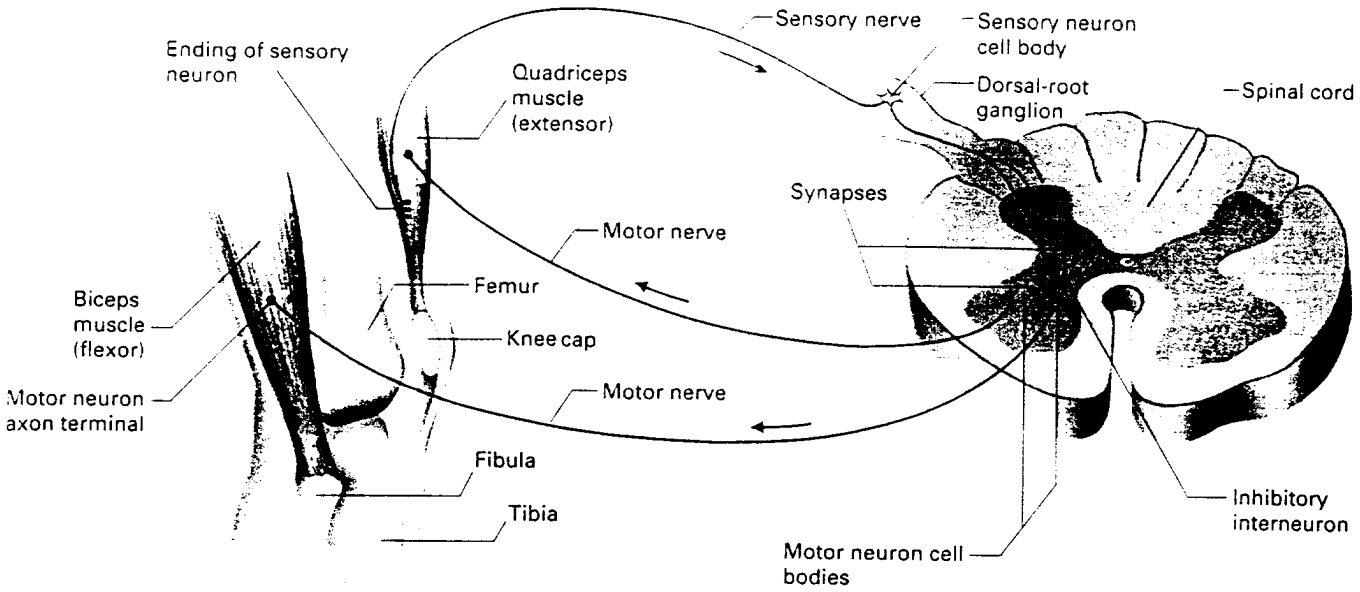


▲ FIGURE 21-6 A modulatory synapse between an inhibitory neuron and the axon terminus of a presynaptic neuron. Stimulation of the inhibitory neuron causes its axon terminal to release neurotransmitter, which reduces the presynaptic axon's ability to transmit a signal to the postsynaptic cell. This type of presynaptic inhibition does not affect the ability of the postsynaptic cell to respond to signals from other neurons.

Neurons communicating by an electric synapse are connected by *gap junctions* (Chapter 24) through which electric impulses can pass directly from the presynaptic cell to the postsynaptic one (Figure 21-7). Electric synapses allow a presynaptic cell to induce an action potential in the postsynaptic cell with greater certainty than chemical synapses and without a lag period.



▲ FIGURE 21-7 An electric synapse. The plasma membranes of the presynaptic and postsynaptic cells are linked by gap junctions; flow of ions through these channels allows electric impulses to be transmitted directly from one cell to the other. (b) Negatively stained, electron microscopic image of the cytosolic face of a region of plasma membrane enriched in gap junctions; each "doughnut" forms a channel connecting two cells. [Part (b) courtesy of N. Gilula.]



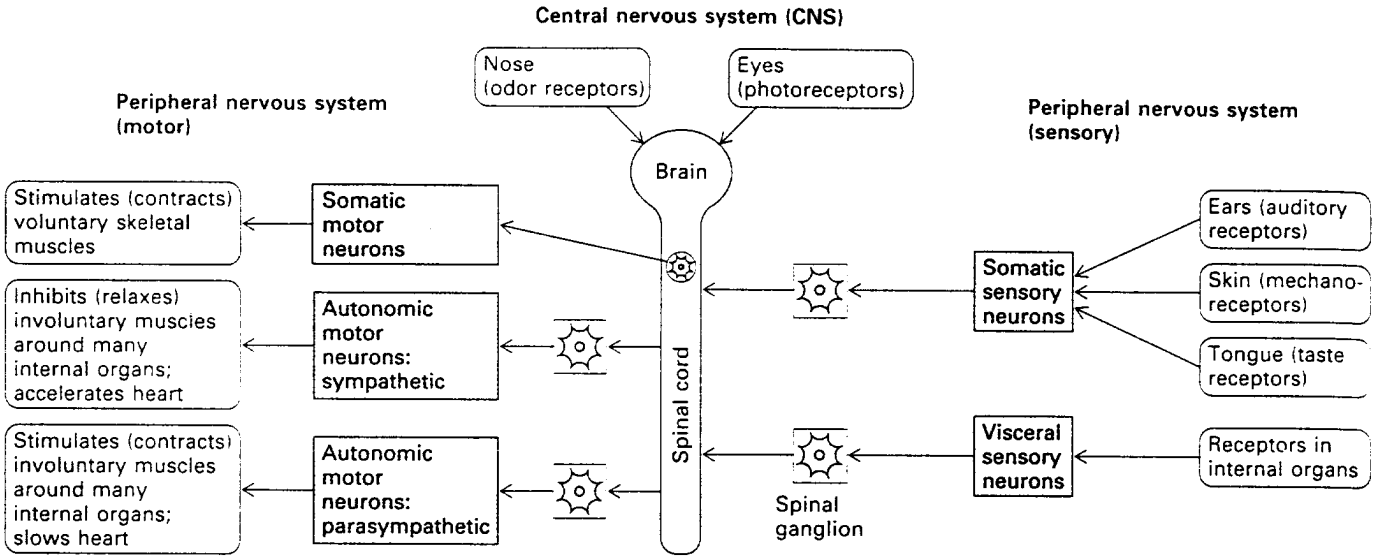
▲ FIGURE 21-8 The knee-jerk reflex arc in the human. Positioning and movement of the knee joint are accomplished by two muscles that have opposite actions: contraction of the quadriceps muscle straightens the leg, whereas contraction of the biceps muscle bends the leg. The knee-jerk response, a sudden extension of the leg, is stimulated by a blow just below the knee cap. The blow directly stimulates sensory neurons (blue) located in the tendon of the quadriceps muscle. The axon of each sensory neuron extends from the tendon to its cell body in a dorsal root ganglion. The sensory axon then continues to the spinal cord, where it branches and synapses with two neurons: (1) a motor neuron (red) that innervates the quadriceps muscle and (2) an inhibitory interneuron (black) that synapses with a motor neuron (red) innervating the biceps muscle. Stimulation of the sensory neuron causes a contraction of the quadriceps and, via the inhibitory neuron, a simultaneous inhibition of contraction of the biceps muscle. The net result is an extension of the leg at the knee joint. (Each cell illustrated here actually represents a nerve, that is, a population of neurons.)

Neurons Are Organized into Circuits

In complex multicellular animals, such as insects and mammals, signaling circuits consist of two or more neurons and, in some cases, highly specialized *sensory receptor cells*, which respond to specific environmental stimuli such as light, heat, stretching, pressure, and concentrations of many chemical substances. In the type of circuit called a reflex arc, interneurons connect multiple sensory and motor neurons, allowing one sensory neuron to affect multiple motor neurons and one motor neuron to be affected by multiple sensory neurons; in this way interneurons integrate and enhance reflexes. For example, the knee-jerk reflex in humans involves a complex reflex arc in which one muscle is stimulated to contract while another is inhibited from contracting (Figure 21-8). Such circuits allow an organism to respond to a sensory input by the coordinated action of sets of muscles that together achieve a single purpose.

The sensory and motor neurons of circuits such as the knee-jerk reflex are contained within the peripheral nervous system. These circuits send information to and receive information from the central nervous system, which comprises the brain and spinal cord (Figure 21-9) and is composed mainly of interneurons. The peripheral nervous system contains two broad classes of motor neurons. The *somatic motor neurons* stimulate voluntary muscles, such as those in the arms, legs, and neck; the cell bodies of these neurons are located inside the central nervous system, in either the brain or the spinal cord. The *autonomic motor neurons* innervate glands, heart muscle, and smooth muscles not under conscious control, such as the muscles that surround the intestine and other organs of the gastrointestinal tract. The two classes of autonomic motor neurons, sympathetic and parasympathetic, generally have opposite effects: one class stimulates the muscle or gland and the other inhibits it. Sensory neurons, which convey information to the central nervous system, have their cell bodies clustered in ganglia, masses of nerve tissue that lie just outside the spinal cord. The cell bodies of the motor neurons of the autonomic nervous system also lie in ganglia. Each peripheral nerve (Figure 21-10) is a bundle of axons; some are parts of motor neurons; others are parts of sensory neurons.

Having surveyed the general features of neuron structure, interactions, and circuits, let us turn to the mechanism by which a neuron generates and conducts electric impulses.



▲ FIGURE 21-9 A highly schematic diagram of the vertebrate nervous system. The central nervous system (CNS) comprises the brain and spinal cord. It receives direct sensory input from the eyes and nose. The peripheral nervous system (PNS) comprises three sets of neurons: (1) somatic and visceral sensory neurons, which relay information to the CNS from receptors in somatic and internal organs; (2) somatic motor neurons, which innervate voluntary skeletal muscles; and (3) autonomic motor neurons, which innervate the heart, the smooth involuntary muscles such as those surrounding the stomach and intestine, and glands such as the liver and pancreas. The sympathetic and parasympathetic autonomic motor neurons frequently cause opposite effects on internal organs. The cell bodies of somatic motor neurons are within the CNS; those of sensory neurons and of autonomic motor neurons are in ganglia adjacent to the CNS.

➤ *The Action Potential and Conduction of Electric Impulses*

We saw in Chapter 15 that an electric potential exists across the plasma membrane of all cells. The potential across the plasma membrane of large cells can be measured with a microelectrode inserted inside the cell and a reference electrode placed in the extracellular fluid. The two are connected to a voltmeter capable of measuring small potential differences (Figure 21-11). In virtually all cases the inside of the cell membrane is negative relative to the outside; typical membrane potentials are between −30 and −70 mV. The potential across the surface membrane of most animal cells generally does not vary with time. In contrast, neurons and muscle cells—the principal types of

◀ FIGURE 21-10 Freeze-fracture preparation of a rat sciatic nerve viewed in a scanning electron microscope. The axon of each neuron in the nerve is surrounded by a myelin sheath (MS) formed from the plasma membrane of a Schwann cell (SN). The axonal cytoplasm contains abundant filaments—mostly microtubules and intermediate filaments—that run longitudinally and serve to make the axon rigid. [From R. G. Kessel and R. H. Kardon, 1979, *Tissues and Organs: A Text-Atlas of Scanning Electron Microscopy*, W. H. Freeman and Company, p. 80.]

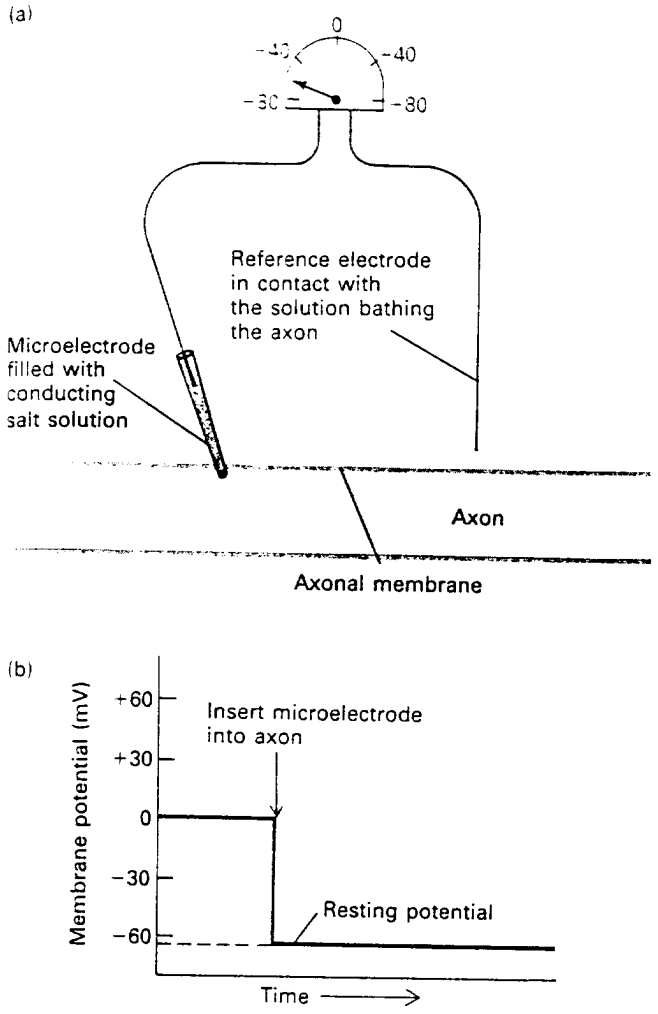
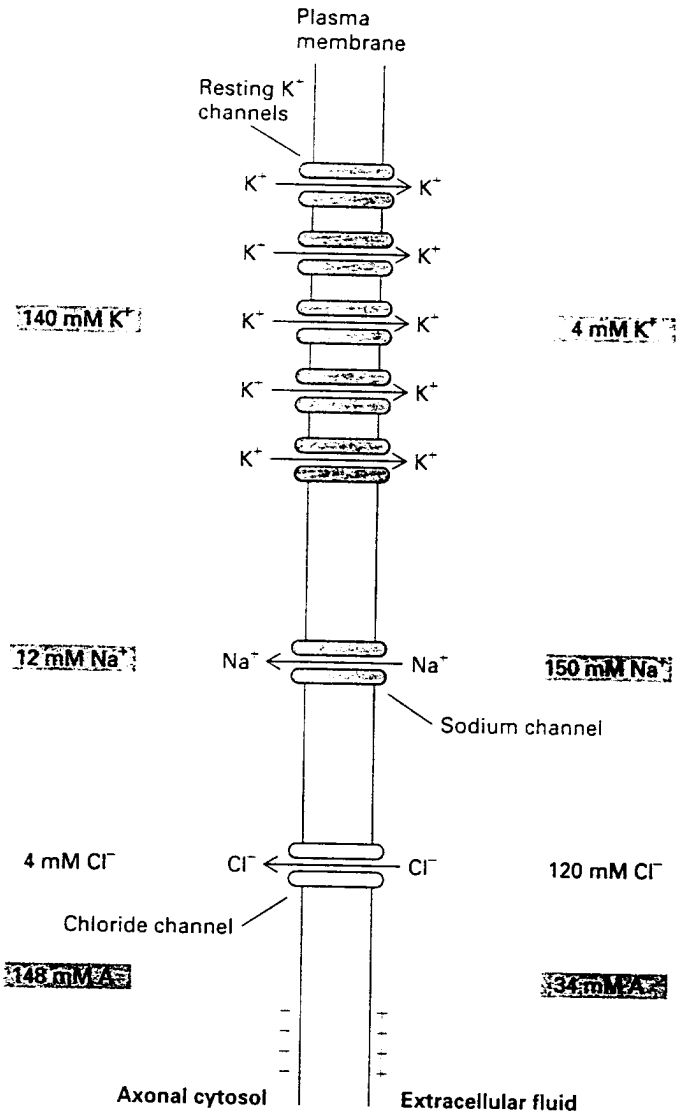


FIGURE 21-11 Measurement of the electric potential across an axonal membrane. (a) A microelectrode, constructed by filling a glass tube of extremely small diameter with a conducting fluid such as KCl, is inserted into an axon in such a way that the surface membrane seals itself around the electrode. A reference electrode is placed in the bathing medium. A potentiometer connecting the two electrodes registers the potential. The potential difference maintained across the cell membrane in the absence of stimulation is called the *resting potential*, in this case, −60 mV. (b) A potential difference is registered only when the microelectrode is inserted into the axon; no potential is registered if the microelectrode is in the bathing fluid.



▲ FIGURE 21-12 Origin of the resting potential in a typical vertebrate neuron. The ionic compositions of the cytosol and of the surrounding extracellular fluid are different. A^- represents negatively charged proteins, which neutralize the excess positive charges contributed by Na^+ and K^+ ions. In the resting neuron there are more open K^+ channels than Na^+ or Cl^- channels; as a consequence more positively charged K^+ ions exit the cell than Na^+ or Cl^- ions enter, and the outside of the plasma membrane acquires a net positive charge relative to the inside.

electrically active cells—undergo controlled changes in their membrane potential (Figure 21-2); they conduct action potentials along their membrane by sequentially opening and closing ion channels that are specific for Na^+ and K^+ ions (Figure 21-3). Thus, we need to explain how the opening and closing of ion channels and the resultant movement of small numbers of ions from one side of the membrane to the other causes changes in the membrane potential.

The Resting Potential Is Generated Mainly by Open Potassium Channels

The concentration of K^+ ions inside typical metazoan cells is about 10 times that in the extracellular fluid, whereas the concentrations of Na^+ and Cl^- ions are much higher outside the cell than inside (Figure 21-12); these concentration gradients are maintained by Na^+-K^+ ATPases with the expenditure of cellular energy (see Figure 15-13). Another

important property of the plasma membrane is that it contains open, so-called "resting", ion channels that allow passage only of Na^+, K^+ or Cl^-, the principal cellular ions. Since the number of open K^+ channels exceeds that of Na^+ or Cl^- channels, more K^+ ions than Na^+ or Cl^- move through the plasma membrane. Each type of ion moves down its concentration gradient. The resting potential—inside negative—is determined mainly by the concentration gradient of K^+ ions: movement of a K^+ ion across the membrane down its concentration gradient leaves an excess negative charge on the cytosolic face and deposits a positive one on the exoplasmic face.

To see this point quantitatively, imagine that the plasma membrane in the neuron depicted in Figure 21-12 has only K^+ channels and thus is impermeable to any other ion; we can use the Nernst equation (see equations 15-7 and 15-9) to determine the membrane potential:

$$E_K = \frac{RT}{Z\mathcal{F}} \ln \frac{K_o}{K_i} = 59 \log_{10} \frac{K_o}{K_i} = 59 \log_{10} \frac{4}{140} = -91 \text{ mV} \quad (21\text{-}1)$$

where E_K is the potassium equilibrium potential, K_o and K_i are the potassium concentrations outside and inside the cell, R is the gas constant, T is the absolute temperature, $\mathcal{F}$ is the Faraday constant, and Z is the valency of the ion (+1 for K^+). E_K is close to the typical resting potential of −60 mV. If in a resting cell one were to vary the extracellular concentration of K^+ and measure the resultant membrane potential, E would always be close to the calculated value of E_K; this is evidence that the resting potential is due mainly to movements of K^+ through open K^+ channels in the plasma membrane.

The actual situation in cells is complicated because there are also open Na^+ and Cl^- channels in the plasma membranes of the resting cell. Cells, of course, contain other ions, such as HPO_4^{2-}, Ca^{2+}, SO_4^{2-}, and Mg^{2+}, but there are few channels that admit these ions. Furthermore, the membrane potential of electrically active cells such as nerves and muscles is affected only by changes in the channels for K^+, Na^+, and Cl^- (and occasionally Ca^{2+}). Thus these three ions are the only ones we need consider here.

To calculate the membrane potential as a function of concentrations of different ions, it is useful to define a permeability constant P for each ion. P is a measure of the ease with which an ion can cross a unit area (1 cm^2) of membrane driven by a 1 M difference in concentration; it is proportional to the number of open ion channels and to the number of ions each channel can conduct per second (the channel conductivity). Thus P_K, P_{Na}, and P_{Cl} are measures of the numbers of K^+, Na^+, and Cl^- ions that move across a unit area of membrane per second. The units of permeability are expressed in centimeters per second (cm/s). Permeabilities are generally not measured directly; rather, both the number of open ion channels and the conductivity of each channel are measured by techniques we discuss later in the chapter.

The resultant membrane potential across a cell-surface membrane is given by a more complex version of the Nernst equation in which the concentrations of the ions are weighted in proportion to their permeability constants:

$$E_K = \frac{RT}{Z\mathcal{F}} \ln \frac{P_K K_o + P_{Na} Na_o + P_{Cl} Cl_i}{P_K K_i + P_{Na} Na_i + P_{Cl} Cl_o} = 59 \log_{10} \frac{P_K K_o + P_{Na} Na_o + P_{Cl} Cl_i}{P_K K_i + P_{Na} Na_i + P_{Cl} Cl_o} \quad (21\text{-}2)$$

where the "o" and "i" subscripts denote the ion concentrations outside and inside the cell. Because of their opposite charges (Z value in the Nernst equation), K_o and Na_o are placed in the numerator, but Cl_o is placed in the denominator; conversely, K_i and Na_i are in the denominator, but Cl_i is in the numerator. The membrane potential at any time and at any position in the neuron can be calculated with this equation if the relevant ion concentrations and permeabilities are known.

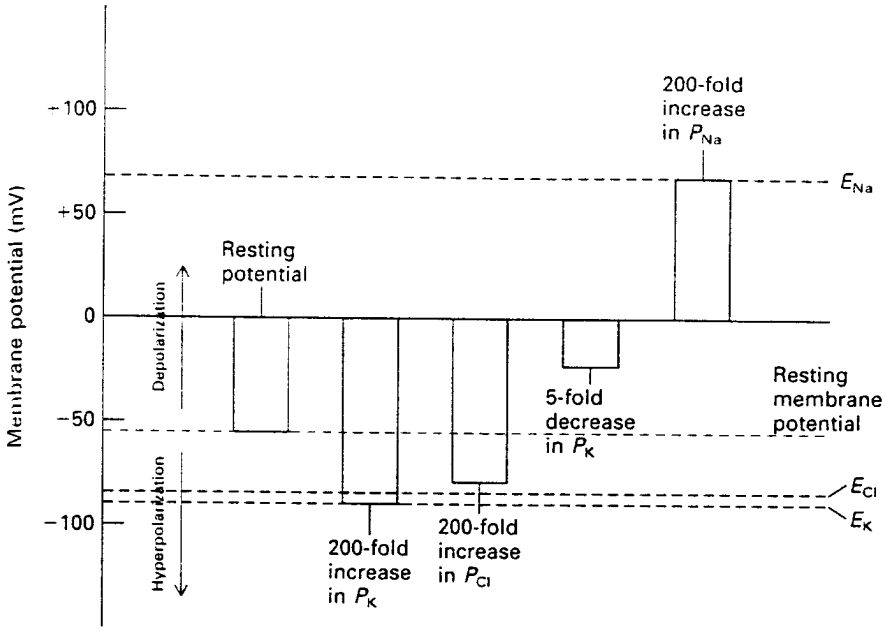
Note that if $P_{Na} = P_{Cl} = 0$, then the membrane is permeable only to K^+ ions and equation 22-2 reduces to equation 22-1. Similarly, if $P_K = P_{Cl} = 0$, then the membrane is permeable only to Na^+ ions and equation 21-2 reduces to the following:

$$E = \frac{RT}{Z\mathcal{F}} \ln \frac{Na_o}{Na_i} = E_{Na} \quad (21\text{-}3)$$

Let us apply equation 21-2 to resting neurons in which the ion concentrations are typically those shown in Figure 21-12. For typical resting neurons (and for most nonexcitable cells), the permeability of the membrane to K^+ ions is much greater than that for Na^+ or Cl^- ions; that is, there are more open channels for K^+ than for Na^+ or Cl^-. The resultant membrane potential is closer to E_K (−91.1 mV) than to E_{Na} (+64.7 mV); E_{Cl} −87.2 mV) is close to E_K. We can see this relationship by substituting into equation 21-2 typical values of the three permeability constants ($P_K = 10^{-7}$ cm/s, $P_{Na} = 10^{-8}$ cm/s, and $P_{Cl} = 10^{-8}$ cm/s) and of the ion concentrations:

$$E = 59 \log_{10} \frac{(10^{-7})(0.004) + (10^{-8})(0.15) + (10^{-8})(0.004)}{(10^{-7})(0.14) + (10^{-8})(0.012) + (10^{-8})(0.12)} = -52.9 \text{ mV}$$

The potential of −53 mV is close to, but less negative than, the equilibrium K^+ potential E_K. The potential is not equal to E_K because the membrane also contains open Na^+ channels; influx of Na^+ ions adds positive charges to the inside of the cell membrane, making the membrane potential more positive (or less negative).



◀ FIGURE 21-13 Effect of changes in ion permeability on membrane potential calculated with equation 21-2 using the permeability constants given in the text and the ion concentrations shown in Figure 21-12. The resting membrane potential is −53 mV; E_{Na}, E_K, and E_{Cl} are the potentials if the membrane contains only channels for Na^+ or K^+ or Cl^-, respectively.

The Opening and Closing of Ion Channels Cause Specific, Predictable Changes in the Membrane Potential

The membrane potential changes predictably if the membrane permeability of an ion changes (Figure 21-13):

1. *Opening of* K^+ *channels (increasing* P_K*) causes hyperpolarization of the membrane;* the membrane potential becomes more negative, approaching E_K. Intuitively, this occurs because more K^+ ions flow outward from the cytosol, leaving excess negative ions on the cytosolic surface of the membrane and putting more positive ones on the outer surface. Conversely, closing K^+, channels, decreasing P_K, causes depolarization of the membrane and a less negative potential.

2. *Opening of* Na^+ *channels (increasing* P_{Na}*) causes depolarization of the membrane;* if the increase is large enough, the potential can become positive inside, approaching E_{Na}. Intuitively, Na^+ ions tend to flow inward from the extracellular medium, leaving excess negative ions on the outer surface of the membrane and putting more positive ions on the cytosolic surface. Conversely, closing Na^+ channels, decreasing P_{Na}, causes membrane hyperpolarization, a more negative potential.

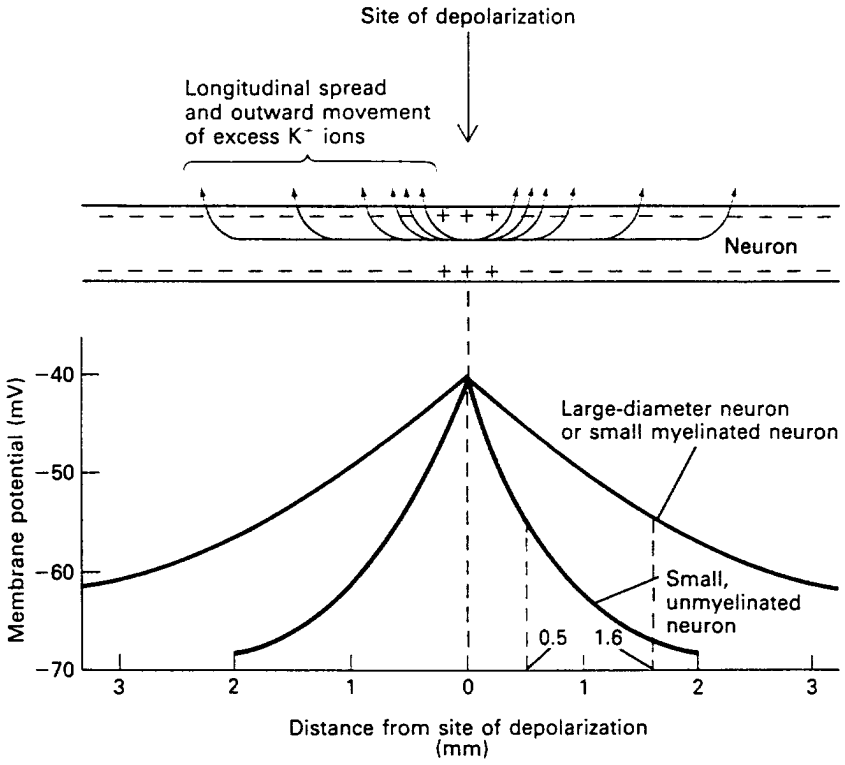
3. *Opening of* Cl^- *channels (increasing* P_{Cl}*) causes hyperpolarization of the membrane,* and the potential approaches E_{Cl}. Intuitively, Cl^- ions tend to flow inward from the extracellular medium, leaving excess positive ions on the outer surface of the membrane and putting more negative ions on the cytosolic surface. Conversely, closing Cl^- channels, decreasing P_{Cl} causes depolarization and a less negative potential.

Neurons, like all cells, contain "resting ion channels," channels that are open at the resting potential and that admit only one type of ion. As discussed, resting K^+ channels generate the resting potential of ≈−50 to −70 mV. Axons are specialized regions of the neuron that conduct action potentials-sequential depolarizations and repolarizations of the plasma membrane over long distances without diminution (see Figure 21-2). To do this they must have, besides the resting channels, other types of Na^- and K^+ channels, called *voltage-gated channels.* At the resting potential, voltage-gated channels are closed; no ions move through them. However, when the region of the plasma membrane becomes depolarized (see Figure 21-3) these channels open for a short period, and allow movement of one type of ion (e.g., Na^+ or K^+) through them. In order to appreciate the role of voltage-gated channels in conducting action potentials in one direction down an axon, let us examine how a plasma membrane with only resting ion channels would conduct an electric depolarization.

Membrane Depolarizations Would Spread Only Short Distances without Voltage-Gated Cation Channels

In its electric properties, a nerve cell with only resting ion channels resembles a long underwater telephone cable. It consists of an electrical insulator, the poorly-conducting cell membrane, separating two media—the cell cytosol and the extracellular fluid—that have a high conductivity for ions.

Suppose that a single microelectrode is inserted into the axon and that the electrode is connected to a source of electric current (e.g., a battery) such that the electric poten-



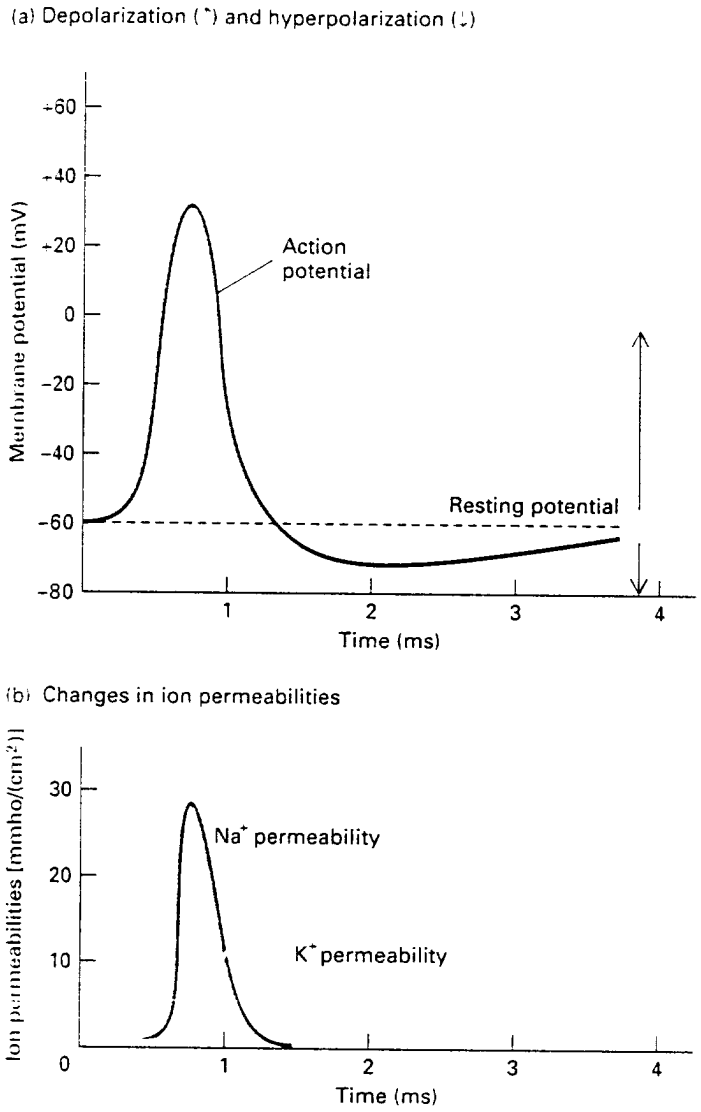
◀ FIGURE 21-14 Passive spread of a depolarization of a neuronal plasma membrane with only resting K^+ and Na^+ ion channels. The neuronal membrane is depolarized from −70 to −40 mV at a single point and clamped at this value. The voltage is then measured at various distances from this site. Because of the outward movement of K^+ ions through resting K^+ channels, the extent of depolarization falls off with distance from the initial depolarization. Passive spread occurs equally in both directions from the site of depolarization. The length constant is the distance over which the magnitude of the depolarization falls to a value of $1/e$ ($e = 2.718$) of the initial depolarization. The length constant for a small neuron with a large number of resting K^+ channels (black curve) can be as small as 0.1 mm; in this example it is about 0.5 mm. For a large axon, or one surrounded with a myelin sheath (Fig. 21-10) (blue curve), the length constant can be as large as 5 mm; in this example it is about 1.6 mm.

tial at that point is suddenly depolarized and maintained at this new voltage. At this site the inside of the membrane will have a relative excess of positive charges, principally K^+ ions. These ions will tend to move away from the initial depolarization site, thus depolarizing adjacent sections of the membrane. This is called the *passive spread of depolarization.* In contrast to an action potential, passive spread occurs equally in both directions. Also, the magnitude of the depolarization diminishes with distance from the site of initial depolarization, as some of the excess cations leak back across the membrane through resting cation channels (Figure 21-14). Only a small portion of the excess cations are carried longitudinally along the axon for long distances. The extent of this passive spread of depolarization is a function of two properties of the nerve cells: the permeability of the membrane to ions and the conductivity of the cytosol.

The passive spread of a depolarization is greater for neurons of large diameter; this is because the conductivity of the cytosol of a nerve cell depends on its cross-sectional area: the larger the area, the greater the number of ions there will be (per unit length of neuron) to conduct current. Thus K^+ ions are able to move, on the average, farther along the axon before they "leak" back across the membrane. As a consequence large-diameter neurons passively conduct a depolarization faster and farther than thin ones. Nonetheless, a membrane depolarization can spread passively for only a short distance, from 0.1 to about 5 mm. Depolarizations in dendrites and the cell body generally spread in this manner, though some dendrites can conduct an action potential. Neurons with very short axons also conduct axonal depolarizations by passive spread. However, passive spread does not allow propagation of electric signals over long distances.

Opening of Voltage-Gated Sodium Channels Depolarizes the Nerve Membrane during Conductance of an Action Potential

The action potential is a cycle of membrane depolarization, hyperpolarization, and return to the resting value. The cycle lasts 1–2 ms, and an action potential can be generated hundreds of times a second (Figures 21-3 and 21-15a). All of these changes in the membrane potential can be ascribed to transient increases in the conductance of a region of the membrane, first to Na^+ ions, then to K^+ ions (Figure 21-15b). More specifically, these electric changes are due to voltage-gated Na^+ and K^+ channels that open and shut in response to changes in the membrane potential. The role of these channels in the generation and conduction of action potentials was elucidated in classic studies done on the giant axon of the squid, in which multiple microelectrodes can be inserted without causing damage to the integrity of the plasma membrane. However, the same basic mechanism is used by all neurons.

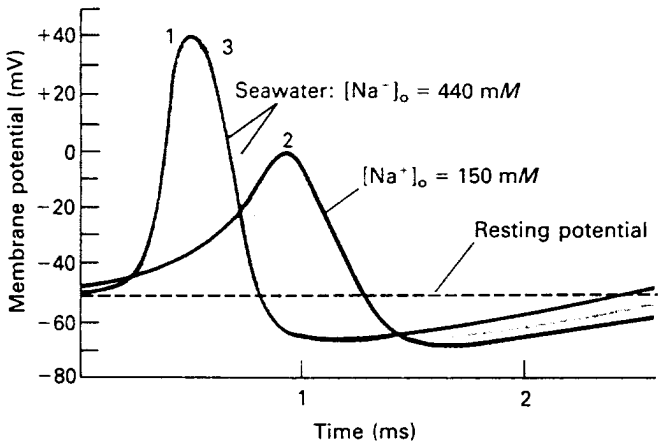


◀ FIGURE 21-15 (a) Changes in membrane potential at a single point on the giant axon of a squid, following stimulation at time 0, as measured by a microelectrode. (b) Changes in permeability (conductance) of the axonal membrane for Na^+ and K^+ associated with the action potential shown in (a). The transient increase in Na^+ influx, resulting from the transient opening of voltage-gated Na^+ channels, causes the membrane to become depolarized. This precedes opening of voltage-gated K^+ channels and the resultant efflux of K^+ ions, which causes the membrane to become hyperpolarized. [See A. L. Hodgkin and A. F. Huxley, 1952, *J. Physiol.* **117**:500.]

The sudden but short-lived depolarization of a region of the plasma membrane during an action potential (Figures 21-2, 21-15a) is caused by a sudden massive, but transient, influx of Na^+ ions through opened voltage-gated Na^+ channel proteins in that region (see Figure 21-15b). At the resting membrane potential these voltage-gated channels are closed. The depolarization of the membrane changes the conformation of these proteins, opening the Na^+-specific channels and allowing Na^+ influx through them.

During conduction of an action potential, the depolarization of a region of membrane spreads passively to the adjacent distal region of membrane. This depolarizes the new region slightly, opening a few of the voltage-dependent Na^+ channels in this segment of the membrane and causing an increase in Na^+ influx. A combination of two forces acting in the same direction drives Na^+ ions into the cell (see Figure 15-9). One is the concentration gradient of Na^+ ions. The other is the resting membrane potential—inside negative—which tends to attract Na^+ ions into the cell. As more Na^+ ions enter the cell, the inside of the cell membrane becomes more positive and thus the membrane becomes depolarized further. This depolarization causes the opening of more voltage-gated Na^+ channels, setting into motion an explosive entry of Na^+ ions that is completed within a fraction of a millisecond. For a fraction of a millisecond, at the peak of the action potential, the permeability of this region of the membrane to Na^+ becomes vastly greater than that for K^+ or Cl^-, and the membrane potential approaches E_{Na}, the equilibrium potential for a membrane permeable only to Na^+ ions (see Figure 21-13).

When the membrane potential almost reaches E_{Na}, further net inward movement of Na^+ ions ceases, since the concentration gradient of Na^+ ions (outside > inside) is



▲ FIGURE 21-16 Effect of changing the external Na^+ concentration on the magnitude of the action potential in a squid giant axon. Curves 1 and 3 are membrane potentials measured in normal seawater before and after the same axon was placed in a low-sodium solution and measured to have the membrane potential plotted by curve 2. [See A. L. Hodgkin and B. Katz, 1949, *J. Physiol.* **108**:37.]

balanced by the membrane potential E_{Na} (inside positive). The action potential is at its peak. The measured peak value of the action potential for the squid giant axon is 35 mV (see Figure 21-15a), which is close to the calculated value of E_{Na} (55 mV) based on Na^+ concentrations of 440 mM outside and 50 mM inside. The relationship between the magnitude of the action potential and the concentration of Na^+ ions inside and outside the cell has been confirmed experimentally. For instance, if the concentration of Na^+ ions in the solution bathing the squid axon is reduced to one-third of normal, the magnitude of the depolarization is reduced by 40 mV, exactly as predicted (Figure 21-16).

Voltage-Dependent Sodium Channel Proteins Propagate Action Potentials Unidirectionally without Diminution

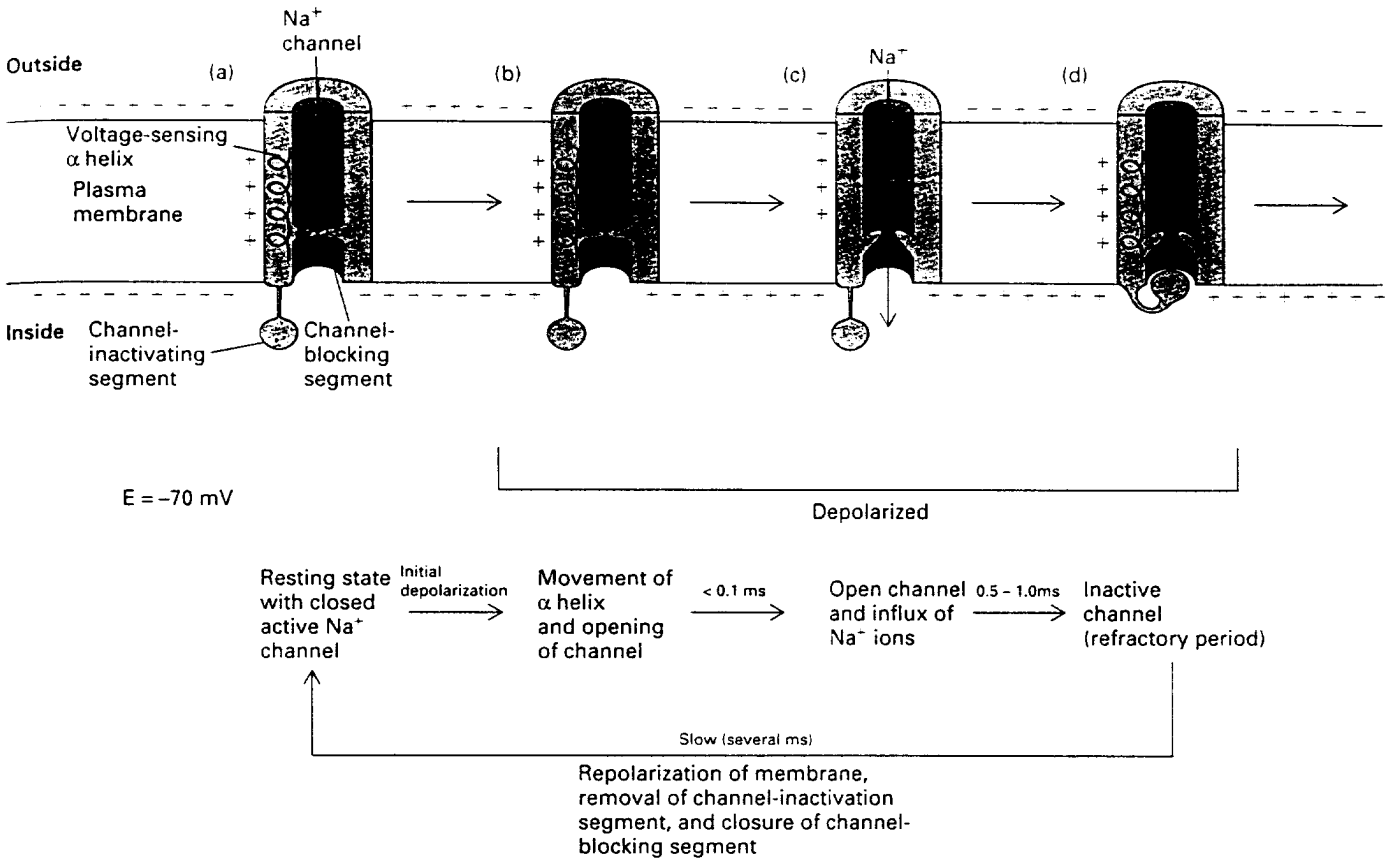
Electrophysiological studies on the squid axon and other axons have established some of the remarkable properties of voltage-gated channel proteins and have helped to explain the generation and propagation of an action potential. These studies have been extended by studies of purified channel proteins incorporated into phospholipid vesicles, and by expression of recombinant channel proteins in living cells.

As we discussed, in the resting state most of the voltage-gated Na^+ channels are closed but capable of being opened if the membrane is depolarized (Figure 21-17a and b). The greater the depolarization, the greater the chance that any one channel will open. Opening is triggered by movement of *voltage-sensing α helices* in response to the membrane depolarization, causing a small conformational change in the *channel-blocking segment* that allows ion flow. Once opened, the channels stay open about 1 ms, during which time about 6000 Na^+ ions pass through them (Figure 21-17c). Then, further ion movements are prevented by the movement of the *channel-inactivating segment* into the channel opening. As long as the membrane remains depolarized the channel is inactivated and cannot be reopened (Figure 21-17d). A few milliseconds after the inside-negative resting potential is reestablished the channels return to the closed state, capable of being opened by depolarization (Figure 21-17a).

At the peak of the action potential the extent of membrane depolarization is sufficient to depolarize a "downstream" segment of membrane, and to open the Na^+ channels in this region. Thus, propagation of the action potential is ensured. The transient inactive state of the Na^+ channels (Figure 21-17d) ensures that the action potential is propagated unidirectionally, in contrast to the passive membrane depolarization that spreads bidirectionally (Figure 21-14). Na^+ channels are momentarily inactive after opening during passage of the action potential (Figure 21-17d), and thus those Na^+ channels "behind" the action potential cannot reopen even though the potential in this segment is still depolarized (Figure 21-18). The inability of Na^+ channels to reopen for several milliseconds ensures that action potentials are propagated unidirectionally from the cell body to the axon terminus, and limits the number of action potentials per second that a neuron can conduct. Reopening of Na^+ channels "behind" the action potential is also prevented by membrane hyperpolarization caused by the opening of voltage-gated K^+ channels, discussed below.

The Opening of Voltage-Gated Potassium Channels Repolarizes the Plasma Membrane during an Action Potential

During the time that the voltage-gated Na^+ channels are closing and fewer Na^+ ions are entering the cell, voltage-gated K^+ channel proteins open. This causes the observed increase in potassium ion permeability (Figure 21-15b), an increase in efflux from the cytosol of K^+ ions, and thus, in accord with Figure 21-13, a repolarization of the plasma membrane—a return to its resting potential. Actually, for a brief instant the membrane potential becomes more negative than the resting potential (see Figure 21-15a); it approaches the potassium equilibrium potential E_K, which is more negative than the resting potential (Figure 21-13). Opening of the K^+ channels is induced by the membrane depolarization of the action potential. Like the voltage-gated Na^+ channels, most types of voltage-gated K^+ channels soon close and remain inactivated as long as the membrane is depolarized. Because the K^+ channels open a fraction of a millisecond or so after the initial depolarization, they are called *delayed* K^+ *channels*. The increase in P_K



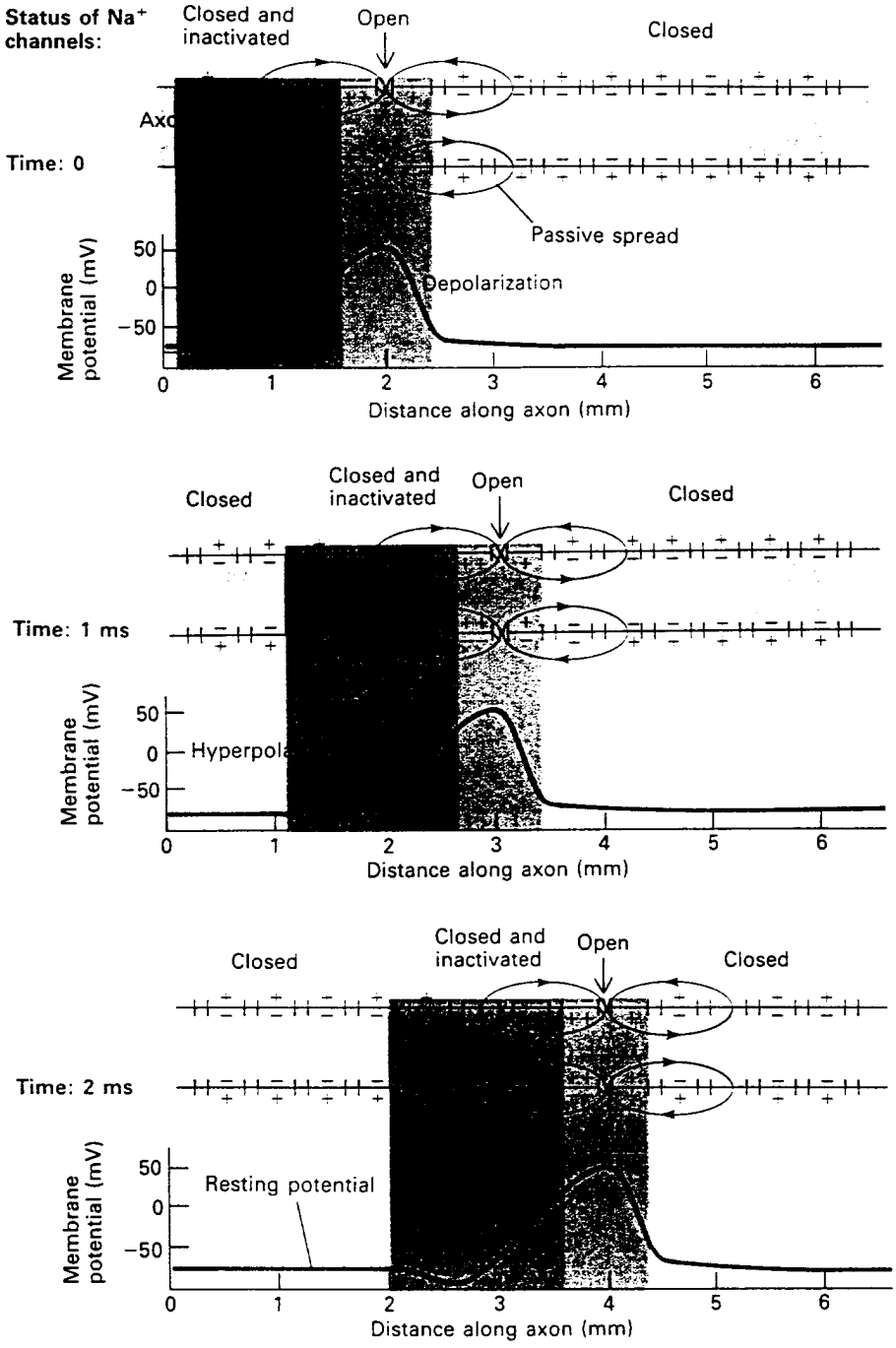
▲ FIGURE 21-17 Operation of voltage-gated Na^+ channels. In the resting state (a), the *channel-blocking segment* of the protein closes the channel, and the *channel-inactivating segment* is free in the cytosol. The channel protein contains four *voltage-sensing α helices* (discussed later in Figure 21-29), which have positively charged side chains every third residue. The attraction of these charges for the negative interior of resting cells helps to ensure that the channel is closed. The channel-blocking segment inhibits movement of Na^+ ions. When the membrane is depolarized (outside becomes negative), the gating helices move (red arrow) toward the outer plasma membrane surface (b). Within a fraction of a millisecond, the channel-blocking segment also moves, opening the channel for influx of Na^+ ions (c). Within a millisecond after opening, the gating helices return to the resting position and the channel-inactivating segment moves into the open channel, preventing further ion movements (d). When the membrane potential is reversed so that the inside is again negative, the channel-blocking segment moves back into the blocking position (not shown). After 1–2 ms the channel-inactivating segment is displaced from the channel opening and the protein reverts to the closed, resting, state (a) where it can be opened again by depolarization. [After C. Miller, 1991, *Science* **252**:1092–1096.]

accounts for the transient hyperpolarization of this region of the membrane. Eventually all voltage-gated K^+ and Na^+ channels close, movement through the membrane of Na^+ and K^+ ions returns to the values characteristic of the resting state, and the membrane potential returns to its resting value.

Movements of Only a Few Sodium and Potassium Ions Generate the Action Potential

The changes in membrane potential characteristic of an action potential are caused by rearrangements in the balances of ions on either side of the membrane, and not by changes in the concentrations of ions in the solutions on either side. The voltage changes are generated by the movements of Na^+ and K^+ across the plasma membrane through voltage-gated channels but the actual number of ions that move is very small relative to the total number in the neuronal cytosol. In fact, measurements of the amount of radioactive sodium entering and leaving single squid axons and other axons during a single action potential show that, depending on the size of the neuron, only about one K^+ ion per 3000–300,000 in the cytosol (0.0003–0.03 percent) is exchanged for extracellular Na^+ to generate the reversals of membrane polarity.

The membrane potential in nerve cells is dependent primarily on a gradient of Na^+ and K^+ ions that is generated and maintained by the Na^+-K^+ ATPase. This ATPase plays no direct role in nerve conduction. If dinitrophenol or another inhibitor of ATP production is added to cells,

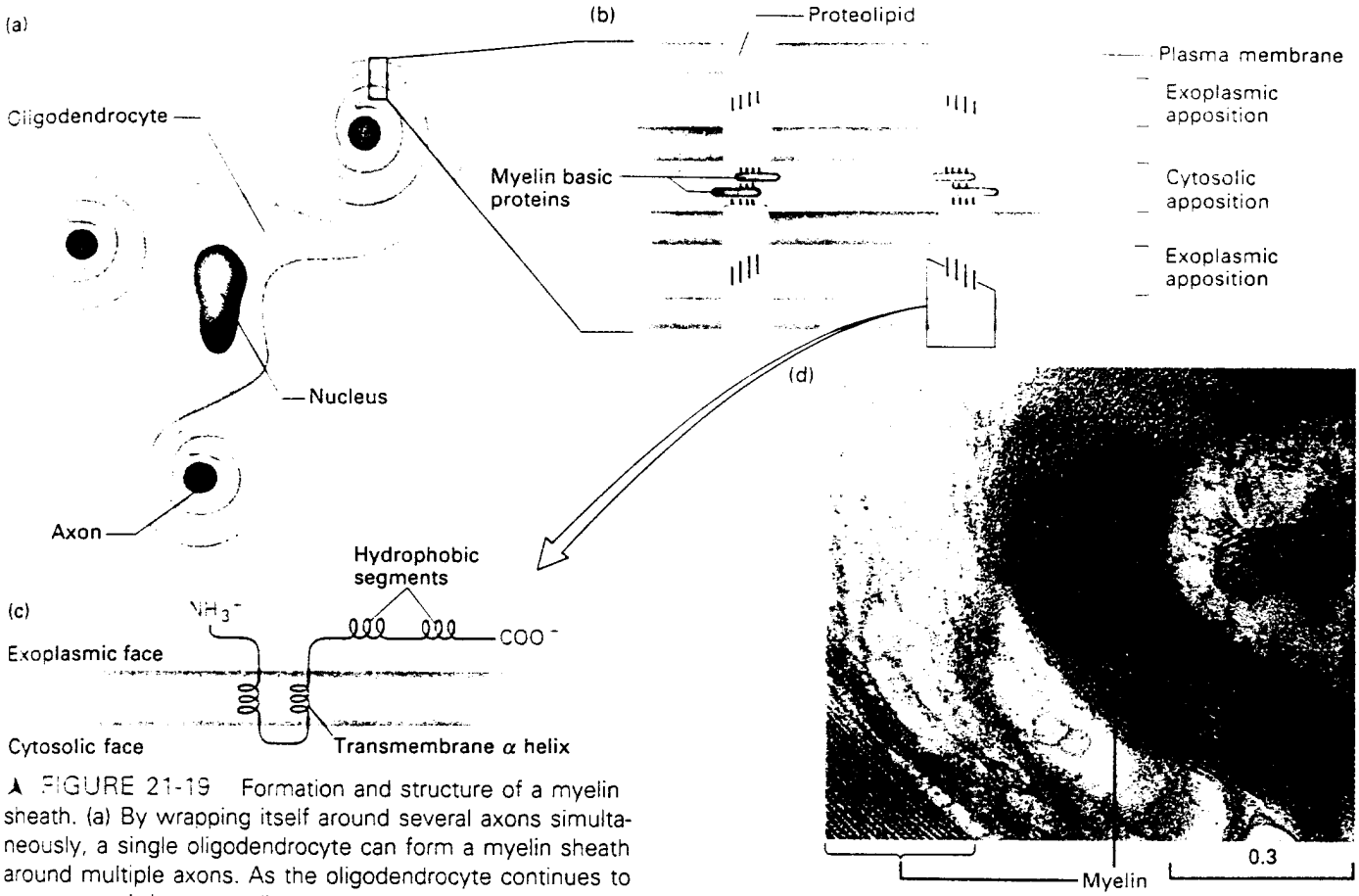


◀ FIGURE 21-18 Because they close and become inactivated shortly after opening, voltage-dependent Na^+ channels ensure unidirectional conduction of an action potential. At time 0, an action potential (light purple) is at the 2-mm position on the axon. The membrane depolarization spreads passively (Figure 21-14) in both directions along the axon but the Na^+ channels at the 1-mm position are still inactivated (light green; see Figure 21-17d) and cannot yet be reopened. Each region of the membrane is refractory (inactive) for a few milliseconds after an action potential has passed. Thus, the depolarization at the 2-mm site at time 0 triggers action potentials downstream only; at 1 ms an action potential is passing the 3-mm position.

the membrane potential gradually falls to zero as all the ions equilibrate across the membrane. In most nerve cells this equilibration is extremely slow, requiring hours. This and similar experiments indicate that the membrane potential is essentially independent of the supply of ATP over the short time spans required for nerve cells to function. Nerve cells normally can fire thousands of times in the absence of an energy supply because the ion movements during each discharge involve only a minute fraction of the cell's K^+ and Na^+ ions.

Myelination Increases the Rate of Impulse Conduction

In man, the cell body of a motor neuron that innervates a leg muscle is in the spinal cord and the axon is about a meter in length. Because the axon is coated with a myelin sheath (Figure 21-10) it takes only about 0.01 second for an action potential to travel the length of the axon (velocity ≈100 meters/second) and stimulate muscle contraction. Without a myelin sheath the velocity would be ≈1 meter/



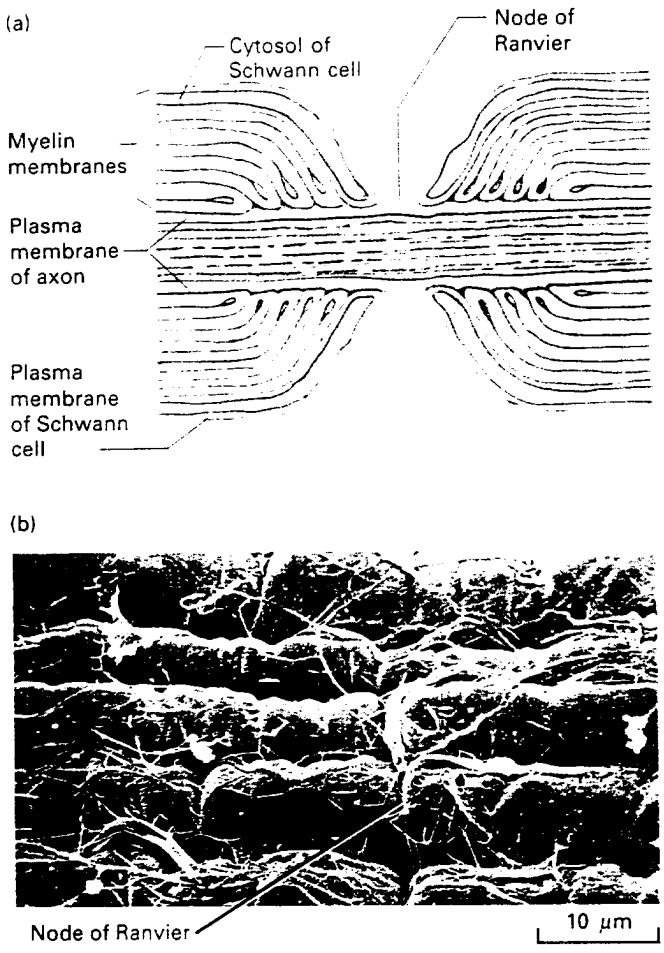
▲ FIGURE 21-19 Formation and structure of a myelin sheath. (a) By wrapping itself around several axons simultaneously, a single oligodendrocyte can form a myelin sheath around multiple axons. As the oligodendrocyte continues to wrap around the axon, all the spaces between its plasma membranes, both cytosolic and exoplasmic, are reduced. Eventually all cytosol is forced out and a structure of compact stacked membranes is formed. This compaction of plasma membranes is generated by proteins that are synthesized only in myelinating cells. (b) Molecular structure of compact myelin. The close apposition of the cytosolic faces of the membrane may result from interactions between myelin basic protein and proteolipid and between myelin basic protein molecules. Apposition of the exoplasmic faces may result from interactions between proteolipid molecules. (c) Each proteolipid molecule (276 amino acids) has two membrane-spanning α helices and two hydrophobic segments (each containing about 30 amino acids) on its exoplasmic face; the latter are thought to generate proteolipid-proteolipid interactions. (d) Electron micrograph of a cross section of the axon of a myelinated peripheral neuron, surrounded by the Schwann cell that produced the myelin sheath. [Part (c) adapted from L. D. Hudson et al., 1989, *J. Cell Biol.* **109**:717. Part (d) from P. C. Cross and K. L. Mercer, 1993, *Cell and Tissue Ultrastructure, A Functional Perspective*, W. H. Freeman and Company, p. 137.]

second (m/s), and coordination of movements such as running would be impossible.

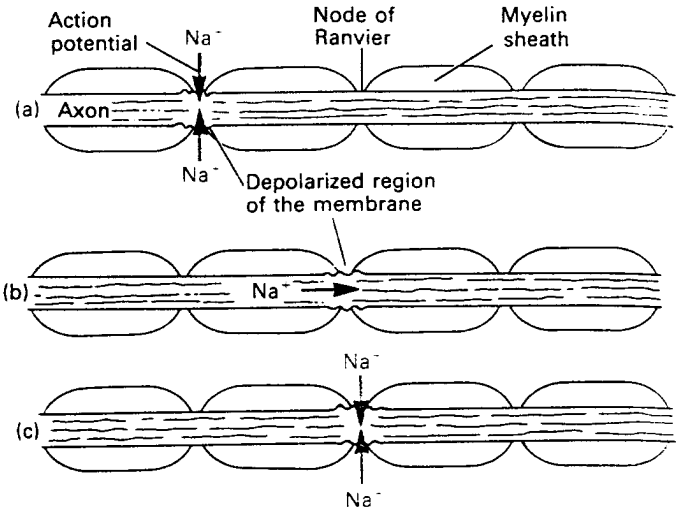
Myelin is a stack of specialized plasma membrane sheets produced by a glial cell that wraps itself around the axon (Figure 14-11). In the peripheral nervous system these glial cells are called *Schwann cells;* in the central nervous system they are called *oligodendrocytes*. Often several axons are surrounded by a glial cell (Figure 21-19a). In both vertebrates and some invertebrates, axons are accompanied along their length by glial cells, but specialization of these glial cells to form myelin occurs predominantly in vertebrates. Vertebrate glial cells that will later form myelin have on their surface a *myelin-associated glycoprotein* and other proteins that bind to adjacent axons and may trigger the formation of myelin.

A myelin membrane, like all membranes, contains phospholipid bilayers, but unlike many other membranes, it contains only a few types of proteins. *Myelin basic protein* and a *proteolipid* found only in myelin in the central nervous system allow the plasma membranes to stack tightly together (Figure 21-19b and c). Myelin in the peripheral nervous system is constructed of other unique membrane proteins. The myelin surrounding each myelinated axon is formed from many glial cells. Each region of myelin formed by an individual glial cell is separated from the next region by an unmyelinated area called the *node of Ranvier* (or simply, node); only at nodes is the axonal membrane in direct contact with the extracellular fluid (Figure 21-20).

The myelin sheath, which can be 50–100 membranes



▲ FIGURE 21-20 (a) Structure of a myelinated axon near a node of Ranvier, the gap that separates the portions of the myelin sheath formed by two adjacent Schwann cells. These nodes are the only regions along the axon where the axonal membrane is in direct contact with the extracellular fluid. (b) A scanning electron micrograph of a peripheral myelinated nerve. The deep folds are the nodes of Ranvier. Numerous strands of collagen surround individual axons and bind them together. [Part (b) from R. G. Kessel and R. H. Kardon, 1979, *Tissues and Organs: A Text-Atlas of Scanning Electron Microscopy*. W. H. Freeman and Company, p. 80.]

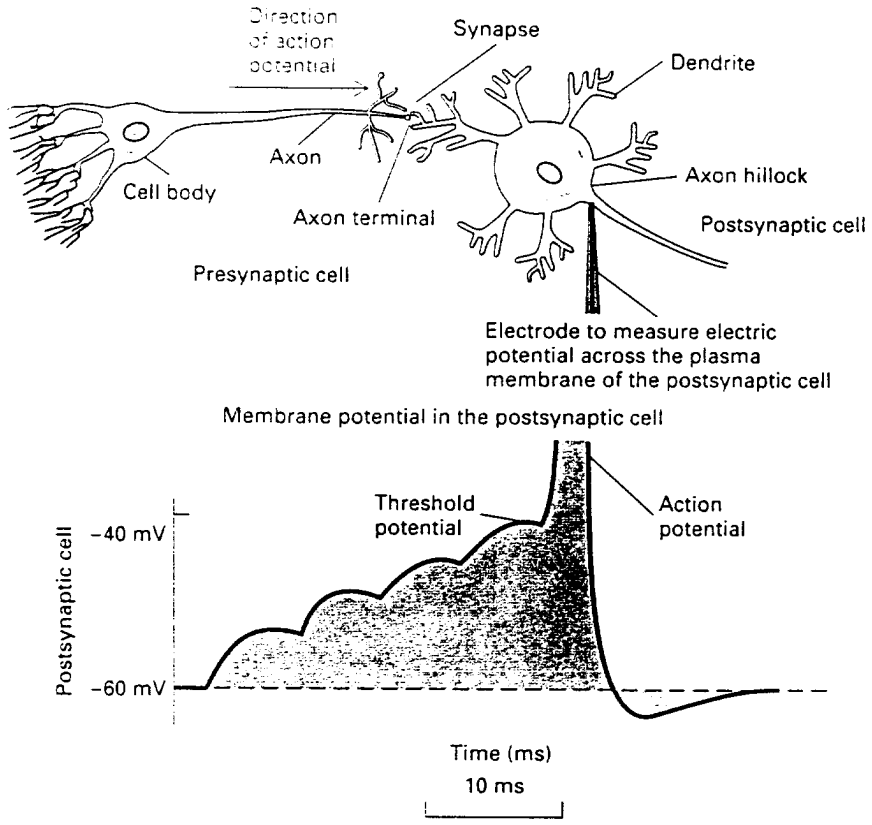


▲ FIGURE 21-21 Regeneration of action potentials at the nodes of Ranvier. (a) The influx of Na^+ ions associated with an action potential at one node results in depolarization of that region of the axonal membrane. (b) Depolarization moves rapidly down the axon because the excess positive ions cannot move outward across the myelinated portion of the axonal membrane. The buildup of these cations causes depolarization at the next node. (c) This depolarization induces an action potential at that node. By this mechanism the action potential jumps from node to node along the axon.

thick, acts as an electric insulator of the axon by preventing the transfer of ions between the axonal cytosol and the extracellular fluids. Thus all electric activity in axons is confined to the nodes of Ranvier, the sites where ions can flow across the axonal membrane. Node regions contain a high density of voltage-gated Na^+ channels, about 10,000 per μm^2 of axonal plasma membrane, whereas the regions of axonal membrane between the nodes have few, if any, channels.

The excess cytosolic positive ions generated at a node during the membrane depolarization associated with an action potential diffuse through the axonal cytosol to the next node with very little loss or attenuation because ions are capable of moving across the axonal membrane only at the myelin-free nodes; myelinated nerves have length constants of several millimeters (Figure 21-14). Thus a depolarization at one node spreads rapidly to the next node, and the action potential "jumps" from node to node (Figure 21-21). For this reason, the conduction velocity of myelinated nerves is much greater than that of unmyelinated nerves of the same diameter. For example, a 12-μm-diameter myelinated vertebrate axon and a 600-μm-diameter unmyelinated squid axon both conduct impulses at 12 m/s. Not surprisingly, myelinated nerves are used for signaling in circuits where speed is important.

One of the leading causes of serious neurologic disease among human adults is multiple sclerosis (MS). This disorder, usually characterized by spasms and weakness in one or more limbs, bladder dysfunctions, local sensory losses, and visual disturbances, is caused by patchy loss of myelin in areas of the brain and spinal cord. It is the prototype *demyelinating disease*. In MS patients, conduction of action potentials by the demyelinated neurons is slowed and the Na^+ channels spread outward from the nodes. The cause of the disease is not known but appears to involve either the body's production of auto-antibodies (antibodies that bind to normal body proteins) that react with myelin basic protein or the secretion of proteases that destroy myelin proteins.



◀ FIGURE 21-22 The threshold potential for generation of an action potential. In this example the presynaptic neuron is generating about one action potential every four milliseconds. Arrival of each action potential at the synapse causes a small change in the membrane potential of the postsynaptic cell, in this example a depolarization of ≈5 mV. Note the voltage scale of 10 mV. This synapse is excitatory; when, due to multiple stimuli, the membrane of this postsynaptic cell becomes depolarized to the threshold potential, here ≈ −40 mV, an action potential is induced.

Action Potentials Are Generated in an All-or-Nothing Fashion by Summation of Electric Disturbances

A single neuron can be affected simultaneously by synapses with many axons (Figure 21-4). Most synapses are *excitatory*, in that they cause a local depolarization in the membrane of the postsynaptic cell. Others are *inhibitory*, in that they generate a local hyperpolarization of the plasma membrane. The various depolarizations and hyperpolarizations move by passive spread along the dendrite plasma membrane from the synapses to the cell body and then to the axon hillock. Whether a neuron generates an action potential in the axon hillock depends on the balance of the timing, amplitude, and localization of all the various inputs it receives. Action potentials are generated whenever the membrane at the axon hillock is depolarized to a certain voltage called the *threshold potential* (Figure 21-22). The generation of an action potential is thus said to be all-or-nothing. Depolarization to the threshold always leads to an action potential; any depolarization that does not reach the threshold potential never induces it.

In a sense, each neuron is a tiny computer that averages all the electric disturbances on its membrane and makes a decision whether to trigger an action potential and conduct it down the axon. An action potential will always have the same *magnitude* in any particular neuron. The *frequency* with which action potentials are generated in a particular neuron is the important parameter in its ability to signal other cells: the more action potentials in a given neuron in a particular period of time the greater the numbers of signals it sends, via synapses, to neurons or to other target cells.

➤ *Molecular Properties of Voltage-Gated Ion Channel Proteins*

Voltage-gated ion channel proteins have three remarkable properties that enable nerve cells to conduct an electric impulse: (1) opening in response to changes in the membrane potential (voltage gating); (2) subsequent channel closing and inactivation; and (3) exquisite specificity for ions that will permeate and those that will not. In this section, we describe the molecular analysis of these voltage-dependent ion channel proteins. The technique of *patch-clamping*, or *single-channel recording*, enables workers to investigate the opening, closing, and ion conductance of individual ion channels—that is, of *single* plasma membrane proteins. One of the surprising results to emerge from molecular cloning of ion channel proteins is that all voltage-gated ion channels, be they Na^+, K^+, or Ca^{2+} channels, are related in structure and in function.

Patch Clamps Permit Measurement of Ion Movements through Single Sodium and Potassium Channels

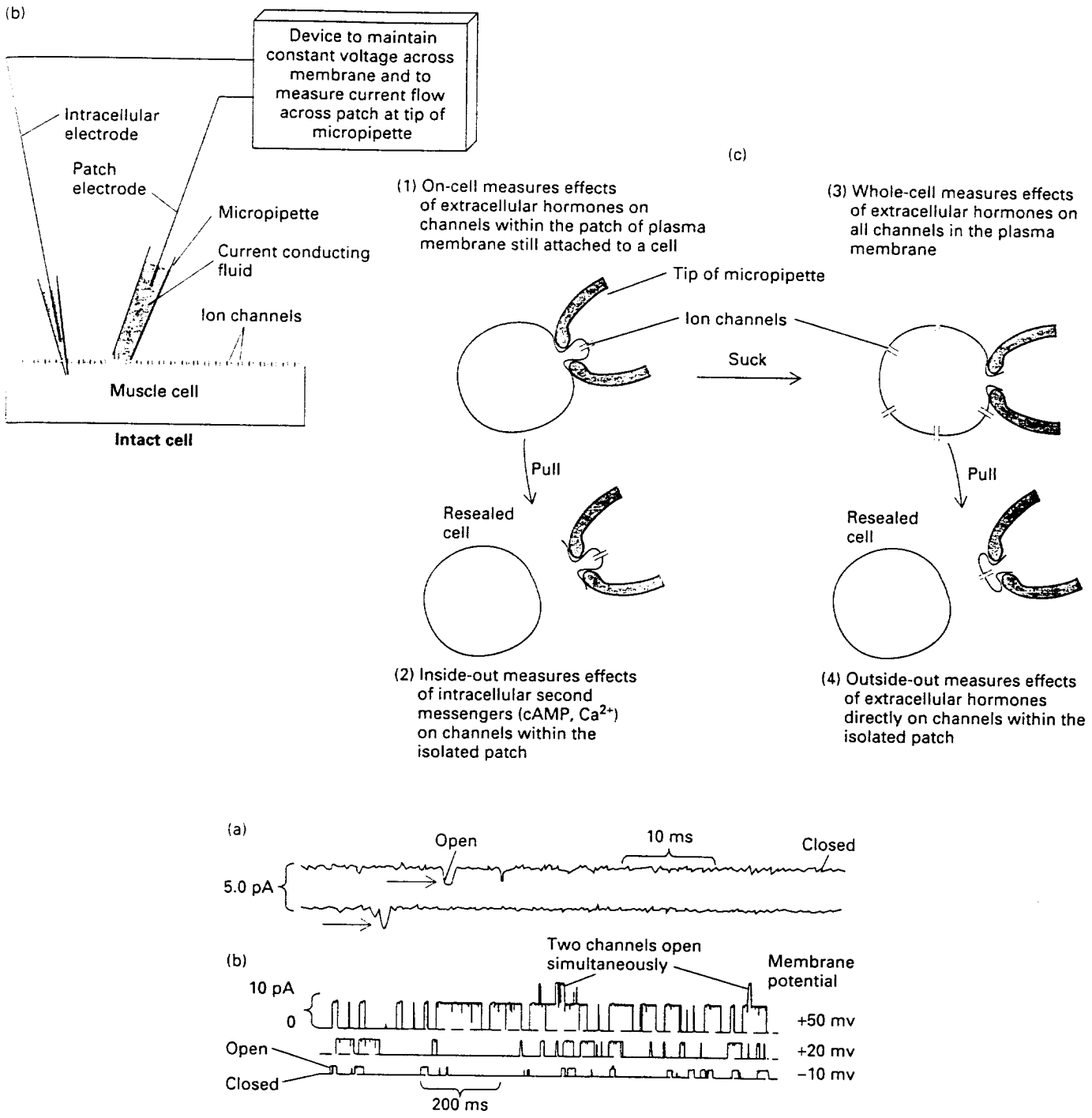
The patch-clamp technique, illustrated in Figure 21-23, measures, across a small patch of isolated membrane, the electric current caused by the movement of ions. In general, the membrane is electrically depolarized or hyperpolarized and maintained (clamped) at that potential by an electronic feedback device. The membrane potential cannot change, in contrast to the situation during an action potential.

The inward or outward movement of ions across a patch of membrane can be quantified from the amount of electric current needed to maintain the membrane potential at the designated "clamped" value. To preserve electroneutrality, the entry of each positive ion (e.g., a Na^+ ion) into the cell across the plasma membrane is balanced by the entry of an electron into the cytosol from the electrode placed in it. Conversely, the movement of each positive ion from the cell (e.g., a K^+ ion) is balanced by the withdrawal of an electron from the cytosol.

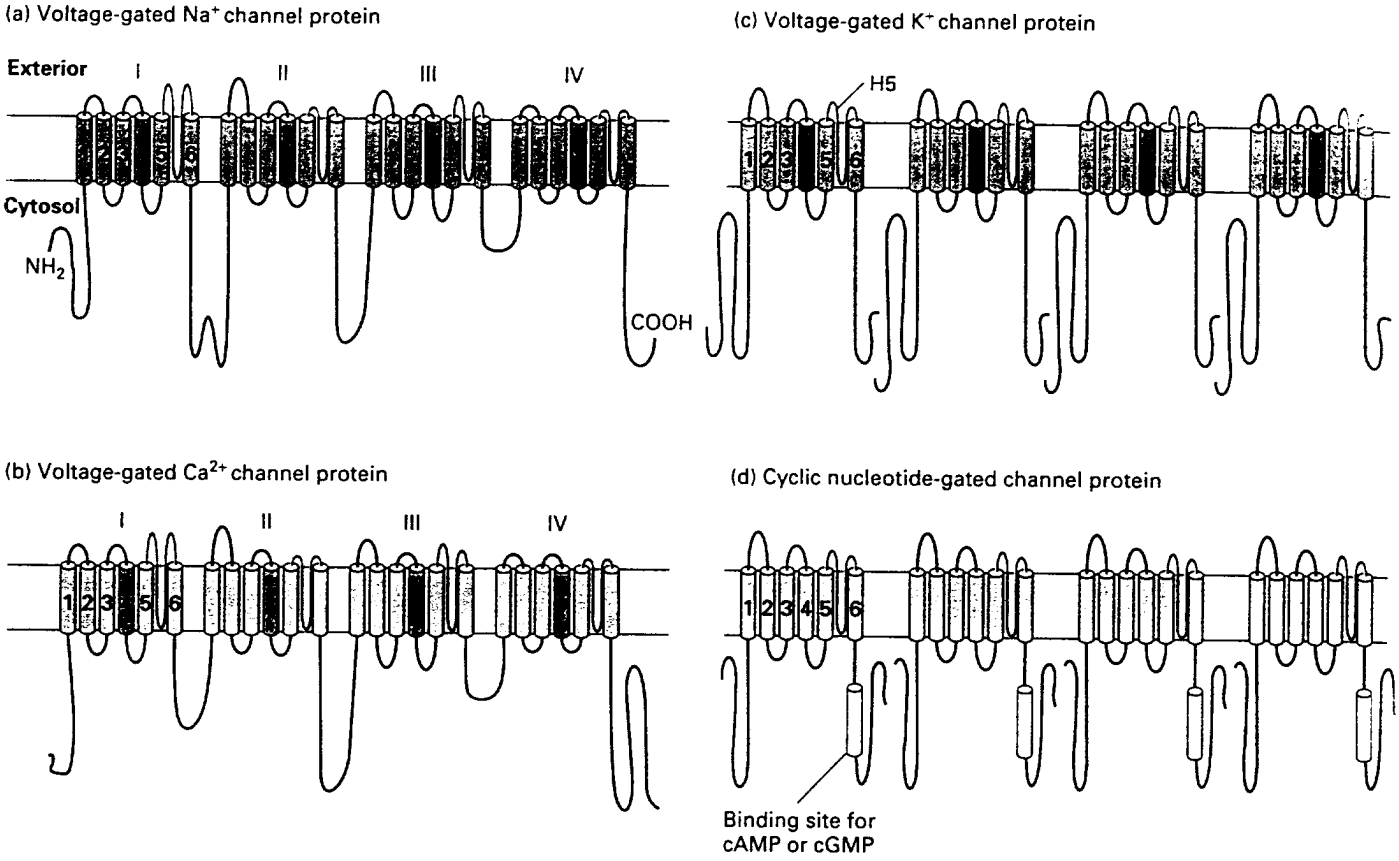
In the study depicted in Figure 21-24a, two patches of muscle membrane, each containing one voltage-gated Na^+ channel, were depolarized about 10 mV and clamped at that voltage. The current flow across each patch of membrane was then monitored. Under these circumstances, the transient pulses of current that cross the membrane result from the opening and closing of individual Na^+ channels. Each channel is either open or closed; there are no graded permeability changes for individual channels. From the recording shown in Figure 21-24a, it can be determined that a channel is open for an average of 0.7 ms and that 9900 Na^+ ions per millisecond move through an open channel. We know that these channels conduct only Na^+, since replacement of NaCl with KCl or choline chloride within the patch pipette (corresponding to the outside of the cell) abolishes current through the channels.

Part (b) illustrates the properties of voltage-gated potassium channels. At the depolarizing voltage of −10 mV the channels in the membrane patch open infrequently and remain open for only a few milliseconds, as judged, respectively, by the number and width of the "upward blips" on the electric recording. Further, the ion flux through them is rather small, as measured by the electric current passing through each open channel (the height of the blips). Depolarizing the membrane further to +20 mV causes the channels to open about twice as frequently. Also, more K^+ ions move through each open channel (the height of the blips is greater) because the force driving cytosolic K^+ ions outward is greater at a membrane potential of +20 mV than at −10 mV. Depolarizing the membrane further to +50 mV, such as at the peak of an action potential, causes the opening of more K^+ channels and also increases the flux of K^+ through them. Sometimes two channels in the same patch open simultaneously. Thus, by opening during the peak of the action potential, these K^+ channels cause the outward movement of K^+ ions and thus the repolarization of the membrane potential towards the resting state.

▲ FIGURE 21-23 Outline of the patch-clamping technique. (a) Photomicrograph of the cell body of a cultured neuron and the tip of a patch pipette touching the cell membrane. The bar is 10 μm, and the diameter of the tip of the micropipette is about 0.5 μm. (b) *Facing page:* Arrangement for measuring current flow through individual ion channels in the plasma membrane of a living cell. The patch electrode is filled with a current-conducting saline solution. It is applied, with a slight suction, to the plasma membrane; a region of membrane 0.5 μm in diameter contains only one or a few ion channels. The second electrode is inserted into the cytosol. The recording device measures current flow only through the channels in the patch of plasma membrane. (c) Different ways in which patch clamping can be used. In the on-cell mode (1), pulling the pipette away from the cell results in a piece of membrane in the mouth of the pipette (2). In this configuration, one can study the effects of intracellular second messengers on individual channels. By clamping the potential across the isolated membrane patch and recording the flow of ion current across it, one can measure the effect of the membrane potential on the opening and closing of single channel proteins. The effects of ion composition of the solution on either side of the membrane can also be examined. In the whole-cell mode (1) gentle suction removes the piece of membrane in the patch (3), allowing measurement of ion movement through all of the plasma membrane ion channels. Pulling the cell away from the pipette results in a piece of plasma membrane being retained in the pore of the pipette, outside-out (4). This allows study of the effects of extracellular substances directly on ion channels within the patch. [Part (a) from B. Sakmann, 1992, *Neuron* **8**:613–629 (Nobel lecture), also published in E. Neher and B. Sakmann, 1992, *Sci. Am.* **266**(3):44. Part (c) after B. Hille, 1992, *Ionic Channels of Excitable Membranes*, Sinauer Associates, p. 89.]



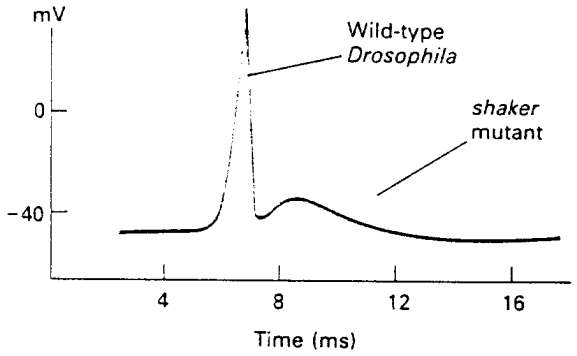
▲ FIGURE 21-24 Use of the patch clamp technique to measure current flux through individual voltage-gated sodium channels (a) or potassium channels (b). In part (a), patches of muscle cell membrane (see Figure 21-23(c), were depolarized by 10 mV and clamped at that value. The transient pulses of electric current (pA = picoamperes), recorded as large downward deviation (arrows), indicate the opening of a Na^+ channel and movement of Na^+ ions across the membrane. The smaller deviations in current represent background noise. The average current through an open channel is 1.6 picoamperes [1.6×10^{-12} amperes; 1 ampere = 1 coulomb (C) of charge per second]. This is equivalent to the movement of about 9900 Na^+ ions per channel per millisecond: $(1.6 \times 10^{-12}$ C/s$)(10^{-3}$ s/ms$)(6 \times 10^{23}$ molecules/mol$) \div$ 96,500 C/mol. (b) Patches of membrane of a cultured muscle cell were clamped at the indicated potentials. Current flux through a patch is recorded as upward deviations since, in contrast to the inward movement of Na^+ through the sodium channels in part (a), K^+ ions are moving outward across the membrane. Increasing the extent of membrane depolarization from −10 mV to +50 mV increases the probability a channel will open, the time it stays open, and the amount of electric current (numbers of ions) that pass through it. [Part (a) see F. J. Sigworth and E. Neher, 1980, *Nature* **287**:447. Part (b) from B. Pallota, K. Magleby, and J. Barrett, 1981, *Nature* **293**:471–474 as modified by B. Hille, 1992, *Ionic Channels of Excitable Membranes*, Sinauer Associates, p. 122.]



▲ FIGURE 21-25 Proposed transmembrane structures of three voltage-gated channels and a cyclic GMP-gated channel. Voltage-gated Na^+ channel proteins (a) contain 1800–2000 amino acids. About 29 percent of the residues are identical in sequence to those in the voltage-gated Ca^{2+} channel protein (b); another 36 percent of the residues in both proteins have similar side chains. Both Na^+ and Ca^{2+} channel proteins have four homologous domains (indicated by Roman numerals), each of which is thought to contain six transmembrane α helices (indicated by Arabic numerals) and a nonhelical domain, H5 (blue line), that lines the ion pore. One α helix in each domain (no. 4, in red) is thought to function as a voltage sensor. These α helices have multiple arginine and lysine side chains. The *shaker* K^+ channel protein (c) isolated from *Drosophila* has only 656 amino acids; it is similar in sequence and transmembrane structure to each of the four domains in the Na^+ and Ca^{2+} channel proteins. The K^+ channel is a tetramer composed of four identical polypeptides, whereas Na^+ and Ca^{2+} channels are single polypeptides. Cyclic AMP- or cyclic GMP-gated ion channels (d) are not voltage-gated and helix 4 does not function as a voltage-sensor. However, these do have a pore-lining H5 segment similar to that in the voltage-gated channels. The segment that faces the cytosol that binds cyclic AMP or cyclic GMP, triggering channel opening, is indicated. These channels are found in abundance in the sensing cells in the visual and olfactory systems (see Figure 21-50). [Adapted from W. A. Catterall, 1988, *Science* **242:**50; L. Heginbotham, T. Abramson, and R. MacKinnon, 1992, *Science* **258:**1152; and T. M. Jessell and E. R. Kandel, 1993, in *Cell* vol. 72/*Neuron* vol. 10 (Suppl), pp. 1–3.]

All Voltage-Gated Ion Channels Have a Similar Molecular Structure

The voltage-gated ion channels that generate the action potential have all been cloned, and all have a remarkably similar structure (Figure 21-25). A typical potassium channel contains four copies of a ≈600-amino-acid polypeptide, each of which has six membrane-spanning α-helices. In contrast, Na^+ and Ca^{2+} channels are single polypeptides of ≈2000 amino acids that contains four homologous transmembrane domains, each similar in sequence and structure to a K^+ channel protein. These domains are connected and flanked by shorter stretches of nonhomologous residues. We will explore how these channels were cloned, how their structures were determined, and how they function.



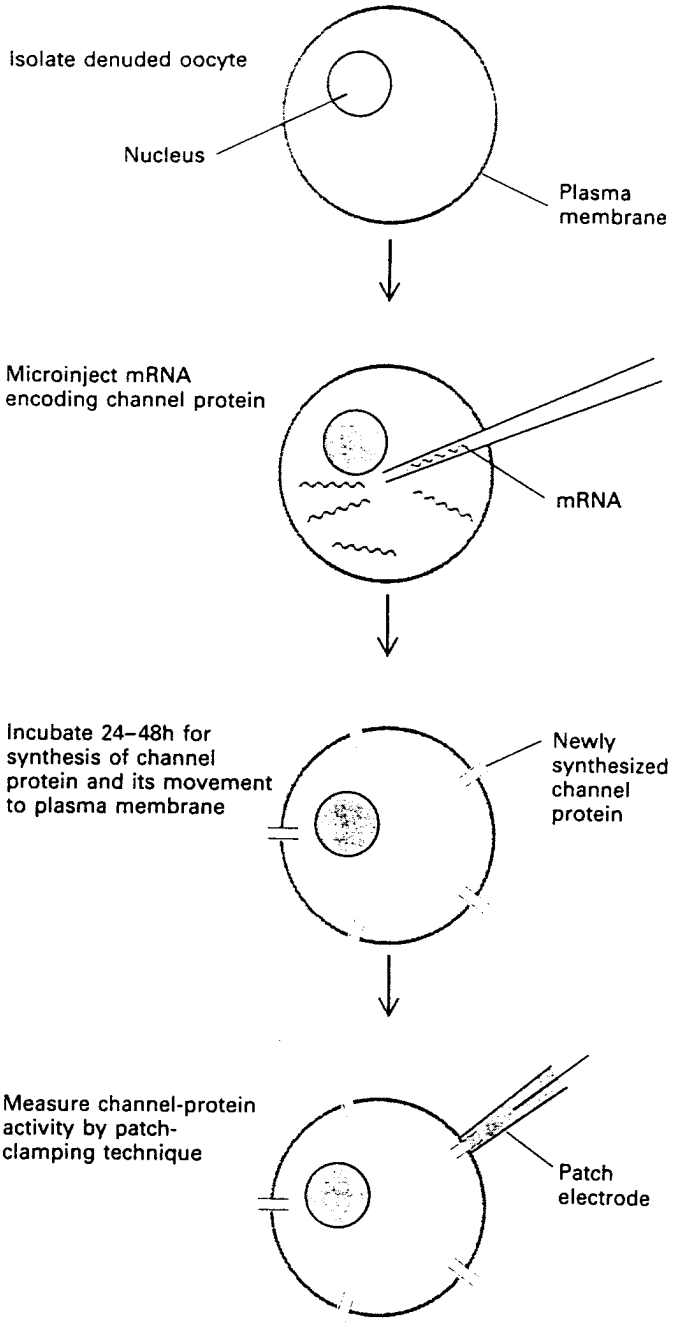
▲ FIGURE 21-26 Action potentials in axons of wild-type *Drosophila* and *shaker* mutants. The *shaker* mutants exhibit an abnormally prolonged action potential because of a defect in the K^+ channel protein that is required for normal repolarization. [See L. A. Salkoff and R. Wyman, 1983, *Trends Neurosci.* **6**:128.]

Study of *shaker* Mutants in *Drosophila melanogaster* Led to the Cloning of a Large Family of Voltage-Gated Potassium Channels

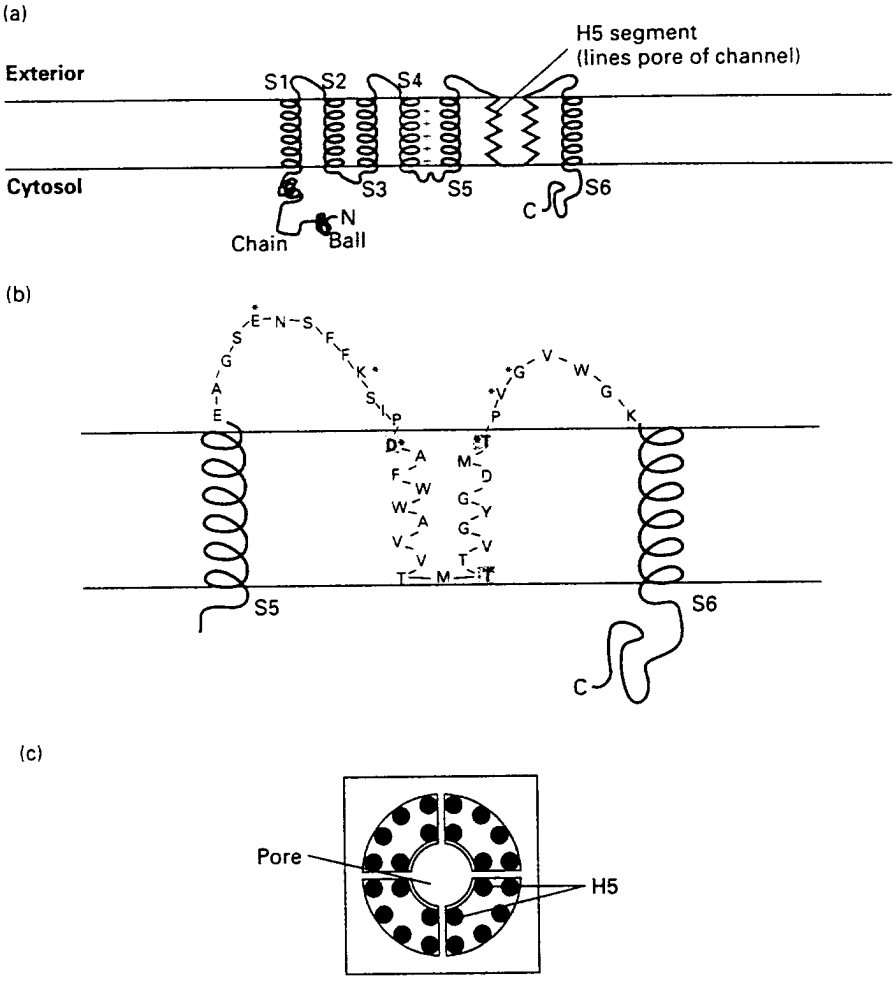
The breakthrough in identification and cloning potassium channels came from a genetic analysis of a mutation of the fruit fly *Drosophila melanogaster* that caused a specific abnormality in certain motor neurons. Flies carrying the *shaker* mutation shake vigorously under ether anesthesia, reflecting a loss of motor control and a defect in excitable cells. The X-linked *shaker* mutation was thought to cause a defect in a K^+ channel protein. Because the axons of giant nerves in *shaker* mutants have an abnormally prolonged action potential (Figure 21-26), it was proposed that defective K^+ channels fail to open normally immediately upon depolarization.

The wild-type *shaker* gene was cloned by chromosome walking (Figure 8-29). To show that the encoded protein indeed was a K^+ channel, the *shaker* cDNA was used as a template to produce *shaker* mRNA in a cell-free system. This mRNA was microinjected into frog oocytes (Figures 15-27 and 21-27) and translated into a new voltage-gated K^+ channel protein on the oocyte plasma membrane. Patch-clamp studies on the channel in oocyte plasma membrane showed that it had properties identical to those of the K^+ channel in the neuronal membrane, demonstrating conclusively that the *shaker* gene encodes that K^+ channel.

The 656-amino-acid polypeptide encoded by the *shaker* gene, like most K^+ channel polypeptides, contains six membrane-spanning α helixes, labeled S1–S6, a segment, H5, between S5 and S6 that lines the pore of the channel, and an amino-terminal segment—the "ball"—that moves into the open channel, inactivating it. The func-



▲ FIGURE 21-27 Expression of a channel protein in a cell that does not normally contain it provides a way to study the activity of normal and mutant channel proteins. A convenient cell for such studies is the oocyte from the frog *Xenopus laevis* (See Figure 15-27). Messenger RNA encoding the channel protein under study is produced in a cell-free transcription reaction using the cloned gene as a template, or a mixture of mRNAs is directly isolated from a tissue. A follicular oocyte is first treated with collagenase to remove the surrounding follicle cells, leaving a denuded oocyte. After microinjection of the mRNA and incubation, the activity of the channel protein is determined by the patch-clamping technique. [Adapted from T. P. Smith, 1988, *Trends Neurosci.* **11**:250.]



▲ FIGURE 21-28 Molecular structure of voltage-gated potassium channels. Each of the four polypeptides that constitute a channel (a) is thought to contain six membrane-spanning α helixes, S1 through S6. S4 (red) contains several positively charged amino acids and is the voltage-sensing α helix (see Figure 21-17). The N-terminus of the polypeptide is in the cytosol, and contains a globular domain, the "ball," that is linked to the first membrane-spanning segment by a flexible peptide "chain." The ball is essential for inactivation of the open channel. The pore-lining segment H5 (blue), shown in detail in (b), is probably not α-helical. Mutant channels with altered amino acids (one letter code is used) at the sites in the pore indicated by * have altered resistance to the channel-blocking toxin charybdotoxin, and those at **bold** sites (orange boxes) have altered sensitivities to the channel blocker tetraethylammonium ion. Thus the entire H5 sequence is thought to line the pore itself or its entrance. A complete channel, shown in cross section in (c), consists of four subunits. Four H5 segments, one from each subunit, constitute the lining of the ion-selective pore. The position of the voltage-sensing S4 helix is not known. [After C. Miller, 1991, *Science* **252**:1092–1096, and 1992, *Curr. Biol.* **2**:573; and C. Stevens, 1991, *Nature* **349**:657–658.]

tional channel is built of four *shaker* polypeptides (Figure 21-28), but the arrangement of the six α helixes in each segment is not known.

Study of Toxin-Resistant Mutants Led to the Identification of Amino Acids That Line the Pore of the Potassium Channel

To identify the amino acid residues that line the pore of the K^+ channel, researchers used a neurotoxin from scorpion venom, charybdotoxin. This toxin physically plugs the channel mouth. Site-specific mutation of any of several amino acids (as depicted in part b of Figure 21-28) in the H5 segment inhibited toxin binding, and thus rendered the channel, when expressed in oocytes, resistant to inhibition by the toxin. Similarly, a tetraethylammonium ion was known to plug most kinds of K^+ channels, and different mutations in the H5 region of the *shaker* protein greatly reduced channel inhibition by tetraethylammonium. Finally, different kinds of K^+ channels have different conductances (number of ions transported per millisecond), and different sensitivities to tetraethylammonium ions. Chimeric proteins were made in which only the H5 segment from one protein was replaced with that from an-

Voltage-gated Na^+ channel

Helix 4:	*domain I*	S	A	L	R	T	F	R	V	L	R	A	L	K	T	I	S	V	I	P	G	L	K
	domain II			G	L	S	V	L	R	S	F	R	L	L	R	V	F	K	L	A	K	S	W	P
	domain III	G	A	I	K	S	L	R	T	L	R	A	L	R	P	L	R	A	L	S	R	F	E
	domain IV	R	V	I	R	L	A	R	I	G	R	I	L	R	L	I	K	G	A	K	G	I	R

Voltage-gated Ca^{2+} channel

Helix 4:	*domain I*	K	A	L	R	T	F	R	V	L	R	P	L	R	V	L	S	G	V	P	S	L	Q
	domain II	L	G	I	S	V	L	R	C	I	R	L	L	R	L	F	K	I	T	K	Y	W	T
	domain III	S	V	V	K	I	L	R	V	L	R	A	L	R	P	L	R	A	I	N	R	A	K
	domain IV	I	S	S	A	F	F	R	L	F	R	V	M	R	L	I	K	L	L	S	R	A	E

Voltage-gated K^+ channel

Helix 4	R	V	I	R	L	V	R	V	F	R	I	F	K	L	S	R	H	S	K	G	L	Q

◀ FIGURE 21-29 Amino acid sequences of the voltage-sensing α helix (S4) of voltage-gated channel proteins. One S4 α helix is present in each of the four transmembrane domains in the Na^+ and Ca^{2+} channel polypeptides; one is present in each K^+ channel polypeptide. These helices have a positively charged lysine (K) or arginine (R) every third or fourth residue (red) whose side chains tend to be localized on one face of an α helix. Amino acids are represented by the single-letter code. [Adapted from W. A. Catterall, 1988, *Science* **242**:50.]

other; in all cases the conductances and sensitivities to tetraethylammonium of the resultant channels correlated with the origin of the H5 segment. Thus, it was concluded that the H5 regions of the *shaker* channel polypeptides constitute the lining of the pore.

A Complete *shaker* Potassium Channel Is Assembled from Four Subunits

Since the pore-lining segment is so small, consisting of about 20–30 amino acids, it would seem that the functional channel would be built of several *shaker* polypeptides, each of which would contribute one segment to line the pore. Indeed, the charybdotoxin-resistant mutant of the *shaker* channel was used to show that the channel comprises four *shaker* polypeptides (Figure 21-28c). In the critical experiment, two kinds of *shaker* mRNA were injected together into frog oocytes; 10 percent coded for the wild-type (toxin-sensitive) channel, and 90 percent for the toxin-resistant mutant. Assuming that the two types of polypeptides mix at random during channel assembly, and that a single copy of the wild-type polypeptide makes a channel toxin sensitive, the fraction of toxin-resistant channels would be 0.9^n, where n is the number of polypeptides that constitute the channel. The determined percentage of toxin-resistant channels was 66 percent, and thus $n = 4$; the channel protein is a tetramer. This result was not surprising, since voltage-gated Na^+ channels contain, in a single polypeptide, four copies of a sequence resembling the *shaker* polypeptide (Figure 21-25), and a single polypeptide generates a functional Na^+ channel.

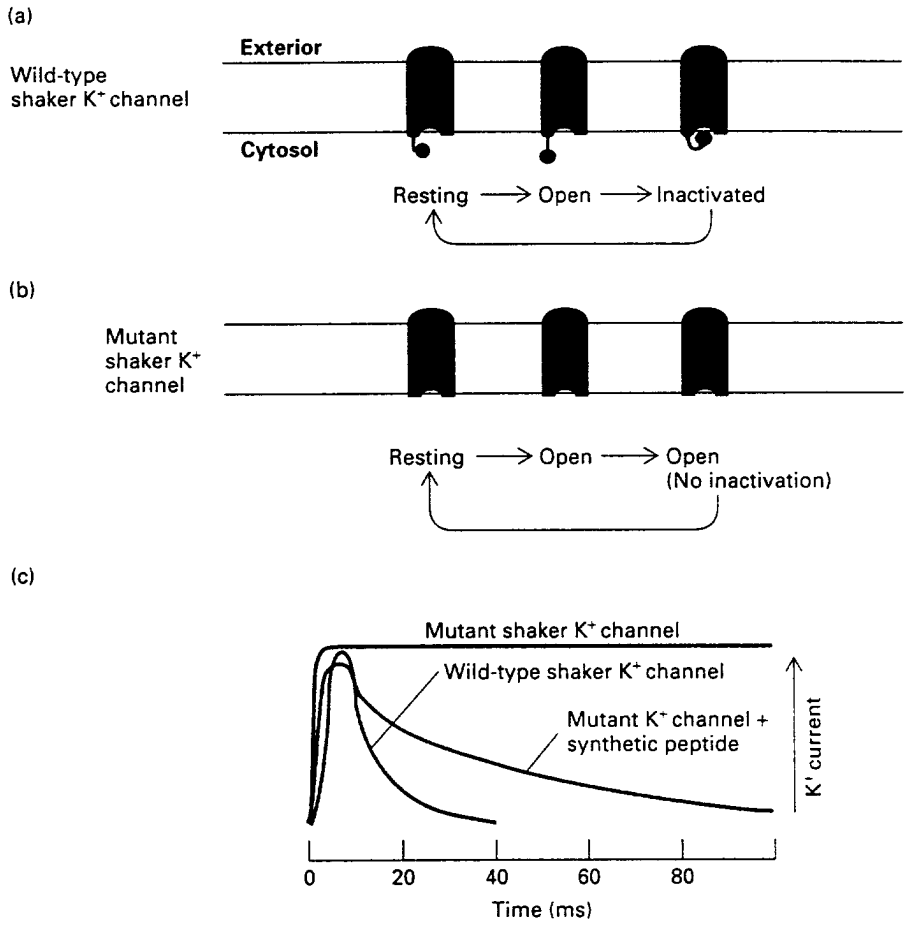
The S4 Segment Is the Voltage Sensor

Since voltage-gated K^+ (and Na^+) channels open when the membrane depolarizes, some segment of the protein must "sense" the change in potential. Sensitive electric measurements suggest that the opening of each K^+ (and Na^+) channel is accompanied by the movement of four to six protein-bound positive charges from the cytosolic to the exoplasmic surface of the membrane; alternatively, a larger number of charges may move a shorter distance across the membrane. The movement of these gating charges (or voltage sensors) under the force of the electric field is believed to trigger a conformational change in the protein that opens the channel. The voltage sensor is thought to be the S4 transmembrane α helix present in each *shaker* polypeptide (Figure 21-28a); similar S4 segments are found in voltage-gated Na^+ and Ca^{2+} channel proteins (Figure 21-25). These voltage-sensing helices, often called *gating helices*, have multiple positively charged side chains (Figure 21-29); when the membrane is depolarized, these amino acids are thought to move toward the exoplasmic surface of the channel (see Figure 21-17).

The role of the S4 helix in voltage sensing was demonstrated in studies with mutant *shaker* K^+ channel proteins produced by site-specific mutagenesis. In these mutant proteins, one or more arginine or lysine residues in the S4 α helix was replaced with neutral or acidic residues. The ability of such mutant proteins, expressed in frog oocytes, to open in response to membrane depolarization was tested using the patch-clamp technique (Figure 21-24b). As expected, when expressed in oocytes, the mutant proteins exhibited altered opening responses to depolarization voltages. In particular, the fewer the positively charged residues in the gating helices, the larger the depolarization required to open the K^+ channels.

The N-Terminal Segment of the *shaker* Protein Causes Channel Inactivation

An important characteristic of all voltage-gated channels is inactivation: soon after opening they close spontaneously, forming an inactive channel that will not reopen until the membrane is repolarized (Figure 21-17). The N-terminus of the *shaker* polypeptide forms a globular ball that swings into the open channel, inactivating it. The key experiments in Figure 21-30 support this model. First, deleting the ball



▲ FIGURE 21-30 (a) The "ball and chain" model for inactivation of a voltage-gated potassium channel. In the resting state the ball at the N-terminus of the wild-type channel is free in the cytosol, and remains there during the first milliseconds after the channel is opened by depolarization. The ball then moves into the open channel, inactivating it. After a few milliseconds (depending on the exact type of channel) the ball is displaced from the channel and the protein reverts to the closed, resting state. Evidence for the ball and chain model was obtained by expressing the wild-type and mutant *shaker* potassium channel (b) in *Xenopus* oocytes. When the plasma membrane, in a patch pipette (Figure 21-23), was depolarized from −70 to +30 mV, the wild-type channel opened for ≈5 milliseconds and then closed (red curve in c). In contrast, a mutant channel lacking the ball amino acids 6 through 46 (b) opened normally, but could not close (green curve). When a chemically synthesized ball peptide was added to the cytosolic face of the patch, the mutant channel opened normally and then closed (purple curve). This demonstrated that the added peptide inactivated the channel after it opened. [Parts (a) and (b) after C. Miller, 1991, *Science* **252**:1092–1096; part (c) from W. N. Zagotta, T. Hoshi, and R. W. Aldrich, 1990, *Science* **250**:568–671.]

domain (b) results in a K^+ channel that opens normally in response to depolarization and has a normal conductivity, but cannot close (green curve in part c). Second, when a chemically synthesized ball peptide is added to the cytosol, the mutant channel opens normally and then closes (purple curve). This demonstrates that the added peptide inactivates the channel after it opens, and that the ball does not have to be tethered to the protein in order to function. Further experiments suggested that there is a chain as well. Deletion of part of the ≈40-amino-acid segment connecting the ball to the first membrane-spanning segment increases the rate of inactivation—the shorter the chain the more rapid the inactivation—as if a ball attached to a shorter chain can move into the open channel more readily. Conversely, addition of random amino acids to the normal chain slows channel inactivation.

Potassium Channel Proteins Are Diverse

A great diversity of K^+ channel types is necessary to account for the electrical activity of different types of neurons. In *Drosophila*, at least five different *shaker* polypeptides are produced by alternative splicing of the primary transcript of the *shaker* gene. In the oocyte expression assay, these polypeptides exhibit different voltage dependencies and K^+ conductivities. Thus differential expression of the *shaker* gene can affect the properties of the action

potential in different neurons. Also, if a single K^+ channel is constructed from two different kinds of channel polypeptides, the properties of the resultant "hybrid" channel differ from those of both of the channels formed from four identical polypeptides.

Using the *shaker* gene as a hybridization probe, workers isolated genes encoding more than two dozen K^+ channel proteins from vertebrates; the encoded channel proteins exhibit different voltage dependencies, conductances, channel open times, and other properties. Many have the property of opening at the strongly depolarizing voltages (as in Figure 21-24b) required for the channel to repolarize the membrane during an action potential. All of the members of this large protein family are identical in essential aspects of mechanism and structure (Figure 21-28).

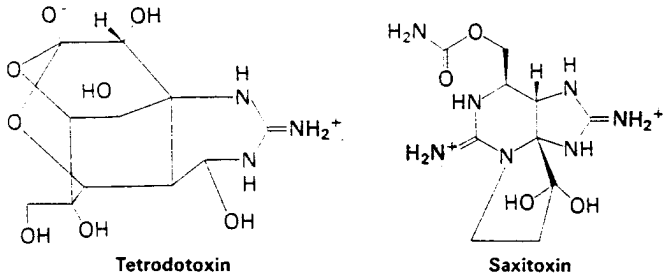
Thus, starting with the isolation of a mutant fruit fly that shakes under ether anesthesia, scientists have derived a remarkably complete molecular picture of how a voltage-gated potassium channel operates. Using the *shaker* gene, workers have isolated a large number of K^+ channel polypeptides expressed in the human brain. As this example illustrates, studies on the nervous systems of invertebrates are indeed directly applicable to an understanding of the human nervous system.

The Sodium Channel Protein Has Four Homologous Transmembrane Domains, Each Similar to a Potassium Channel Polypeptide

Like K^+ channels, the density of voltage-gated Na^+ channels is very low. Depending on the type of unmyelinated axon, there are only 5–500 voltage-gated Na^+ channels per square micrometer of membrane; roughly one membrane protein molecule in a million is a voltage-gated Na^+ channel protein. Despite its low concentration, the Na^+ channel protein has been purified. The use of neurotoxins that bind tightly and specifically to Na^+ channels made this purification possible (Figure 21-31).

Tetrodotoxin, for example, is a powerful poison concentrated in the ovaries, liver, skin, and intestines of the puffer fish. A toxin with related properties, saxitoxin, is produced by certain red marine dinoflagellates. These toxins specifically bind to and inhibit the voltage-gated Na^+ channels in neurons, preventing action potentials from forming. One molecule of either toxin binds to one Na^+ channel with exquisite affinity and selectivity. Measurements of the amount of radioactive tetrodotoxin or saxitoxin that binds to a typical unmyelinated neuron have shown that an axon contains 5–500 Na^+ channels per square micrometer of membrane. This agrees with the numbers of channels estimated from patch-clamp studies. The Na^+ channels in these membranes are thus spaced, on average, about 200 nm apart.

The Na^+ channel protein was purified by affinity chromatography of detergent-extracted membrane proteins on columns of immobilized toxin that specifically bind Na^+ channels. Rat brain and the electroplax of the electric eel



▲ FIGURE 21-31 Structures of two sodium-channel blockers—tetrodotoxin and saxitoxin. The positively charged groups on these neurotoxins may bind to negatively charged carboxylate groups in the Na^+ channel protein. The size of these toxins prevents their passage through the channel and blocks the passage of Na^+ ions.

are rich and convenient sources of this protein. The major component of the Na^+ channels from these sources is a single polypeptide with a molecular weight of 250,000–270,000. Purified Na^+ channel protein(s) can be incorporated into artificial lipid membranes, which then exhibit many of the predicted properties of the Na^+ channel proteins, such as voltage dependence. From the cloned cDNA, the sequence of the major polypeptide of the Na^+ channel has been determined. It contains four homologous transmembrane domains, each similar in sequence and structure to the central part of the *shaker* protein. These domains are connected and flanked by shorter stretches of nonhomologous residues (Figure 21-25a). Other subunits of the Na^+ channel are regulatory; they affect the rate at which the channel opens and becomes inactivated, and the voltage dependence of channel inactivation.

All Voltage-Gated Channel Proteins Probably Evolved from a Common Ancestral Gene

The similarities among the voltage-gated Na^+, Ca^{2+}, and K^+ channels suggest that all three proteins evolved from a common ancestral gene. These similarities include the following:

1. Na^+, K^+, and Ca^{2+} channels all open when the membrane is depolarized.
2. The voltage-sensing S4 α helices in all three ion channels have a positively charged lysine or arginine every third or fourth residue (see Figure 21-29).
3. The Na^+ and Ca^{2+} channel proteins have extensive sequence homology throughout their length. Each contains four transmembrane domains with six α helices and one H5 pore-lining segment per domain. The sequence and transmembrane structure of the much smaller K^+ channels, such as the *shaker* protein from *Drosophila*, are similar to those of each domain in the Na^+ and Ca^{2+} channels (see Figure 21-25). Each K^+ channel comprises four copies of such a polypeptide.

4. All of these channels have an H5 pore-lining sequence in a similar position. The specificity of the ion that passes through the channel is determined by amino acid side chains in the pore-lining regions of the proteins, as was shown by analysis of site-specific mutations of several of these channels.

Voltage-gated K^+ channels have been found in all yeasts and protozoa studied. In contrast, voltage-gated Ca^{2+} channels (important in synaptic transmission, discussed below) are present in only a few of the more complex protozoa, such as *Paramecium*, and only multicellular organisms have voltage-gated Na^+ channels. Thus voltage-gated K^+ channel proteins probably arose first in evolution. The Ca^{2+} and Na^+ channel proteins are believed to have evolved by repeated duplication of an ancestral one-domain K^+ channel gene.

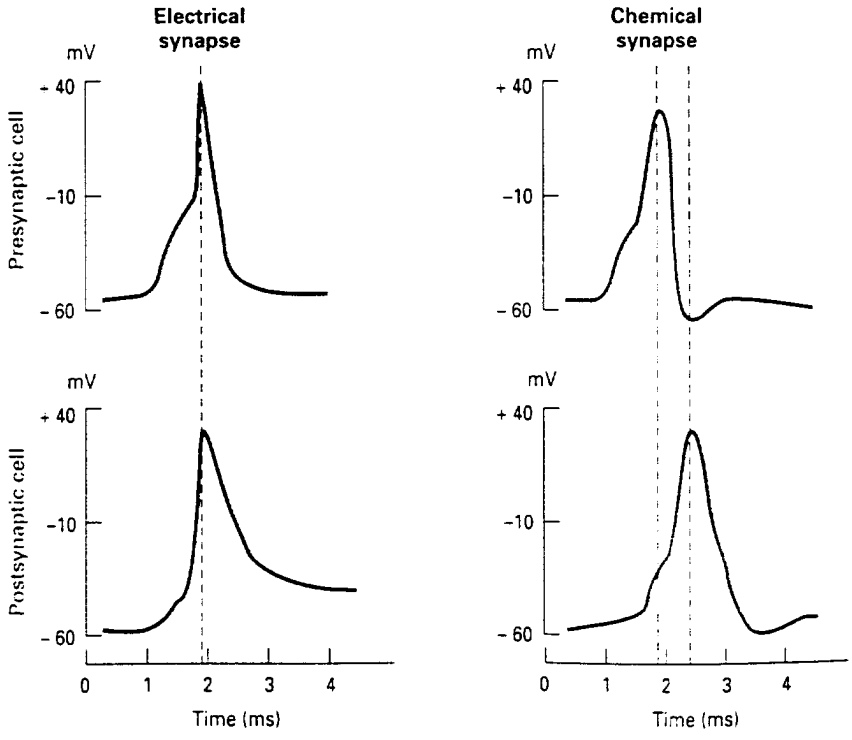
➤ *Synapses and Impulse Transmission*

As noted earlier, synapses are the junctions where neurons pass signals to target cells, which may be other neurons, muscle cells, or gland cells. In most nerve-to-nerve signaling and all known nerve-to-muscle and nerve-to-gland signaling, the neuron releases chemical neurotransmitters at the synapse that act on the target cell. Much rarer, but simpler in function, are electric synapses in which the action potential is transmitted directly and very rapidly from the presynaptic to the postsynaptic cell.

Impulse Transmission across Electric Synapses Is Nearly Instantaneous

In an electric synapse, ions move directly from one neuron to another via gap junctions (see Figure 21-7). The membrane depolarization associated with an action potential in the presynaptic cell passes through the gap junctions, leading to a depolarization, and thus an action potential, in the postsynaptic cell. Such cells are said to be electrically coupled. Gap junctions are also found in nonneuronal cells where, as discussed in Chapter 24, they enable small molecules such as cAMP and amino acids to pass from cell to cell.

Electric synapses have the advantage of speed; the direct transmission of impulses avoids the delay of about 0.5 ms that is characteristic of chemical synapses (Figure 21-32). In certain circumstances, a fraction of a millisecond can mean the difference between life and death. Elec-



➤ FIGURE 21-32 Transmission of action potentials across electric and chemical synapses. In both cases, the presynaptic neuron was stimulated and the membrane potential was measured in both the presynaptic and postsynaptic cells (see Figure 21-11a). Signals are transmitted across an electric synapse within a few microseconds because ions flow directly from the presynaptic cell to the postsynaptic cell through gap junctions. In contrast, signal transmission across a chemical synapse is delayed about 0.5 ms—the time required for secretion and diffusion of neurotransmitter and the response of the postsynaptic cell to it.

tric synapses in the goldfish brain, for example, mediate a reflex action that flaps the tail, which permits a fish to escape from predators. Examples also exist of electric coupling between groups of cell bodies and dendrites, ensuring simultaneous depolarization of an entire group of coupled cells. The large number of electric synapses in many cold-blooded fishes suggests that they may be an adaptation to low temperatures, as the lowered rate of cellular metabolism in the cold reduces the rate of impulse transmission across chemical synapses.

The efficiency with which an electric signal is transmitted across an electric synapse is proportional to the number of gap junctions that connect the cells. The permeability of the gap junction is regulated by the level of cellular H^+ and Ca^{2+} ions; changes in the concentration of these ions might modulate the efficiency of impulse transmission at electric synapses.

Chemical Synapses Can Be Fast or Slow, Excitatory or Inhibitory, and Can Exhibit Signal Amplification and Computation

In a chemical synapse, neurotransmitters are stored in small membrane-bounded vesicles, called synaptic vesicles, in the axon terminals of the presynaptic cell (see Figure 21-5a and b). As we shall explain in detail, the arrival of an action potential at an axon terminal causes the opening of voltage-gated Ca^{2+} channels. This causes a rise in the cytosolic Ca^{2+} concentration, which triggers exocytosis of the synaptic vesicles and release of neurotransmitter. Synaptic vesicles are uniformly sized organelles, 40–50 nm in diameter, that store one of the "classic" small-molecular-weight neurotransmitters, most of which are depicted in Table 21-1. Except for acetylcholine, they are either amino acids, derivatives of amino acids, or nucleosides or their derivatives such as adenosine and ATP. Each neuron generally contains one type of "classic" neurotransmitter. Synaptic vesicles, which are clustered over the synaptic zone, release their neurotransmitters into the region called the *synaptic cleft* that is adjacent to the postsynaptic cell. The neurotransmitter then binds to specific receptors on the plasma membrane of this cell, causing a change in its permeability to ions.

Many neurons secrete neuropeptides (some of which are listed in Table 21-2) in addition to classic neurotransmitters. The neuropeptides are stored in a different type of vesicle. Exocytosis of neuropeptides is also triggered by a rise in cytosolic Ca^{2+}, but they are released outside the synaptic zone. The effects of the neuropeptide transmitters are very diverse and often long-lived (hours to days), and the following discussions will be confined mainly to the actions of the classic neurotransmitters listed in Table 21-1.

Excitatory and Inhibitory Synapses One way of classifying synapses is whether the action of the neurotransmitter tends to promote or inhibit the generation of an action potential in the postsynaptic cell. Many nerve-nerve and most nerve-muscle chemical synapses are excitatory. The binding of a neurotransmitter to an excitatory receptor changes the permeability of the plasma membrane of the postsynaptic cell, generally by opening Na^+ channels (Figure 21-13), such that the postsynaptic plasma membrane becomes depolarized. This depolarization promotes the generation of an action potential (Figure 21-33a).

The binding of a neurotransmitter to an inhibitory receptor on the postsynaptic cell also causes a localized change in ion permeability—generally by opening K^+ or Cl^- channels (Figure 21-13)—that tends to hyperpolarize the membrane and thus block the generation of an action potential (Figure 21-33b).

Fast Synapses and Ligand-Gated Ion Channels One class of neurotransmitter receptors are ligand-gated ion channels (Figure 21-3). Binding of the neurotransmitter causes an immediate conformational change in the protein that opens the channel and allows ions to cross the membrane. The membrane potential of the postsynaptic cell changes very rapidly—in 0.1–2 milliseconds. Such rapid changes can be excitatory or inhibitory, depending on the neurotransmitter receptor. Certain receptors for acetylcholine—the nicotinic acetylcholine receptors—and for glutamate are ligand-gated (i.e., ligand-opened) cation channels that transduce an excitatory signal (see Figure 21-33a). Binding of the ligand to one of these receptors opens a cation channel that allows passage of both Na^+ and K^+ ions and thus depolarizes the membrane of the postsynaptic cell. In contrast, receptors for the neurotransmitters γ-aminobutyric acid and glycine form ligand-gated Cl^- channels. Binding of the ligand hyperpolarizes the postsynaptic plasma membrane; thus these receptors transduce a rapid inhibitory signal (Table 21-3).

Slow Synapses and Receptors Coupled to G Proteins Many functions of the nervous system operate with time courses of seconds or minutes; regulation of the heart rate, for instance, requires that action of neurotransmitters extend over several beating cycles measured in seconds. In general, the types of neurotransmitter receptors utilized in slow synapses are not ligand-gated ion channels. Rather, these receptors are coupled to G proteins (Table 21-2). The sequence is similar to what happens in nonneuronal cells: Binding of a neurotransmitter to its receptor activates a G protein that, in most cases, directly binds to a separate ion channel protein and causes an increase or decrease in its ion conductance. In other cases, the receptor-activated G protein activates adenylate cyclase or phospholipase C, triggering a rise in cytosolic cAMP or Ca^{2+}, respectively,

TABLE 21-1 Some Small Molecules Identified as Neurotransmitters

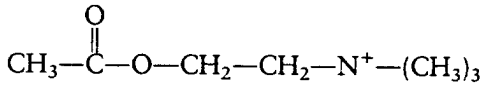
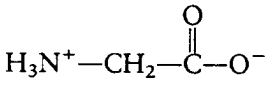
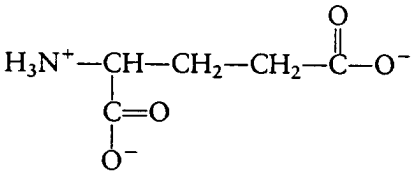
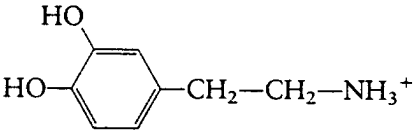
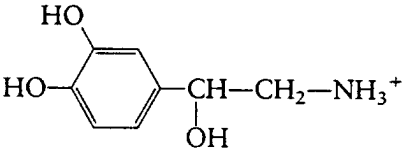
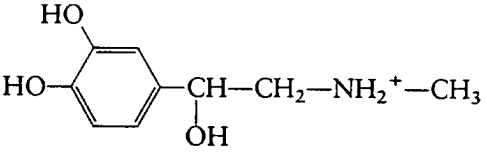
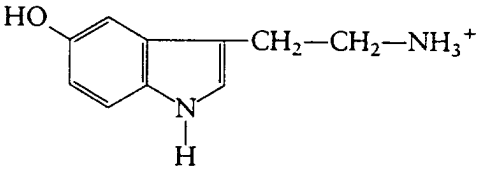
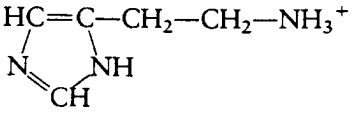
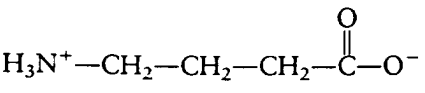
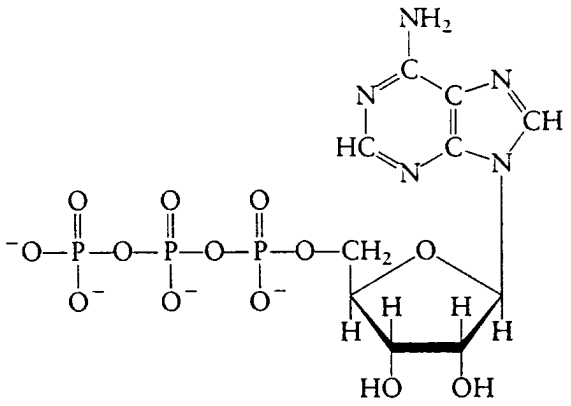
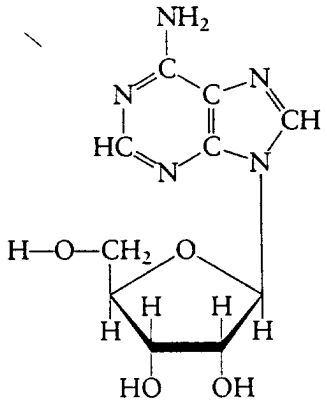
Name	Structure	Derivation or Group
Acetylcholine		
Glycine		Amino acid
Glutamate		Amino acid
Dopamine		Derived from tyrosine
Norepinephrine		Derived from tyrosine
Epinephrine		Derived from tyrosine
Serotonin (5-hydroxytryptamine)		Derived from tryptophan
Histamine		Derived from histidine
γ-Aminobutyric acid (GABA)		Derived from glutamate

TABLE 21-1 *(Continued)*

Name	Structure	Derivation or Group
ATP		Nucleotide
Adenosine		Nucleoside

which in turn affects the ion permeability of a channel protein. G protein-coupled responses are intrinsically slower and longer lasting than those induced by ligand-gated channels; in the heart, for example, the postsynaptic hyperpolarization response to acetylcholine lasts several seconds (Figure 21-33b).

Signal Amplification and Computation Chemical synapses have two important advantages over electric ones. The first is signal amplification, which is common at nerve-muscle synapses. An action potential in a single presynaptic motor neuron can cause contraction of multiple muscle cells because release of relatively few signaling molecules at a synapse is all that is required to stimulate contraction. The second advantage is signal computation, which is common at synapses involving interneurons, especially in the central nervous system. Many interneurons can receive signals at multiple excitatory and inhibitory synapses. Some of the resultant changes in ion permeability are short-lived—a millisecond or less; others may last several seconds. Whether an action potential is generated depends upon a complex function of all of the incoming signals; this signal computation differs for each type of interneuron.

Many Types of Receptors Bind the Same Neurotransmitter

The diversity of receptors for and responses to a single kind of neurotransmitter is illustrated by acetylcholine. Synapses in which acetylcholine is the neurotransmitter are termed *cholinergic* synapses. The type of acetylcholine receptor that causes excitatory responses lasting only milliseconds are called *nicotinic acetylcholine receptors.* They are so named because nicotine is an agonist of this type of receptors—like acetylcholine, nicotine causes a rapid depolarization (see Figure 21-33a). As noted already, these

TABLE 21-2 Some Neurotransmitter Receptors That Are Coupled to G Proteins

Receptor Class	Typical Ligands
Small neurotransmitters	Epinephrine, norepinephrine
	Dopamine
	Serotonin* (for $5HT_1$, $5HT_2$, $5HT_4$ class receptors)
	Histamine
	Acetylcholine* (for muscarinic receptors)
	GABA* (for B-class receptors)
	Glutamate*
	ATP
	Adenosine
Neuropeptides	Opiods (e.g. β-endorphin)
	Tachykinins (e.g. substance P)
	Bradykinin
	Luteinizing hormone–releasing hormone (LHRH)
	Thyrotropin-releasing hormone (TRH)
	Vasoactive intestinal peptide (VIP)
	Adrenocorticotropic hormone (ACTH)
	Cholcystokinin (CCK)
	Gastrin
	Endothelin
	Vasopressin
	Oxytocin
Environmental signals	Light (receptor is the visual pigment rhodopsin)
	Odorants (hundreds of different receptors)

* Ligands with an asterisk are also known to have other receptors that are ligand-gated ion channels, as listed in Table 21-3.

SOURCE: B. Hille, 1992, *Neuron* 9:187–195.

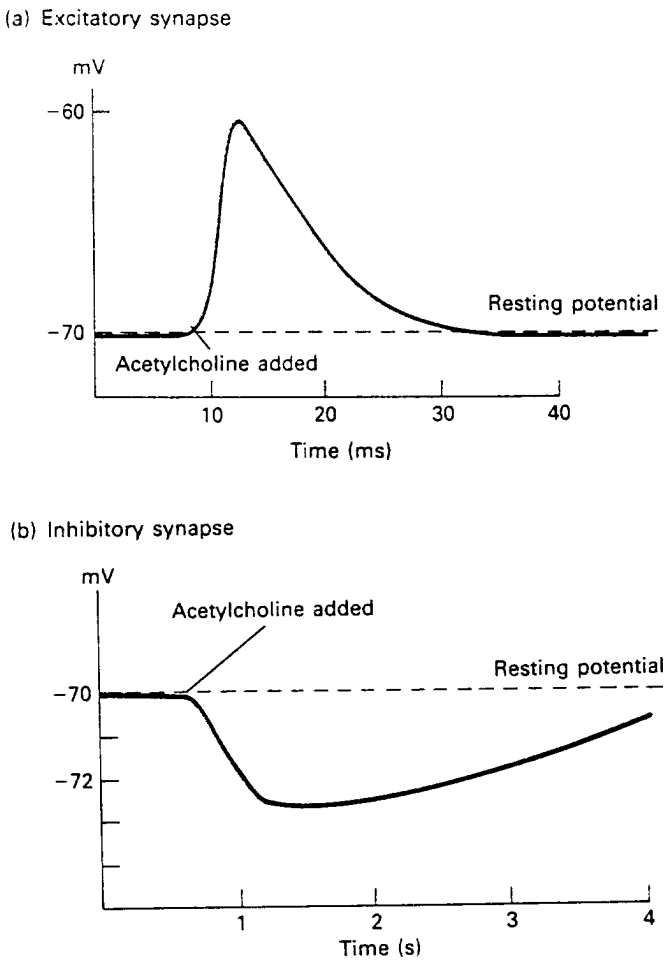
receptors are ligand-gated channels for Na^+ and K^+ ions. The other types of acetycholine receptors are called *muscarinic acetylcholine receptors* because muscarine (a mushroom alkaloid) causes the same response as does acetylcholine. There are several subtypes of muscarinic acetylcholine receptors, all of which are coupled to G proteins but which induce different responses. The M2 receptor present in heart muscle activates a G_i protein that causes the opening of a K^+ channel and thus a hyperpolarization lasting seconds (Figure 21-33b). The M1, M3, and M5 subtypes of muscarinic acetylcholine receptors, found in other cells, are coupled to the types of G proteins known as G_o or G_q and activate phospholipase C, while the M4 subtype activates G_i and inhibits adenylyl cyclase. Thus, a single neurotransmitter induces very different responses in different target nerve and muscle cells, depending on the type of receptor found in the cells.

➤ *Synaptic Transmission and the Nicotinic Acetylcholine Receptor*

The diversity of neurotransmitters and their receptors is extensive. Because the muscle nicotinic acetylcholine receptor was the first ligand-gated ion channel to be purified, cloned, and characterized at a molecular level, we know more about its structure and mechanism than any other. It provides a paradigm for other ligand-gated ion channels. Thus in this section we discuss in detail the muscle nicotinic acetylcholine receptor and the events at synapses containing this receptor—how synaptic vesicles align with the plasma membrane, how exocytosis of synaptic vesicles is triggered by opening of voltage-gated calcium channels and a rise in cytosolic calcium, and how hydrolysis of acetylcholine terminates the depolarization signal. In the following sections, we briefly consider the properties of other neurotransmitters, their receptors, and their transporters.

Acetylcholine Is Synthesized in the Cytosol and Stored in Synaptic Vesicles

The membrane-bounded vesicles that store acetylcholine are about 40 nm in diameter and accumulate, often in rows, in presynaptic axon terminals near the plasma membrane (Figures 21-34 and 21-35). A single axon terminus of a frog motor neuron may contain a million or more synaptic vesicles, each containing 1000–10,000 molecules of acetylcholine; such a neuron might form synapses with a single skeletal muscle cell at several hundred points.



◀ FIGURE 21-33 Excitatory and inhibitory responses in postsynaptic cells. (a) An excitatory response: application of acetylcholine (or nicotine) to frog skeletal muscle produces a rapid postsynaptic depolarization of about 10 mV, which lasts 20 ms. These cells contain nicotinic acetylcholine receptors, a ligand-gated Na^+ and K^+ channel protein. (b) An inhibitory response: application of acetylcholine (or muscarine) to frog heart muscle produces, after a lag period of about 40 ms (not visible in graph), a hyperpolarization of 2–3 mV, which lasts several seconds. These cells contain muscarinic acetylcholine receptors. Thus depending on the type of receptor present in the postsynaptic cell, acetylcholine can either increase muscle contraction (skeletal muscle) or decrease it (cardiac muscle). Note the difference in time scales in the two graphs. [See H. C. Hartzell, 1981, *Nature* **291**:539.]

TABLE 21-3 Neurotransmitter Receptors That Are Ligand-Gated Ion Channels

Functional Type	Ligand†	Ion Channel
Excitatory Receptors*	Acetylcholine (for nicotinic receptor)	Na^+/K^+
	Glutamate (for NMDA class receptors)‡	Na^+/K^+ and Ca^{2+}
	Glutamate (for non-NMDA class receptors)§	Na^+/K^+
	Serotonin (for $5HT_3$ class receptors)	Na^+/K^+
Inhibitory Receptors*	GABA (γ-aminobutyric acid) (for A-class receptors)	Cl^-
	Glycine	Cl^-

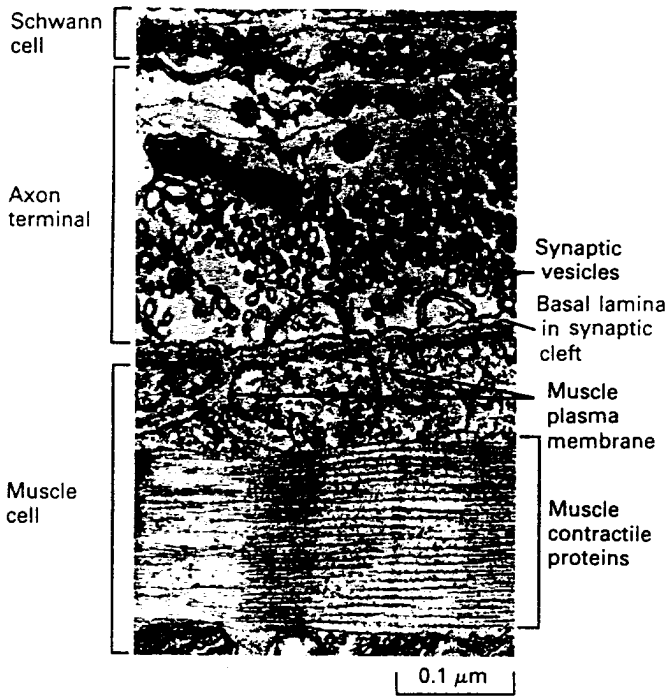
* All of these receptor proteins have a very similar structure, as depicted for the nicotinic receptor for acetycholine in Figures 21-39 and 21-40.

† Most of these ligands also bind to receptors that are coupled to G proteins (Table 21-2); thus it is important to define the class of receptors for each ligand.

‡ Glutamate receptors of this class are selectively activated by the nonnatural amino acid N-methyl-D aspartate (NMDA); receptors of this class require both glutamate (or NMDA) and a slightly depolarized membrane in order to open the ion channel. These receptors are thought to be important in long-term potentiation, a form of memory (Figure 21-56).

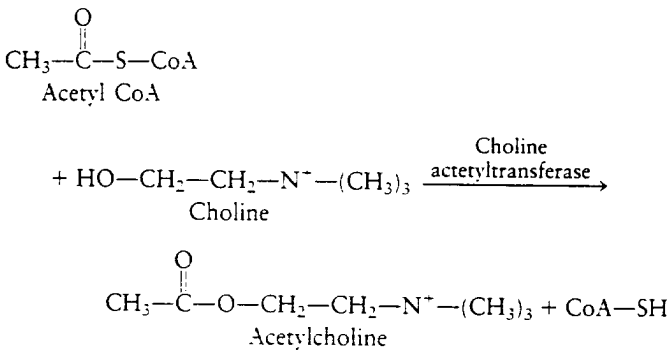
§ For glutamate receptors of this class, opening of the cation channel only requires binding of glutamate.

SOURCE: H. Lester, 1988, *Science* 241:1057; E. Barnard, 1988, *Nature* 335:301; N. Unwin, 1993, *Cell* vol. 72/*Neuron* vol. 10 (Suppl), pp. 31–41; A. Maricq, A. Peterson, A. Brake, R. Myers, and D. Julius, 1991, *Science* 254:432.



◀ FIGURE 21-34 Longitudinal section through a frog nerve-muscle synapse. The plasma membrane of the muscle cell is extensively folded. The basal lamina lies in the synaptic cleft separating the neuron from the muscle membrane. The axon terminal is surrounded by a Schwann cell, which periodically interdigitates between the terminal and the muscle. Acetylcholine receptors are concentrated in the postsynaptic muscle membrane at the top and part way down the sides of the folds in the membrane. Synaptic vesicles in the axon terminal are clustered near specializations lying just inside the presynaptic plasma membrane where exocytosis occurs. From J. E. Heuser and T. Reese, 1977, in E. R. Kandel, ed., *The Nervous System*, vol. 1, *Handbook of Physiology*, Williams & Wilkins, p. 266.]

Acetylcholine is synthesized in the cytosol of axon terminals from acetyl coenzyme A (CoA) and choline by the enzyme choline acetyltransferase:

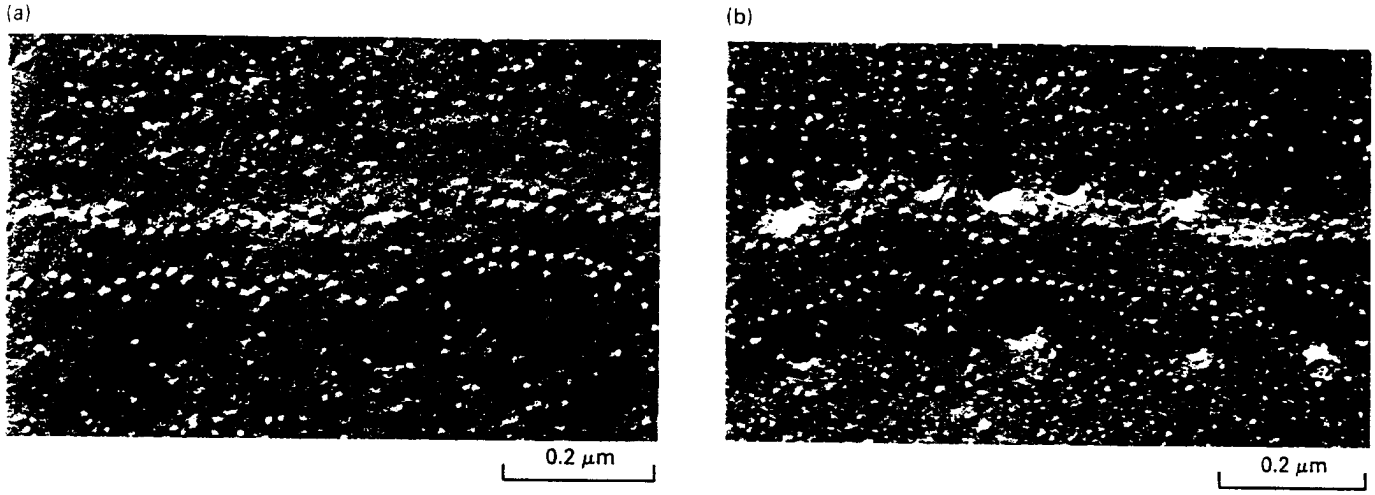


The synaptic vesicles take up and concentrate acetylcholine from the cytosol against a steep concentration gradient, using a proton-acetylcholine antiport transporter in the vesicle membrane (Figure 21-36).

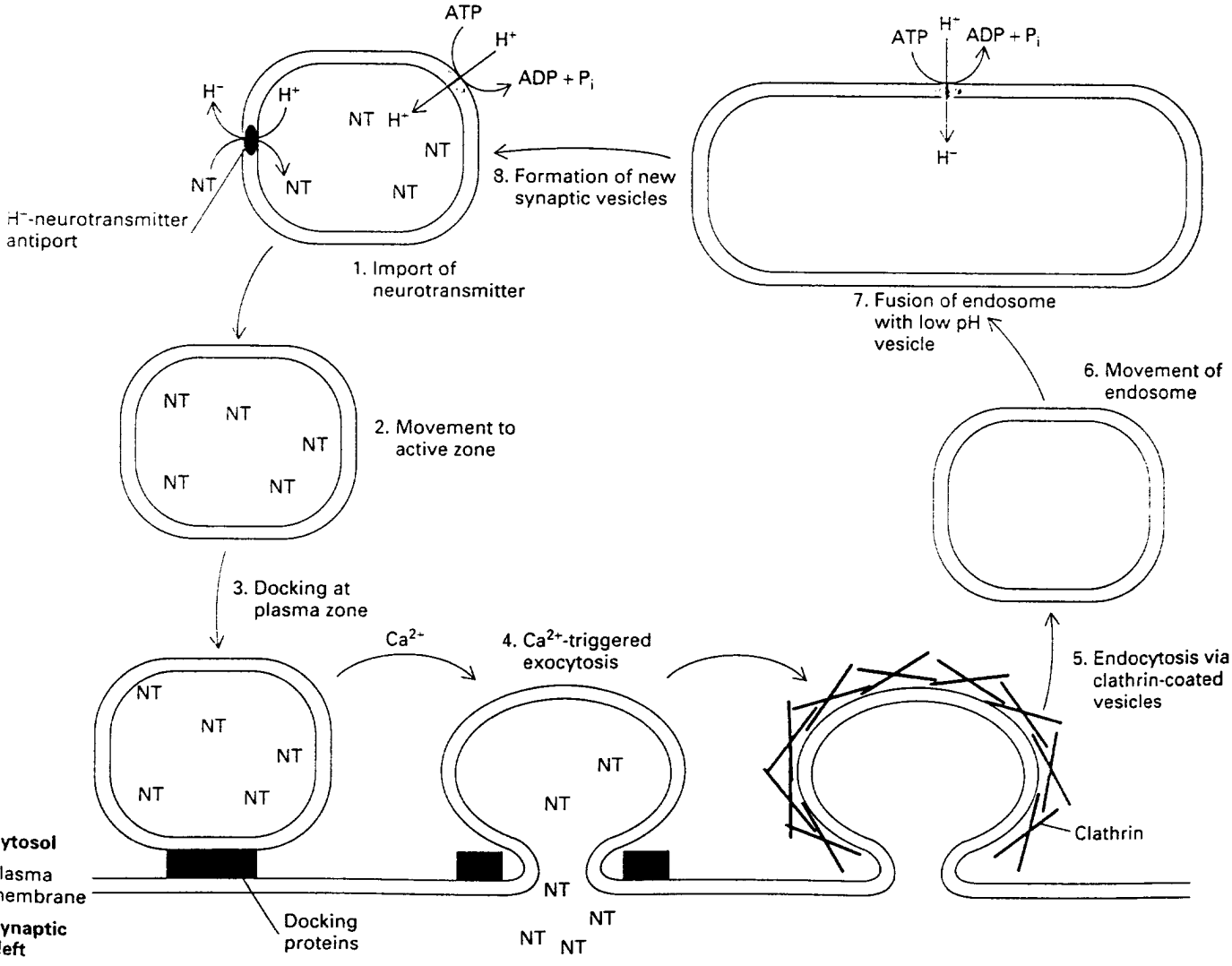
Exocytosis of Synaptic Vesicles Is Triggered by the Opening of Voltage-Gated Calcium Channels and a Rise in Cytosolic Calcium

The arrival of an action potential in the axon terminal depolarizes the plasma membrane, opening voltage-gated Ca^{2+} channels and thus causing an influx of Ca^{2+} ions into the cytosol from the extracellular medium. The amount of Ca^{2+} that enters an axon terminal through voltage-gated Ca^{2+} channels is sufficient to raise the level of Ca^{2+} from <0.1 μM, characteristic of the cytosol in the resting state, to several micromolar in the region of the cytosol near the synaptic vesicles. The Ca^{2+} ions bind to proteins that connect the synaptic vesicle with the plasma membrane, inducing membrane fusion and thus exocytosis of the neurotransmitter into the synaptic cleft. The extra Ca^{2+} ions are

➤ FIGURE 21-36 Release of neurotransmitters and the endocytic cycle of synaptic vesicles. Vesicles import neurotransmitters from the cytosol (step 1) using a proton-neurotransmitter antiport. The low intravesicular pH powers neurotransmitter import, and is generated by a V-type ATPase in the vesicle membrane. The vesicles then move to the "active zone" near the plasma membrane (step 2) where they dock at defined sites (step 3). Exocytosis of the docked vesicles (step 4) is triggered by the rise in cytosolic Ca^{2+}, with release of neurotransmitters into the synaptic cleft. The synaptic vesicle membrane proteins are then specifically recovered by endocytosis in clathrin-coated vesicles (step 5) and move away from the active zone (step 6). Non-clathrin-coated vesicles (not shown) may also be used for endocytosis. The clathrin coat is depolymerized, and the vesicles fuse with larger, low pH, endosomes (step 7). New synaptic vesicles then form (step 8) by budding from these endosomes and then are filled with neurotransmitters (step 1), completing the cycle. [From T. Südhof and R. Jahn, 1991, *Neuron* **6**:665–677.]



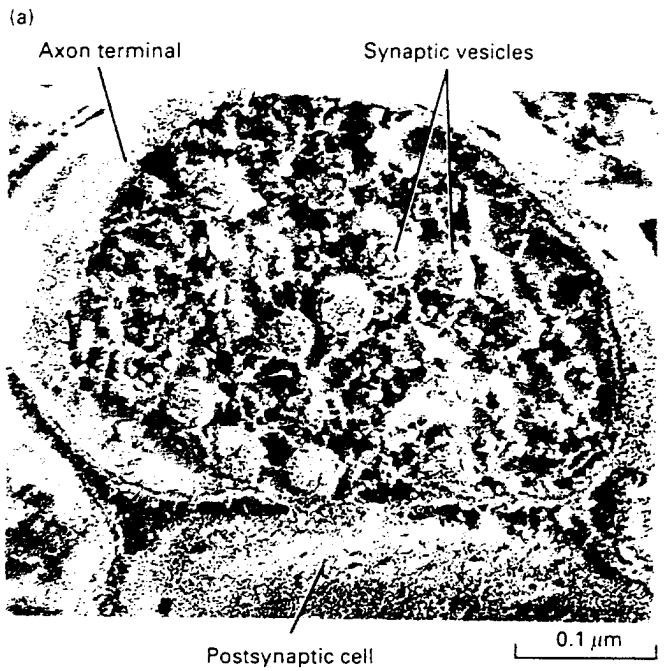
▲ FIGURE 21-35 Freeze-fracture image of the external face of the axonal plasma membrane at a neuron-muscle synapse, viewed from the vantage point of the muscle. (a) In the resting state, the membrane contains rows of particles that are aligned with rows of synaptic vesicles. The function of these particles is not known, but it is suspected that they are voltage-gated Ca^{2+} channels. (b) During stimulation, large pits in the membrane, resulting from exocytosis of synaptic vesicles, are visible near the rows of particles. [From J. E. Heuser and T. Reese, 1977, in E. R. Kandel, ed., *The Nervous System*, vol. 1, *Handbook of Physiology*, Williams & Wilkins, p. 268.]



rapidly pumped out of the cell by Ca^{2+} ATPases, lowering the cytosolic Ca^{2+} level and preparing the terminal to respond again to an action potential.

The presence of voltage-gated Ca^{2+} channels in axon terminals has been demonstrated in neurons treated with drugs that block Na^+ channels and thus prevent conduction of action potentials. When the membrane of axon terminals in such treated cells is artificially depolarized, an influx of Ca^{2+} ions into the neurons occurs and exocytosis is triggered. Patch-clamping experiments show that voltage-gated Ca^{2+} channels, like voltage-gated Na^+ channels, open transiently upon depolarization of the membrane. Several voltage-gated Ca^{2+} channels have been purified and cloned. Their sequences and structures are similar to those of voltage-gated Na^+ channels (see Figure 21-25).

More generally, voltage-gated Ca^{2+} channels can be viewed as the transducer of the electric signal in a nerve membrane; that is, the action potential is transduced into a chemical signal. Depolarization of the plasma membrane cannot, in itself, cause synaptic vesicles to fuse with the plasma membrane. The electric signal must be converted into a rise in Ca^{2+} concentration, which, in turn, induces synaptic vesicle fusion.

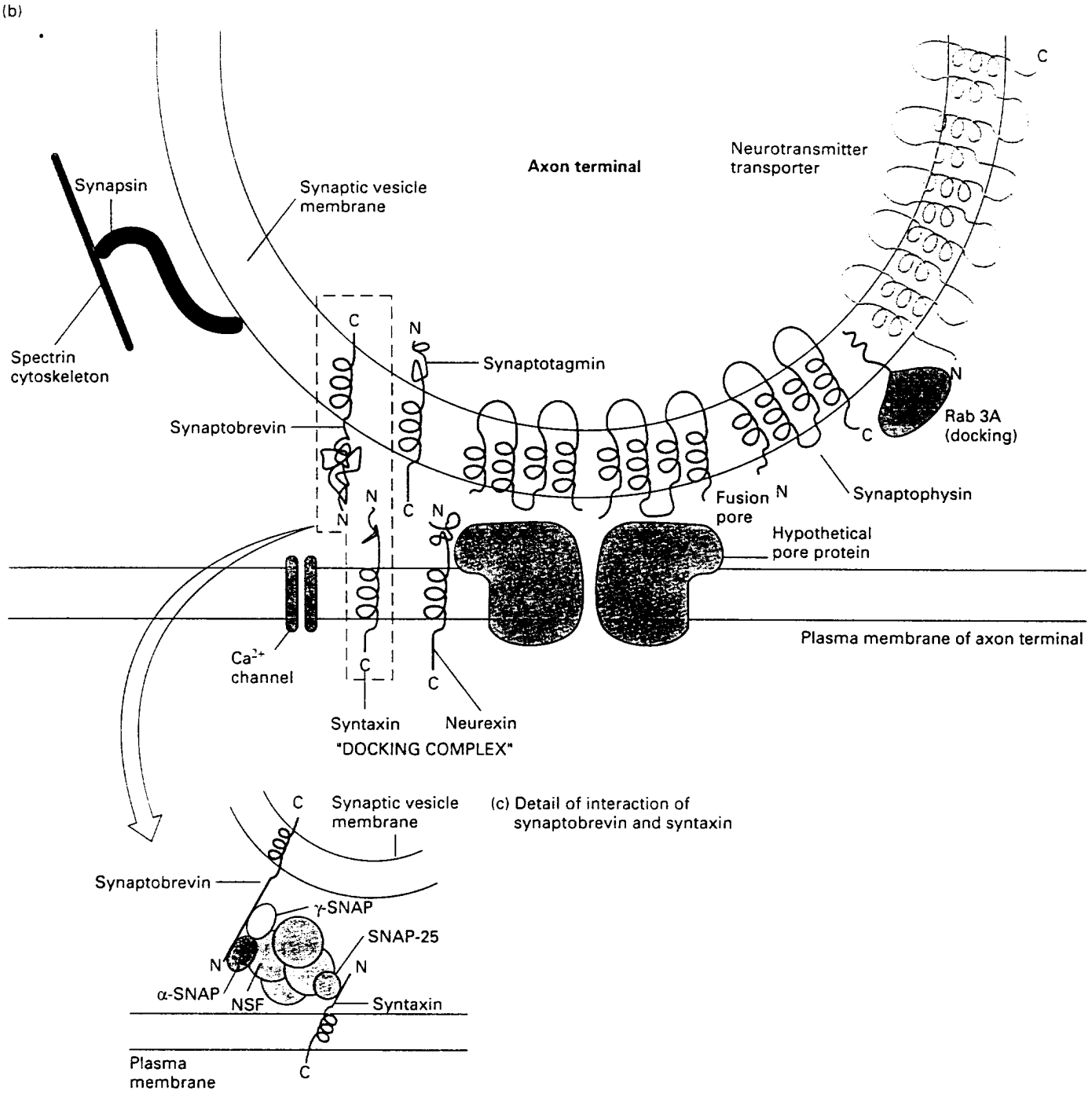


▲ FIGURE 21-37 (a) Micrograph of an axon terminal obtained by the rapid-freezing deep-etch technique. (b) *Facing page:* Structure and function of synaptic vesicle proteins and their interactions with proteins in the plasma membrane of the axon terminal. Synapsins interconnect synaptic vesicles and also connect vesicles to spectrinlike filaments extending inward from the plasma membrane. At least two vesicle proteins, synaptobrevin and synaptotagmin, are important for docking the vesicle to the underside of the plasma membrane; a complex of proteins including NSF, α-SNAP, γ-SNAP, and SNAP-25 binds synaptobrevin to the plasma membrane protein syntaxin, as detailed in (c). Neurexin, Ca^{2+} channels, and other plasma membrane proteins localized to the synaptic region also interact with synaptic vesicle proteins. Synaptophysin contains four membrane-spanning α helixes and may form part of the fusion pore between the synaptic vesicle and plasma membrane. Rab3A is important for localizing the synaptic vesicles to the fusion zone, but the proteins with which it interacts are not known. The synaptic vesicle membrane also contains transporters for specific neurotransmitters and, not shown here, V-type proton ATPases. [Part (a) from D. M. D. Landis et al., 1988, *Neuron* **1**:201. Parts (b) and (c) after T. M. Jessell and E. R. Kandel, 1993, *Cell* vol. 72/*Neuron* vol. 10 (Suppl), pp 1–30 and T. Söllner et al., 1993, *Nature* **362**:318–324.]

Multiple Proteins Align Synaptic Vesicles with the Plasma Membrane and Participate in Vesicle Exocytosis and Endocytosis

There are two pools of synaptic vesicles, those docked at the plasma membrane that can be readily exocytosed and those that provide a reservoir near the plasma membrane in the active zone. The triggering of vesicle exocytosis by a rise in Ca^{2+} is but one of a series of steps involved in forming synaptic vesicles, filling them with neurotransmitter, moving them to the active zone near the plasma membrane, docking them at the plasma membrane, and then, after vesicle fusion with the plasma membrane, recycling their membrane components by endocytosis (Figure 21-36). Recycling of synaptic vesicle membrane proteins is rapid, as indicated by the ability of many neurons to fire fifty times a second, and quite specific, in that several membrane proteins unique to the synaptic vesicles are specifically internalized by endocytosis.

The axon terminal exhibits a highly organized arrangement of cytoskeletal fibers that appear essential for localizing the vesicles to the plasma membrane at the synaptic cleft (Figure 21-37a). The vesicles themselves are linked together by the fibrous phosphoprotein synapsin (Figure 21-37b). Synapsin is localized in the cytosolic surface of all synaptic vesicle membranes and constitutes 6 percent of vesicle proteins; it is related in structure to band 4.1 protein in erythrocyte membranes, a protein that binds both actin and spectrin (see Figure 14-38). Thicker filaments composed of a spectrinlike protein radiate from the plasma membrane and bind to vesicle-associated synapsin. Probably these interactions keep the synaptic vesicles close to



the part of the plasma membrane facing the synapse. Synapsins are substrates of cAMP-dependent and calcium-calmodulin-dependent protein kinases, and the rise in cytosolic Ca^{2+} triggers their phosphorylation. This apparently causes the release of synaptic vesicles from the cytoskeleton and increases the number of vesicles available for fusion with the plasma membrane.

Synaptic vesicles contain several proteins important for their function. Six synaptophysin polypeptides (MW 38,000) form a complex in the synaptic vesicle membrane. This complex may be part of the fusion pore between the synaptic vesicle and plasma membrane together with as yet unidentified proteins in the plasma membrane. V-type ATPases (see Figure 15-10) generate a low intravesicular pH, and proton-coupled antiporters for neurotransmitters catalyze import of neurotransmitters from the cytosol where they are synthesized (Figure 21-36). Rab3A is a neuron-specific GTP-binding protein similar in sequence

and function to the rab proteins that control vesicular traffic in the secretory pathway (see Figure 16-43). It appears to be essential for localization of the synaptic vesicles to the active zone, as indicated by an experiment in which a mutant Rab3A protein that cannot bind GTP was expressed in cultured neurons: the synaptic vesicles became randomly localized in the axon terminals. Synaptotagmin binds the plasma membrane protein neurexin (Figure 21-37b) and may also participate in vesicle docking.

Two experimental observations established that synaptobrevin, another abundant synaptic vesicle protein, is essential for interaction of the vesicle with the plasma membrane. First, synaptobrevin binds a protein complex containing NSF and SNAPs, proteins that are also required for fusion of other intracellular vesicles with their target membranes (for instance, for fusion of ER-to-Golgi transport vesicles with the *cis* region of the Golgi—see Figure 16-42c). The NSF-SNAP complex also binds syntaxin, a plasma membrane protein that is localized to the active zone of axon terminals, and thus the synaptobrevin-NSF-syntaxin complex may anchor the vesicle to the plasma membrane so that they can fuse together when Ca^{2+} is increased. Other evidence for the role of synaptobrevin came from a study of *botulinum-B toxin,* a bacterial protein that can cause the paralysis and death characteristic of botulism, a type of food poisoning. The toxin is composed of two polypeptides, one of which binds to cholinergic motor neurons and facilitates the entry of the other into the cytosol. The second polypeptide is a protease with a remarkable specificity—the only protein it cleaves is synaptobrevin, and by destroying synaptobrevin it blocks acetylcholine release at the neuromuscular synapse.

The synaptic vesicle protein synaptotagmin contains two binding sites for Ca^{2+} in its cytosolic domain; it is thought to be the key Ca^{2+} sensing protein that triggers vesicle exocytosis. In one set of experiments, injection of an antibody specific for the cytosolic domain of synaptotagmin into a neuron inhibited Ca^{2+}-stimulated vesicle exocytosis. Other experiments used a series of synaptotagmin mutants of *Drosophila.* Embryos that completely lacked synaptotagmin failed to hatch and exhibited very reduced, uncoordinated muscle contractions. Larvae with partial lack-of-function mutations of synaptotagmin survived, but their neurons were defective in Ca^{2+}-stimulated vesicle exocytosis. How synaptotagmin functions is beginning to be understood. In resting cells (with low cytosolic Ca^{2+}) synaptotagmin apparently binds to the complex of synaptobrevin and syntaxin; the presence of synaptotagmin blocks the binding of the fusion protein α-SNAP to the complex and thus prevents vesicle fusion. When synaptotagmin binds Ca^{2+} it is displaced from the complex, allowing α-SNAP to bind and thus initiating membrane fusion. Thus synaptotagmin operates as a "clamp" to prevent fusion from proceeding in the absence of a Ca^{2+} signal.

The Nicotinic Acetylcholine Receptor Protein Is a Ligand-Gated Cation Channel

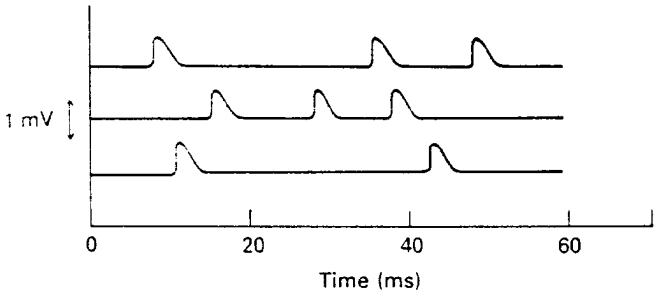
Once acetylcholine molecules have been released from neurons, they diffuse across the synaptic cleft and combine with *nicotinic acetylcholine receptor* molecules in the membrane of the postsynaptic cell. The interaction of acetylcholine with the nicotinic acetylcholine receptor produces within 0.1 ms a large transient increase in the permeability of the membrane to both Na^+ and K^+ ions. Thus the nicotinic acetylcholine receptor is a ligand-gated cation channel.

Since the resting potential of the muscle plasma membrane is near E_K, the potassium equilibrium potential, opening of acetylcholine receptor channels causes little increase in the efflux of K^+ ions; Na^+ ions, on the other hand, flow into the muscle cell. The simultaneous increase in permeability to Na^+ and K^+ ions produces a net depolarization to about -15 mV from the resting potential of about -60 mV. This depolarization of the muscle membrane generates an action potential, which—like an action potential in a neuron—spreads along the membrane surface via voltage-gated Na^+ channels (see Figure 21-18). As detailed in Chapter 22, the resultant depolarization triggers Ca^{2+} movement from its intracellular store, the sarcoplasmic reticulum, into the cytosol, and then contraction.

The snake venom toxin α-bungarotoxin binds specifically and irreversibly to nicotinic acetylcholine receptors. When radioactive α-bungarotoxin is added to sections of muscle cells, the toxin—as detected by autoradiography (see Figure 6-24)—becomes localized in the membranes of postsynaptic striated muscle cells immediately adjacent to the terminals of presynaptic neurons. Thus, the nicotinic acetylcholine receptors are clustered in this domain of the muscle plasma membrane. Patch-clamping studies on isolated outside-out patches of muscle plasma membranes (Figure 21-23c), showed that, in response to acetylcholine, a cation channel in the receptor opened and remained open for several milliseconds before closing spontaneously. When open, each channel is capable of transmitting 15,000–30,000 Na^+ or K^+ ions a millisecond. The time required for an acetylcholine molecule to induce the opening of a channel is too short to be measured directly but is probably a few microseconds.

Spontaneous Exocytosis of Synaptic Vesicles Produces Small Depolarizations in the Postsynaptic Membrane

Careful monitoring of the membrane potential of the muscle membrane at a synapse with a cholinergic motor neuron has demonstrated spontaneous, intermittent depolarizations of about 0.5–1.0 mV in the absence of stimulation



➤ FIGURE 21-38 Spontaneous depolarizations in the postsynaptic membrane of a cholinergic synapse. Recordings were taken from intracellular electrodes in an unstimulated frog muscle cell near the neuron-muscle synapse (see Figure 21-11a). Such depolarizations—each generated by the spontaneous release of acetylcholine from a single synaptic vesicle—seem to occur at random intervals.

of the motor neuron (Figure 21-38). Each of these depolarizations is caused by the spontaneous release of acetylcholine from a single synaptic vesicle. Indeed, demonstration of the spontaneous small depolarizations led to the notion of quantal release of acetylcholine (later applied to other neurotransmitters) and thereby led to the hypothesis of vesicle exocytosis at synapses. The release of one vesicle of acetylcholine results in the opening of about 3000 ion channels in the postsynaptic membrane—sufficient to depolarize the region of membrane by about 1 mV but insufficient to reach the threshold for an action potential.

The Nicotinic Acetylcholine Receptor Contains Five Subunits, Each of Which Contributes to the Cation Channel

In most nerve and muscle tissues the nicotinic acetylcholine receptor constitutes a minute fraction of the total membrane protein. Electric eel and electric ray (torpedo) electric organs are particularly rich in this receptor and are the sources used for its purification. Computer-generated averaging of many high-resolution electron microscopic images of purified acetylcholine receptors, viewed from several angles, has led to the pentameric model shown in Figure 21-39a. Each molecule has a diameter of about 9 nm and protrudes from the membrane surfaces about 6 nm into the extracellular space and about 2 nm into the cytosol. A tapered central pore, with a maximum diameter of 2.5 nm, is the cation channel. The channel opens when the receptor cooperatively binds two acetylcholine molecules.

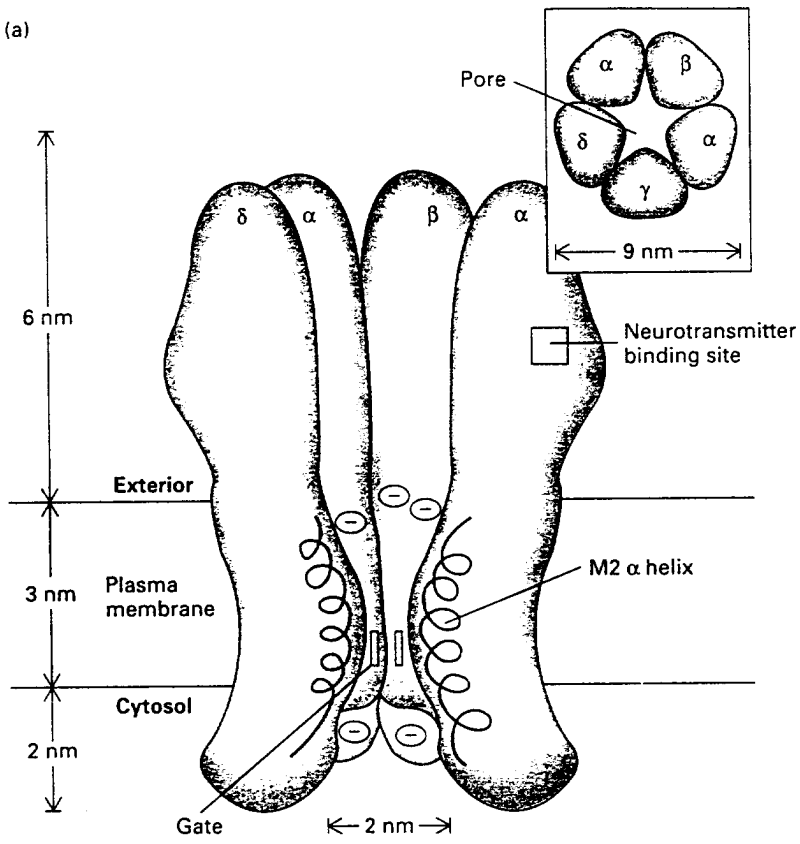
The nicotinic acetylcholine receptor can be solubilized from these membranes by nonionic detergents. The crucial step in its purification is affinity chromatography on columns of immobilized cobra toxin, which binds the receptor but from which it can be subsequently eluted and recovered. The molecular weight of the receptor protein is 250,000–270,000. The receptor consists of four different polypeptides with the composition $\alpha_2\beta\gamma\delta$.

Messenger RNAs encoding all four receptor subunits have been cloned. When all four are microinjected into a single frog oocyte (see Figure 21-27), functional nicotinic acetylcholine receptors form. No channels, or poorly functional ones, are obtained if the mRNA for one subunit is omitted. Thus all four subunit polypeptides—and only these—are needed for receptor function.

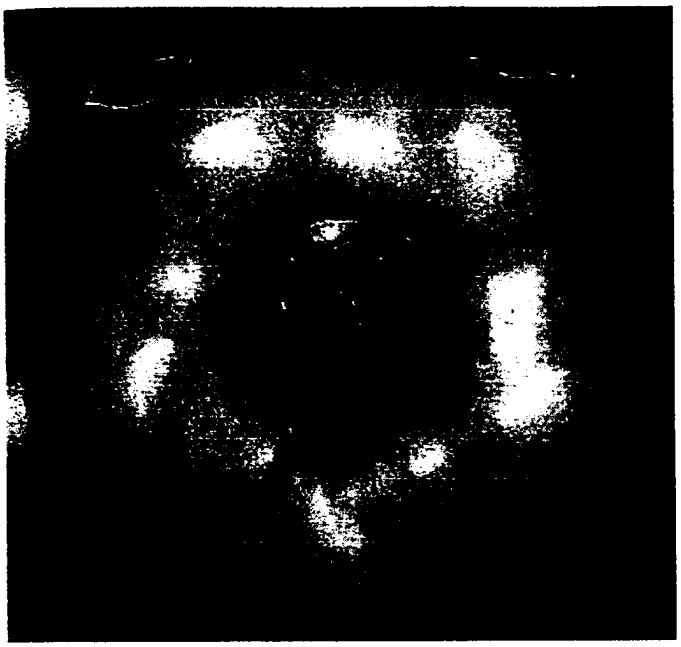
The α, β, γ, and δ subunits have considerable sequence homology; on average, about 35–40 percent of the residues in any two subunits are homologous. Each subunit is thought to contain one transmembrane α helix, called M2; the bulk of the receptor consists of multiple β strands. The complete receptor has a fivefold symmetry, and the actual cation channel is thought to be formed by segments from each of the five subunits (Figures 21-39 and 21-40). Studies measuring the permeability of different small cations have suggested that the ion channel is, at its narrowest, about 0.65–0.80 nm in diameter, sufficient to allow passage of both Na^+ and K^+ ions with their tightly bound shell of water molecules (see Figure 2-12).

The structure of the channel is not known in molecular detail. However, much evidence indicates that the channel is lined by five transmembrane M2 α helices, one from each of the five subunits. One reason for thinking that M2 helices form the channel is that certain positively charged organic molecules, such as chlorpromazine, inhibit receptor function by "plugging" the ion channel. Chlorpromazine can be chemically cross-linked to serine residues (number 254 in the β subunit, 262 in δ) in the middle of the M2 helices (Figure 21-39b).

A second line of evidence for the role of M2 helices comes from expression of mutant receptor subunits in frog oocytes. Amino acid residues with negatively charged side chains (glutamate, aspartate) are on both sides of the membrane-spanning M2 helices. If just one of these residues in one subunit is mutated to a lysine, and the mutant mRNA is injected together with mRNAs for the other three wild-type subunits, a functional channel forms but its ion conductivity—the number of ions that can cross it during its open state—is reduced. The greater the number of glutamate or aspartate residues mutated (in one or another subunit), the greater the reduction in conductivity. It is



▲ FIGURE 21-39 Proposed structure of the nicotinic acetylcholine receptor. (a) Schematic model of the pentameric receptor, which contains four different polypeptides and has the composition $\alpha_2\beta\gamma\delta$; for clarity, the γ subunit is not shown. This model is based on amino acid sequence data, information from site-specific mutations, and analysis of electron micrographs. Most of the mass of the protein protrudes from the outer (synaptic) surface of the plasma membrane. Each α subunit contains an acetylcholine binding site and most of the mass of the receptor is composed of β sheets. The M2 α helix (red) in each subunit is part of the lining of the ion channel. The gate, which is opened by binding of the acetylcholine neurotransmitter, lies within the pore. Inset: Cross section of the exoplasmic face of the receptor showing the arrangement of subunits around the central pore. At its narrowest (in the membrane), the ion channel is about 0.65–0.80 nm in diameter. (b) Amino acid sequences of the M2 helix in the β subunit and δ subunit. Negatively charged glutamate or aspartate residues (blue) are present at both ends of the M2 helices, on either side of the pore. They help to screen out anions, and assist in binding Na^+ and K^+ ions in the channel. Cross-linking of chlorpromazine to serine residues (yellow) in the middle of the M2 helices inhibits receptor function, indicating that these serines are in the pore. [See N. Unwin, 1993, *Cell* vol. 72/*Neuron* vol. 10 (Suppl.), pp. 31–41.]



◀ FIGURE 21-40 A view of the three-dimensional structure of the nicotinic acetylcholine receptor from above, looking into the synaptic entrance of the channel. The tunnel made by the synaptic entrance narrows abruptly after a length of about 6 nm, exposing the surfaces proposed by the model in Figure 21-39b to be the negatively charged rings at the ends of the M2 α-helical segments. [From N. Unwin, 1993, *Cell* vol. 72/*Neuron* vol. 10 (Suppl.), pp. 31–41.]

thought that the aspartate and glutamate residues form rings—one residue from each of the five chains—on either side of the channel and participate in attracting Na^+ or K^+ ions as they enter the pore.

The most dramatic evidence came from study of the $\alpha 7$ subunit of a neuronal nicotinic acetylcholine receptor. In neurons, it probably forms a receptor of the composition $\alpha^7{}_2\beta_3$. However, in *Xenopus* oocytes the $\alpha 7$ mRNA alone directs the expression of a functional acetylcholine-gated cation channel, presumably of composition $(\alpha 7)_5$. The α polypeptide of the GABA receptor is similar in sequence to the acetylcholine receptor α polypeptides; however, the GABA receptor is selective for anions, such as Cl^- (Table 21-3). Replacement of only three amino acids in the M2 helix of the α^7 acetylcholine receptor by the analogous amino acids in the GABA receptor converted the protein from an acetylcholine-gated *cation* channel to an acetylcholine-gated *anion* channel. Since the specificity of the channel for anions or cations is determined by the amino acid side chains in the M2 α helix, it is reasonable to conclude that the M2 helices line the ion channel.

Hydrolysis of Acetylcholine Terminates the Depolarization Signal

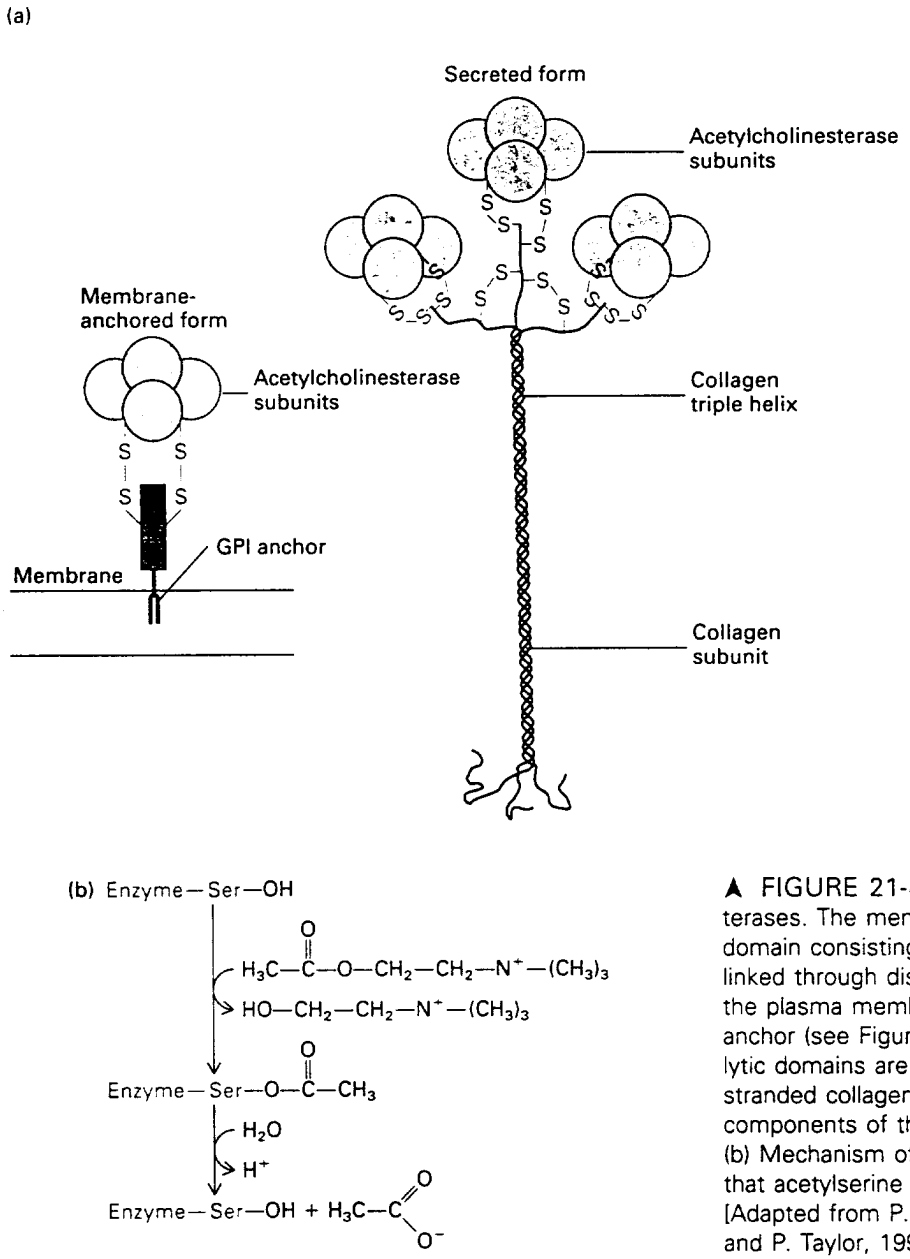
To restore a synapse to its resting state, the neurotransmitter must be removed or destroyed. There are three main ways to end the signaling: (1) the transmitter may diffuse away from the synaptic cleft; (2) the transmitter may be taken up by the presynaptic neuron; and (3) the transmitter may be enzymatically degraded. Signaling by acetylcholine is terminated by enzymatic degradation of the transmitter, but uptake is used to terminate signaling by most other neurotransmitters.

Acetylcholine is hydrolyzed to acetate and choline by the enzyme *acetylcholinesterase*, which is localized in the synaptic cleft between the neuron and muscle cell membranes. There are several forms of the enzyme (Figure 21-41a), one of which is bound to a network of collagen forming the basal lamina that fills this space (see Figure 21-34).

During hydrolysis of acetylcholine by acetylcholinesterase, a serine at the active site reacts with the acetyl group forming an enzyme-bound intermediate (Figure 21-41b). A large number of nerve gases and other neurotoxins inhibit the activity of acetylcholinesterase by reacting with the active-site serine. Physiologically, these toxins prolong the action of acetylcholine, thus prolonging the period of membrane depolarization. Such inhibitors can be lethal if they prevent relaxation of the muscles necessary for breathing.

➤ *Functions of Other Neurotransmitters, Their Receptors, and Their Transporters*

Because it causes a rapid, short-lived, and dramatic excitatory response in skeletal muscle cells, the nicotinic acetylcholine receptor was one of the first neurotransmitter re-



▲ FIGURE 21-41 (a) Alternative forms of acetylcholinesterases. The membrane-anchored form contains one catalytic domain consisting of four enzymatically active polypeptides, linked through disulfide bonds to a subunit that is inserted in the plasma membrane by a glycosylphosphatidylinositol anchor (see Figure 14-20). In the secreted form three catalytic domains are connected, by disulfide bonds, to a three-stranded collagenlike subunit that anchors the enzyme to components of the extracellular matrix in the synaptic cleft. (b) Mechanism of action of acetylcholinesterase, showing that acetylserine is an essential enzyme-bound intermediate. [Adapted from P. Taylor et al., 1987, *Trends Neurosci.* **10**:93 and P. Taylor, 1991. *J. Biol. Chem.* **266**:4025–4028.]

ceptors to be characterized definitively. Many other receptors are now understood in molecular detail; our study of these begins with several that trigger inhibitory, rather than excitatory, responses in postsynaptic cells.

GABA and Glycine Receptors Are Ligand-Gated Anion Channels Used at Many Inhibitory Synapses

Synaptic inhibition in the vertebrate central nervous system is mediated primarily by two amino acids, glycine and γ-aminobutyric acid (GABA); the latter is formed from glutamate by loss of a carboxyl group. The concentration of GABA in the human brain is 200—1000 times higher than that of other neurotransmitters such as dopamine, norepinephrine, and acetylcholine. Glycine is the major inhibitory neurotransmitter in the spinal cord and brain stem; GABA predominates elsewhere in the brain. Both glycine and GABA activate ligand-gated Cl^- channels.

The opening of Cl^- channels tends to drive the membrane potential toward the Cl^- equilibrium potential E_{Cl}, which in general is slightly more negative than the resting

membrane potential (see Figure 21-13). In other words, the membrane becomes slightly hyperpolarized. If many Cl^- channels are opened, the membrane potential will be held near E_{Cl}, and a much larger than normal increase in the sodium permeability will then be rquired to depolarize the membrane. The effect of GABA or glycine on Cl^- permeability is induced rapidly (a fraction of a millisecond) but can last for a second or more, a long time compared with the millisecond required to generate an action potential. Thus GABA or glycine induces a long lasting inhibitory postsynaptic response.

GABA and glycine receptors have been purified, cloned, and sequenced. In overall structure and sequence both resemble the nicotinic acetylcholine receptors (see Table 21-3). All are pentamers of similar subunits, although functional GABA and glycine receptors are built of only one or two different types of subunits. As noted already, the M2 helices of nicotinic acetylcholine receptors are thought to line the ion channel and to discriminate cations from anions. The negatively charged glutamate and aspartate side chains at the ends of the M2 helices in acetylcholine receptors may participate in cation binding (see Figure 21-39). Strikingly, the M2 helices of the GABA and glycine receptor subunits have lysine or arginine residues at these positions; positively charged side chains of these residues may attract Cl^- ions specifically.

Cardiac Muscarinic Acetylcholine Receptors Activate a G Protein and Open Potassium Channels

The response of skeletal muscle cells to the release of acetylcholine at the neuron-muscle junction is very rapid—the permeability changes and resulting membrane depolarization are completed within a few milliseconds (see Figure 21-33a). But many other synapses do not work as rapidly, and many functions of the nervous system operate with time courses of seconds or minutes. Responses that begin after a lag period of milliseconds following addition of transmitter are called slow postsynaptic potentials. Binding of acetylcholine to muscarinic acetylcholine receptors in frog aorta cardiac muscle generates a slow inhibitory response. Stimulation of the cholinergic nerves in heart muscle causes a long-lived (several seconds) hyperpolarization of the membrane (see Figure 21-33b) and slows the rate of heart muscle contraction.

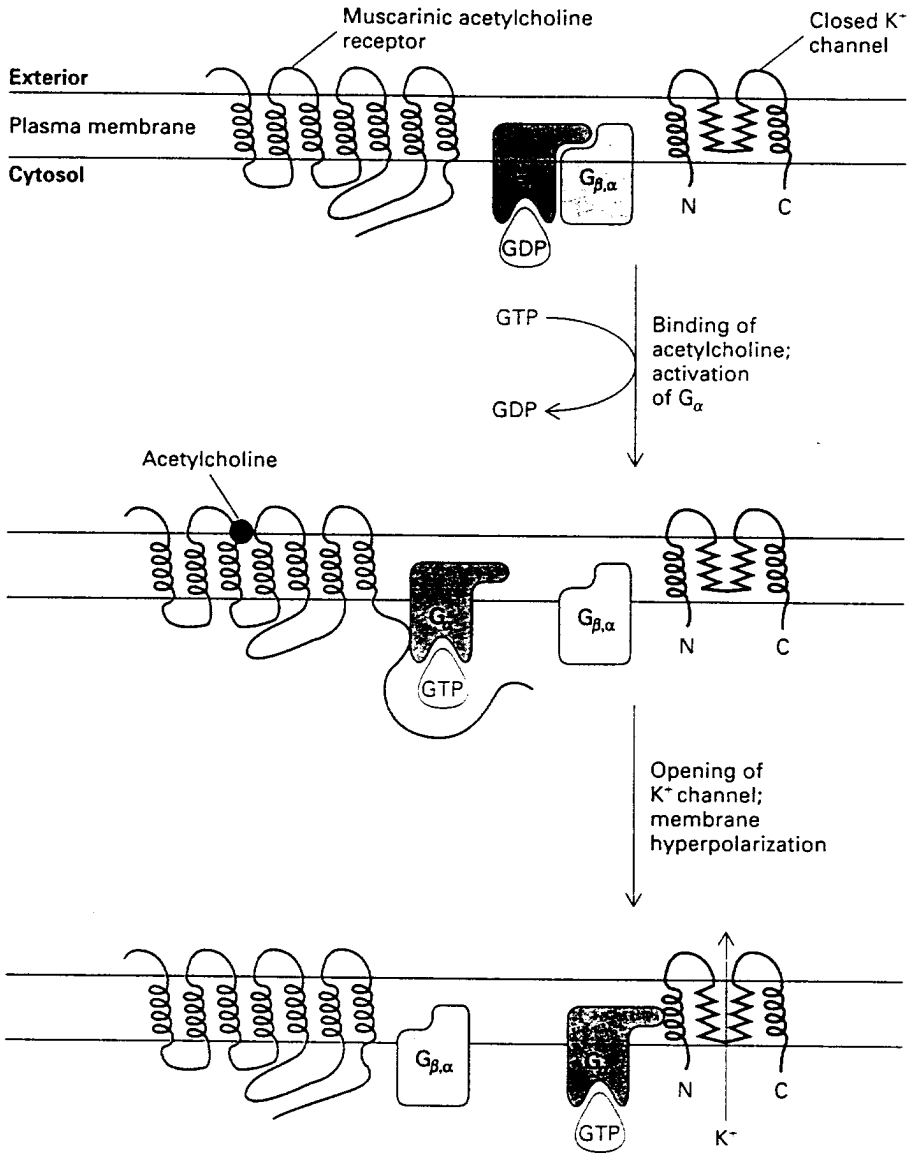
Figure 21-42 shows how activation of the cardiac muscarinic receptor leads to opening of K^+ channels and subsequent hyperpolarization of the plasma membrane. Binding of acetycholine to the receptor activates a transducing G protein; the active G_α-GTP subunit then directly binds to and opens a particular K^+ channel protein. That G_α-GTP directly activates the K^+ channel has been shown by single-channel recording experiments: when purified G_α-GTP was added to the cytosolic face of a patch of heart muscle plasma membrane (see Figure 21-23c), potassium channels opened immediately and in the absence of acetylcholine or other neurotransmitters. The $G_{\beta,\gamma}$ subunits, generated by receptor activation, also bind to and open these K^+ channels.

The cardiac muscarinic receptor illustrates one way in which G protein–coupled receptors affect ion channels: the active G_α-GTP and $G_{\beta,\gamma}$ subunits bind to a channel protein. Receptors for other neurotransmitters activate G proteins that, in turn, affect the activity of enzymes that synthesize or degrade intracellular second messengers. These, in turn, can affect the activity of channel proteins, as several examples will illustrate.

Different Catecholamine Receptors Affect Different Intracellular Second Messengers

Epinephrine and norepinephrine function as both systemic hormones and neurotransmitters. Norepinephrine is the transmitter at synapses with smooth muscles that are innervated by sympathetic autonomic motor neutrons, the neurons of the peripheral nervous system that increase the activity of the heart and internal organs in "fight or flight" reactions. Norepinephrine is also found at synapses in the central nervous system. Epinephrine is synthesized and released into the blood by the adrenal medulla, an endocrine organ that has a common embryologic origin with neurons of the sympathetic system. Unlike neurons, the medulla cells do not develop axons or dendrites.

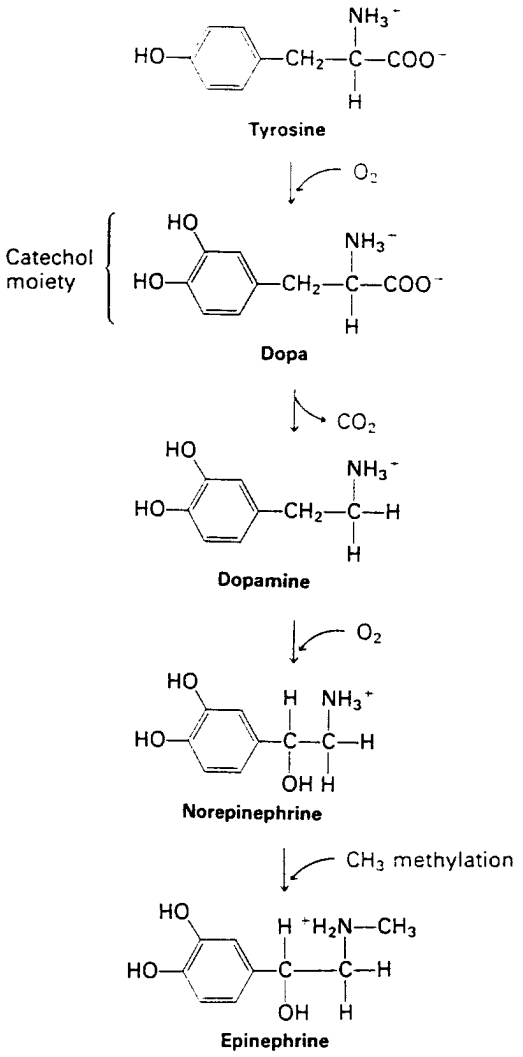
The neurotransmitters epinephrine, norepinephrine, and dopamine all contain the catechol moiety and are synthesized from tyrosine (Figure 21-43). These transmitters are referred to as catecholamines, and nerves that synthesize and use epinephrine or norepinephrine are termed *adrenergic*. All known receptors for catecholamines are coupled to G proteins, yet different ones affect different G proteins and thus different intracellular second messengers. The binding of agonists to β-adrenergic receptors on nerve cells causes activation of G_s and an increase in cAMP synthesis, the same mechanism by which β-adrenergic receptors function in nonneuronal cells (see Figures 20-14 and 20-15). As happens with the multiple neuronal muscarinic receptors, activation of other neuronal adrenergic receptors inhibits the synthesis of cAMP or increases the level of other intracellular second messengers, such as inositol *tris* phosphate, diacylglycerol, and arachidonic acid. The existence of multiple receptor-signaling pathways for the same neurotransmitter allows for great flexibility in nerve-nerve signaling.



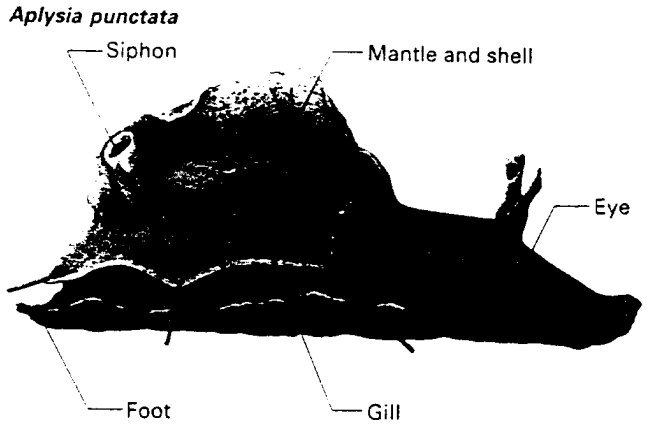
◀ FIGURE 21-42 Opening of K^+ channels in heart muscle plasma membrane. Binding of acetylcholine by muscarinic acetylcholine receptors triggers activation of a transducing G protein by catalyzing exchange of GTP for GDP on the α subunit. The active G_α-GTP and (not shown here) the released $G_{\beta\gamma}$ subunit bind to and open a K^+ channel. The increase in K^+ permeability hyperpolarizes the membrane, which reduces the frequency of heart muscle contraction. Though not shown here, the activation is terminated when the GTP bound to G_α is hydrolyzed to GDP and G_α · GDP recombines with $G_{\beta\gamma}$. The K^+ channel contains an H5 pore-lining segment, but is different from those in the *shaker* family (Figure 21-28) in that it has only two membrane-spanning α helixes. It, like other K^+ channels, probably is a tetramer. It is similar in sequence to the resting K^+ channels that maintain the resting membrane potential of all cells. [See K. Dunlap, G. Holz, and S. G. Rane, 1987, *Trends Neurosci.* **10**:241; E. Cerbai, U. Klockner, and G. Isenberg, 1988, *Science* **240**:1782; K. Ho et al., 1993, *Nature* **362**:31–37; and Y. Kubo et al., 1993, *Nature* **362**:127–133.]

A Serotonin Receptor Modulates Potassium Channel Function via the Activation of Adenylate Cyclase

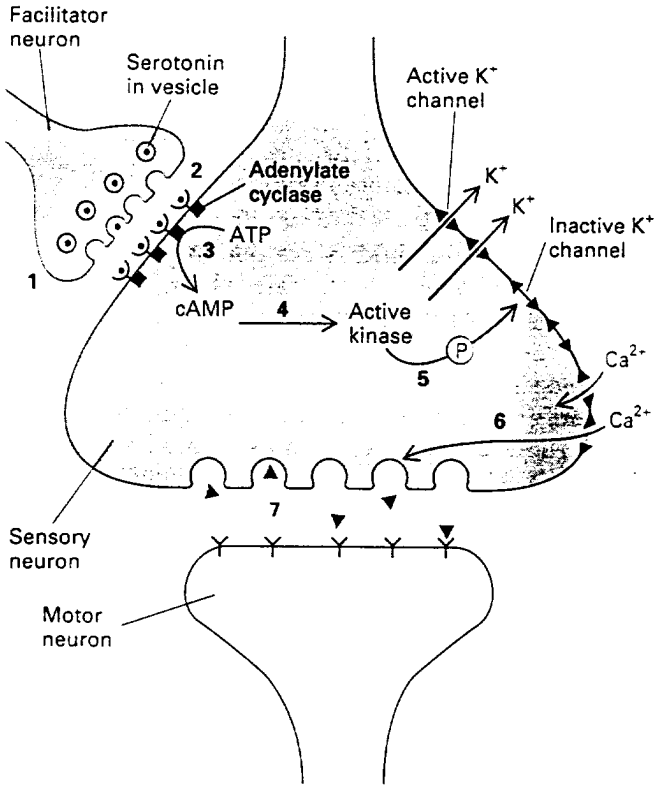
The effect of an increase in cAMP on channel function can best be seen in one well-studied synapse of the sea slug *Aplysia* (Figure 21-44). A particular type of interneuron, called a facilitator neuron (involved in a neural circuit we discuss later, Figure 21-54), forms a synapse with the axon terminal of a sensory neuron that stimulates a motor neuron via an unknown transmitter (Figure 21-45). Stimulation of the facilitator neuron increases the ability of the sensory neuron to stimulate the motor neuron. When the facilitator neuron is stimulated, it secretes serotonin, which



▲ FIGURE 21-43 Pathway for synthesis of the catecholamine neurotransmitters from tyrosine. The first step produces the catechol moiety, which is retained in all three transmitters.



▲ FIGURE 21-44 The sea slug *Aplysia punctata*. The gill is under the protective mantle; it can be seen if the overlying tissue is pulled aside. [Adapted from E. R. Kandel, 1976, *Cellular Basis of Behavior*, W. H. Freeman and Company, p. 76.]



▲ FIGURE 21-45 Pathway by which serotonin, released by stimulation of a facilitator neuron, increases the ability of a sensory neuron to activate a motor neuron in the sea slug *Aplysia*. The effect of serotonin is mediated through adenylate kinase and cAMP. Phosphorylation of the voltage-gated K^+ channel protein or a channel-binding protein, indicated by the circled P, prevents the K^+ channels from opening, leading to prolonged depolarization. See text for discussion. [See E. R. Kandel and J. Schwartz, 1982, *Science* **218**:433; M. B. Boyle et al., 1984, *Proc. Nat'l. Acad. Sci. USA* **81**:7642; and M. J. Schuster et al., 1985, *Nature* **313**:392.]

binds to serotonin receptors on the sensory neuron (Figure 21-45, steps 1 and 2). This binding activates adenylate cyclase, triggering the synthesis of cAMP in the sensory neuron (step 3). cAMP then activates a cAMP-dependent protein kinase, which phosphorylates a voltage-gated K^+ channel protein or an associated protein leading to an inability of the K^+ channels to open during an action potential (steps 4 and 5). This decreases the outward flow of K^+ ions that normally repolarizes the membrane of the sensory neuron after an action potential reaches the axon terminal. The resulting prolonged membrane depolarization increases the influx of Ca^{2+} ions through voltage-gated Ca^{2+} channels (step 6). The increased Ca^{2+} level leads to greater exocytosis of synaptic vesicles in the sensory neuron (step

7), and hence greater activation of the motor neuron each time an action potential reaches the terminal.

As evidence for this model, direct administration of serotonin through a micropipette to the sensory neuron causes decreased efflux of K^+ ions and prolonged depolarization of the membrane. Also, the *Aplysia* sensory neuron is large enough that proteins such as the active catalytic subunit of the cAMP-dependent protein kinase can be injected into it. Such treatment mimics the effect of applying the natural transmitter serotonin to the nerve. Additional supporting evidence that serotonin acts by means of cAMP and a protein kinase has come from patch-clamping studies on isolated inside-out pieces of sensory neuron plasma membrane (see Figure 21-23c). When both ATP and the purified active catalytic subunit of cAMP-dependent protein kinase were added to the cytosolic surface of the patches, the K^+ channels closed. Thus the protein kinase indeed acts on the cytosolic surface of the membrane to phosphorylate the channel protein itself or a membrane protein that regulates channel activity. We shall return to this particular synapse later, as these modifications in synapse efficiency are part of a simple learning response.

Neurotransmitter Transporters Are the Proteins Affected by Drugs Such as Cocaine

After their release into synapses, catecholamines and amino acid neurotransmitters are removed by transport into the axon terminals that released them. Re-uptake is the most widely used mechanism of transmitter removal; enzymatic degradation is used only for acetylcholine and neuropeptides. Transporters for GABA, norepinephrine, dopamine, and serotonin were the first to be cloned and studied. These four transporters are encoded by gene family, and are 60–70 percent identical in amino acid sequences. Each transporter is thought to have 12 membrane-spanning α helices. All four are Na^+-neurotransmitter symports and frequently Cl^- is transported along with the neurotransmitter. As with other Na^+ symports, the movement of Na^+ into the cell down its electrochemical gradient provides the energy for uptake of the neurotransmitter (see Figure 15-17). Study and cloning of these transporters was facilitated by the observation that, following microinjection of mRNA from regions of the brain into frog oocytes, functional transporters for these neurotransmitters were expressed on the oocyte plasma membrane.

The norepinephrine, serotonin, and dopamine transporters are all inhibited by cocaine. Cocaine inhibits dopamine uptake and thus prolongs signaling at key brain synapses; the dopamine transporter is the principle brain "cocaine receptor." Therapeutic agents such as the antidepressant drugs fluoxetine, imipramine, and Prozac, block the serotonin transporter, and the tricyclic antidepressant desipramine blocks norepinephrine uptake. However, the precise role of transporters in the antidepressant action of these drugs is not yet clear.

Some Peptides Function as Both Neurotransmitters and Neurohormones

Many of the small peptides found in nervous tissue function as synaptic neurotransmitters. Others act in a paracrine fashion (see Figure 20-1c) as "diffusible" hormones that affect many neurons over great distances. Yet other neuropeptides also act as regulators of nerve cell growth and division. Many of the peptides listed in Table 21-2 are found both in the brain and in nonneural tissues. However, in contrast to capillaries in other parts of the body, capillaries in the brain are essentially impermeable to peptides. Thus, any peptide hormones traveling through the body in the blood will be excluded from the brain: this constitutes the blood-brain barrier. Hormones in the blood do not "confuse" the functioning of the central nervous system.

Neurons that secrete peptide hormones, called neurosecretory cells, were first discovered in the hypothalamus. Secretion of peptide hormones by the anterior cells of the pituitary gland is controlled by the hypothalamus, which in turn is regulated by other regions of the brain. The hypothalamus is connected to the anterior pituitary by a special closed system of blood vessels (See Figure 20-5). Hypothalamic neurons secrete hypothalamic peptide hormones into these vessels, and the hormones then bind to receptors on the anterior pituitary cells. One such hypothalamic hormone, thyrotropin-releasing hormone (TRH), stimulates secretion by the anterior pituitary of prolactin and thyrotropin. Another hypothalamic hormone—luteinizing hormone–releasing hormone (LHRH)—causes other cells in the anterior pituitary to secrete follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which are important in regulating the growth and maturation of oocytes in the ovary (see Figure 20-5). The receptors for many neuropeptides have been cloned and all contain seven membrane-spanning α-helical segments and are coupled to G proteins (Table 21-2). Thus, the intracellular signaling pathways induced by neuropeptides are the same as those induced by the classical neurotransmitters.

Neuropeptide hormones, like all neuronal proteins, are synthesized in the cell body, packaged into secretory vesicles, and sent by axonal transport to the axon terminals. Peptide hormones are packaged into different vesicles than those used to store the "classical" neurotransmitters, such as serotonin, produced in the same neuron. In at least one organism, the sea slug *Aplysia*, different peptide hormones produced by proteolysis from the same precursor are packaged into different secretory vesicles; exocytosis of the different types of secretory vesicles is regulated. In contrast to

serotonin but like acetylcholine, peptide neurohormones and neurotransmitters are used only once and then degraded by proteases; they are not recycled.

Endorphins and Enkephalins Are Neurohormones That Inhibit Transmission of Pain Impulses

The activity of neurons in both the central and peripheral nervous systems is affected by a large number of neurohormones that act on cells quite distant from their site of release. Neurohormones can modify the ability of nerve cells to respond to synaptic neurotransmitters. Several small polypeptides with profound hormonal effects on the nervous system have been discovered; examples are Met-enkephalin, with the sequence Tyr-Gly-Gly-Phe-Met; Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu) and the 31-amino-acid peptide β-endorphin. These three contain a common tetrapeptide sequence, Tyr-Gly-Gly-Phe, that is essential to their functions. Enkephalins and endorphins function as natural pain killers, or opiates, and decrease the pain responses in the central nervous system.

Enkephalins were discovered during research in the early 1970s on opium addiction. Several groups of researchers discovered that brain plasma membranes contain high-affinity binding sites for purified opiates such as the alkaloid morphine. The sites were presumed to be the receptors that mediated the effects of these narcotic, analgesic drugs. Since such receptors exist in the brains of all vertebrates from shark to man, the question was raised why vertebrates should have highly specific receptors for alkaloids produced by opium poppies. Since none of the neurotransmitters and neuropeptides then known could serve as agonists or antagonists for the binding of opiates to brain receptors, a search was begun for natural compounds that could. This led to the discovery of two pentapeptides, Metenkephalin and Leu-enkephalin, both of which bind to the "opiate" receptors in the brain and have the same effect as morphine (a profound analgesia) when injected into the ventricles (cavities) of brains of experimental animals. Enkephalins and endorphins appear to act by inhibiting neurons that transmit pain impulses to the spinal cord; presumably these neurons contain abundant endorphin or enkephalin receptors.

Sensory Transduction: The Visual and Olfactory Systems

The nervous system receives input from a large number of sensory receptors (see Figure 21-9). Photoreceptors in the eye, taste receptors on the tongue, odorant receptors in the nose, and touch receptors on the skin monitor various aspects of the outside environment. Stretch receptors surround many muscles and fire when the muscle is stretched. Internal receptors monitor the levels of glucose, salt, and water in body fluids. The nervous system, the brain in particular, processes and integrates this vast barrage of information and coordinates the response of the organism.

The "language" of the nervous system is electric signals. Each of the many types of receptor cells must convert, or transduce, its sensory input into an electric signal. A few sensory receptors are themselves neurons that generate action potentials in response to stimulation. However, most are specialized epithelial cells that do not generate action potentials but synapse with and stimulate adjacent neurons that then generate action potentials. The key question that we will consider is how a sensory cell transduces its input into an electric signal.

In some cases, the sensory receptor protein is a Na^+ channel, and activation of the receptor causes an influx of Na^+ ions and thus membrane depolarization. Examples include the stretch and touch receptors that are activated by stretching of the cell membrane, and that have been identified in a wide array of cells, ranging from vertebrate muscle and epithelial cells to yeast, plants, and even bacteria.

The cloning of genes encoding touch receptors began with the isolation of mutant strains of the nematode *Caenorhabditis elegans* that were insensitive to touch. Three of the genes in which mutations were isolated, *MEC4*, *MEC6*, and *MEC10*, encode three similar subunits of a Na^+ channel in the touch receptor cells. These channels are necessary for touch sensitivity, and may open directly in response to mechanical stimulation. Similar kinds of channels are found in prokaryotes and lower eukaryotes; by opening in response to membrane stretching they may play a role in osmoregulation and the control of a constant cell volume. It is thought that such receptors were among the first sensory receptors to evolve. Similarly, of the four taste stimuli used by vertebrates (salty, sweet, bitter, and sour), the "receptors" for salt are the best understood. They are simply Na^+ channels in the apical membrane of taste receptor cells; entry of Na^+ through these channels depolarizes the membrane.

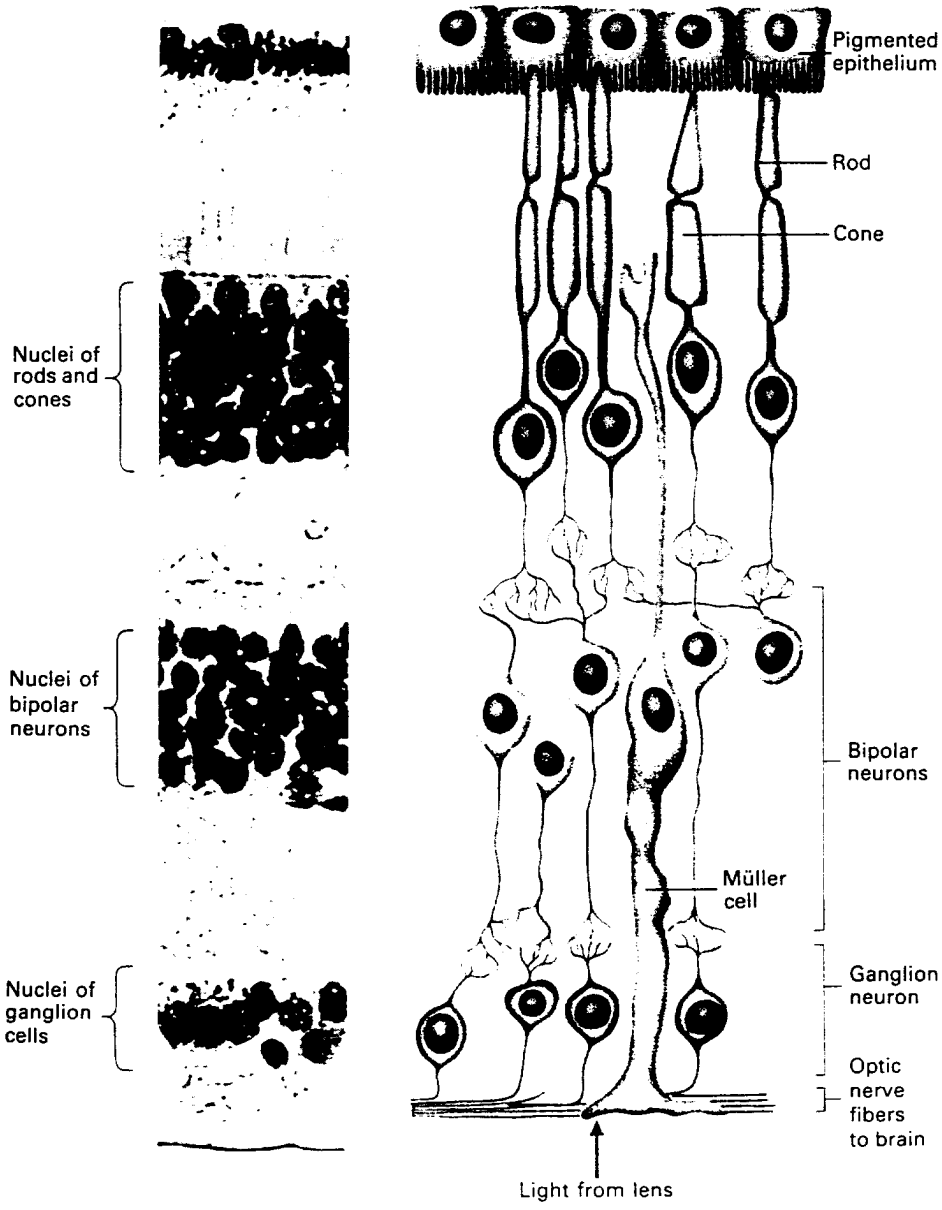
More often, though, the connection between the sensory receptor protein and the ion channel is indirect; the sensory receptor activates a G protein that, in turn, directly or indirectly induces the opening or closing of ion channels. The light receptors in the rod cells in the mammalian retina function in this manner, as do the olfactory (odor) receptors in the nose. We will discuss the light receptor cells in detail, as they are one of the best understood sensory systems, and they illustrate how a sensory system adapts to varying intensities of stimuli—here it is levels of ambient light that vary.

The Light-Triggered Closing of Sodium Channels Hyperpolarizes Red Cells

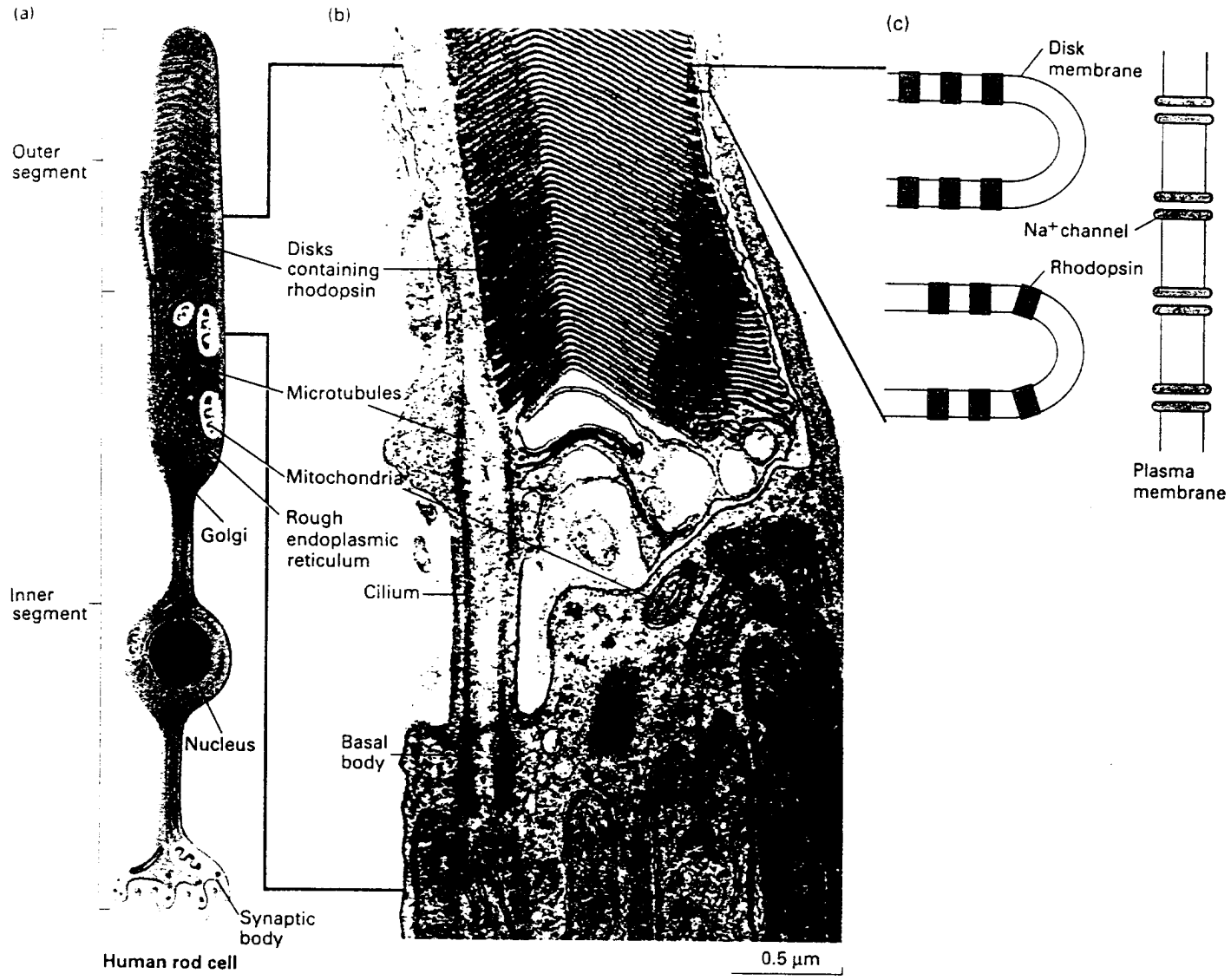
The human retina contains two types of photoreceptors, rods and cones (Figure 21-46). The cones are involved in color vision, and are discussed on page 977. The rods are stimulated by weak light over a range of wavelengths; in bright light, such as sunlight, the rods become inactive, for reasons that we will discuss. In the outer segment of the rod cell are membrane disks that contain the photoreceptor rhodopsin (Figure 21-47). Rod cells form synapses with neurons that in turn synapse with others that transmit impulses to the brain.

In the dark, the membrane potential of a rod cell is about −30 mV, considerably less than the resting potential (−60 to −90 mV) typical of neurons and other electrically active cells. As a consequence of this depolarization, rod cells in the dark are constantly secreting neurotransmitters, and the bipolar neurons with which they synapse are continually being stimulated. A pulse of light causes the membrane potential in the outer segment of the rod cell to become slightly hyperpolarized—that is, more negative (Figure 21-48). The light-induced hyperpolarization causes a decrease in release of neurotransmitters.

The depolarized state of the plasma membrane of resting, dark-adapted rod cells is due to the presence of a large



◀ FIGURE 21-46 Some of the cells in the neural layer of the human retina. The outermost layer of cells (in the rear of the eyeball) forms a pigmented epithelium in which the tips of the rod and cone cells are buried. Light focused from the lens passes through all of the cell layers of the retina and is absorbed by the photoreceptor proteins in the rods and cones. The axons of these cells synapse with many bipolar neurons. These, in turn, synapse with cells in the ganglion layer that send axons—optic nerve fibers—through the optic nerve to the brain. By synapsing with multiple rod cells, certain bipolar cells integrate the responses of many cells. They are involved in recognizing patterns of light that fall on the retina—for instance, a band of light that excites a set of rod cells in a straight line. Müller cells are supportive nonneural cells that fill much of the retinal spaces. Other types of cells are not depicted; all of the cells depicted here make many more synapses than are shown. [From R. G. Kessel and R. H. Kardon, 1979, *Tissues and Organs: A Text-Atlas of Scanning Electron Microscopy*, W. H. Freeman and Company, p. 87.]

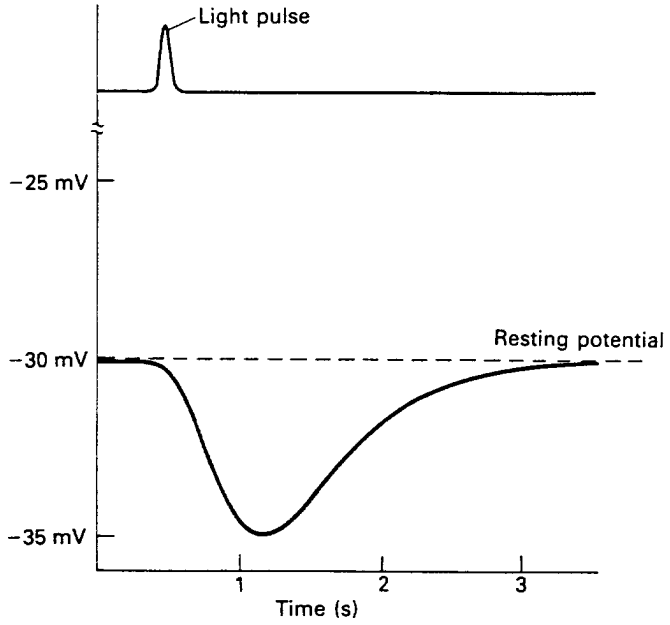


▲ FIGURE 21-47 (a) Diagram of the structure of a human rod cell. At the synaptic body, the rod cell forms a synapse with one or more bipolar neurons. (b) Electron micrograph of the region of the rod cell indicated by the bracket in (a); this region includes the junction of the inner and outer segments. (c) A diagram of a small region of the rod cell, showing that the disks, which contain rhodopsin, are not in contact with the Na^+ channels or other proteins in plasma membrane. [Part (b) from R. G. Kessel and R. H. Kardon, 1979, *Tissues and Organs: A Text-Atlas of Scanning Electron Microscopy*, W. H. Freeman and Company, p. 91.]

number of open Na^+ channels. The effect of light is to close Na^+ channels; as shown in Figure 21-13, the closing of Na^+ channels causes the membrane potential to become more negative. The more photons absorbed, the more Na^+ channels are closed, the more negative the membrane potential becomes, and the less neurotransmitter is released.

Remarkably, a single photon absorbed by a resting rod cell produces a measurable response, a hyperpolarization of about 1 mV, which lasts a second or two. Humans are able to detect a flash of as few as five photons. A single photon blocks the inflow of about 10 million Na^+ ions due to the closure of hundreds of Na^+ channels. Only about 30–50 photons need to be absorbed by a single rod cell in order to cause half-maximal hyperpolarization. The photoreceptors in rod cells, like many other types of receptors, exhibit the phenomenon of adaptation. That is, more photons are required to cause hyperpolarization if the rod cell is continuously exposed to light than if it is kept in the dark.

Let us now turn to three key questions: how is light

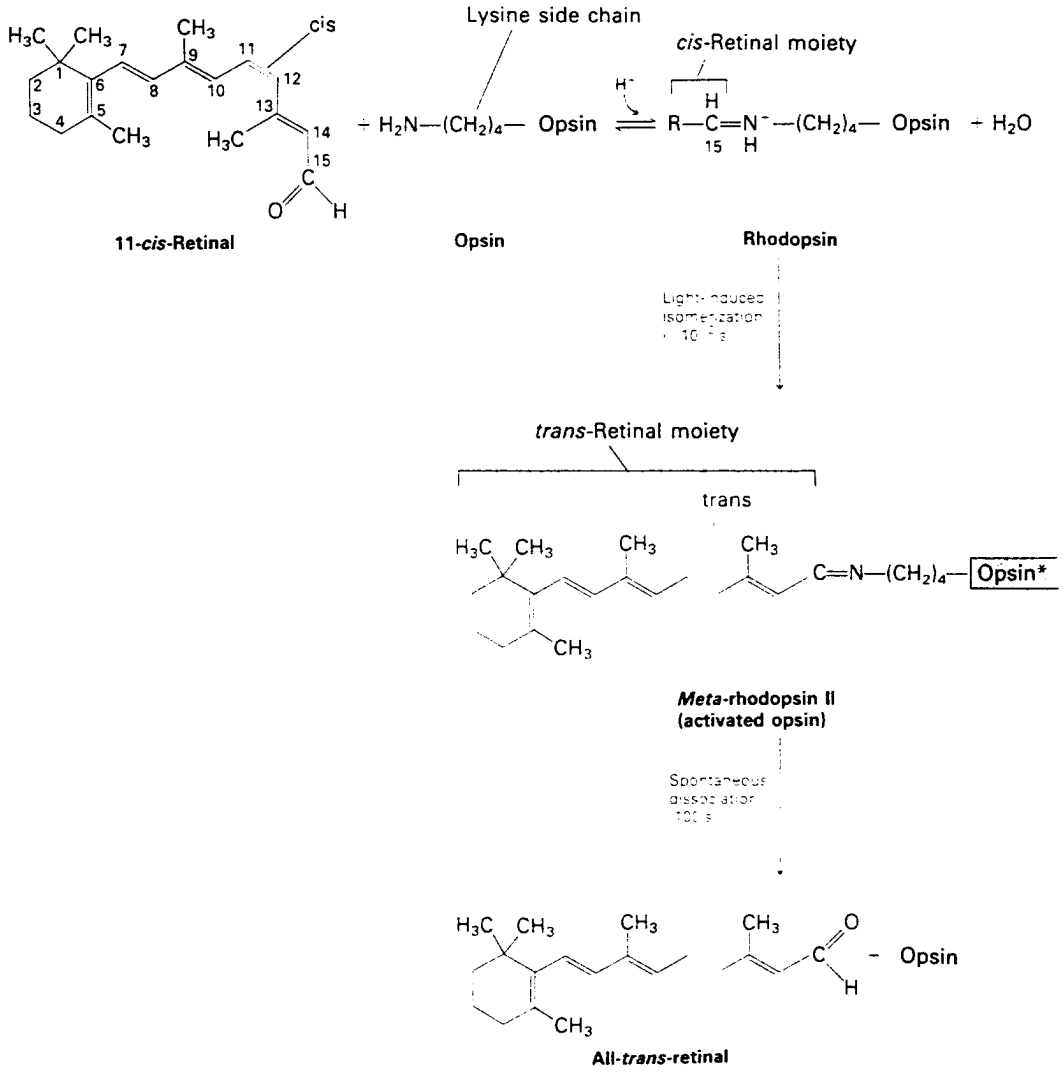


◀ FIGURE 21-48 A brief pulse of light causes a transient hyperpolarization of the rod-cell membrane. The membrane potential is measured by an intracellular microelectrode (see Figure 21-11a).

absorbed; how is the signal transduced into the closing of Na^+ channels; and how does the rod cell adapt to 100,000-fold or greater variations in light intensity?

Absorption of a Photon Triggers Isomerization of Retinal and Activation of Opsin

The photoreceptor in rod cells, rhodopsin, consists of the transmembrane protein opsin bound to the light-absorbing pigment 11-*cis*-retinal (Figure 21-49). Opsin has seven membrane-spanning α helices, similar to other receptors



➤ FIGURE 21-49 The photoreceptor in rod cells is rhodopsin, which is formed from 11-*cis*-retinal and opsin, a transmembrane protein. Absorption of light causes rapid photoisomerization of the *cis*-retinal to the *trans* isomer, forming the unstable intermediate *meta*-rhodopsin II, or activated opsin. The latter dissociates spontaneously to give all-*trans*-retinal and opsin. [See J. Nathans, 1992, *Biochemistry* **31**:4923–4931.]

that interact with transducing G proteins. Rhodopsin is localized to the thousand or so flattened membrane disks that make up the rod's outer segment; a human rod contains about 4×10^7 rhodopsin molecules.

The pigment 11-*cis*-retinal absorbs light in the visible range (400–600 nm). The primary photochemical event is isomerization of the 11-*cis*-retinal moiety in rhodopsin to all-trans-retinal, which has a different conformation than the *cis* isomer; thus the energy of light is converted into atomic motion. The stable intermediate in which opsin is bound to all-*trans*-retinal is called *meta*-rhodopsin II, or *activated opsin*. The light-induced formation of activated opsin is both extremely efficient and rapid. Approximately 20 percent of photons at a wavelength of 500 nm, that of maximum rhodopsin absorption, that strike the retina lead to a signal transduction event, an efficiency comparable to that of the best photomultiplier tubes. Of the 57 kcal/mole of energy of photons of 500 nm, 27 kcal/mole, or 47 percent is stored in the conformationally activated *meta*-rhodopsin II intermediate, making it an effective and reliable trigger of the next signaling step. An absorbed photon triggers opsin activation in less than 10 ms. In contrast, the spontaneous isomerization of 11-*cis*-retinal is extremely slow—about once per thousand years. This means that there is very little spontaneous activation of opsin, and that the system has a very good ratio of signal to noise.

Activated opsin is unstable and spontaneously dissociates, releasing opsin and all-*trans*-retinal. In the dark, all-*trans*-retinal is isomerized back to 11-*cis*-retinal in a reaction catalyzed by enzymes in rod-cell membranes; the *cis* isomer can then rebind to opsin, re-forming rhodopsin.

Cyclic GMP Is a Key Transducing Molecule

The key transducer molecule 3′,5′ cyclic GMP links activated opsin to the closing of Na^+ channels. Rod outer segments contain an unusually high concentration of 3′,5′-cGMP, about 0.07 mM, and its concentration *drops* upon illumination. Rod outer segments contain a specific phosphodiesterase for cGMP that is activated by a cascade triggered by light:

$$3',5'\text{-cGMP} + H_2O \xrightarrow[\text{phosphodiesterase}]{\text{cGMP}} 5'\text{-GMP}$$

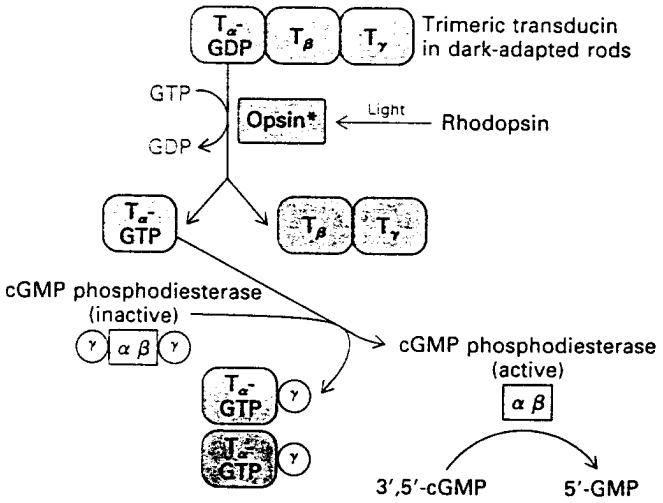
However, light appears to have no immediate effect on the synthesis of cGMP from GTP:

$$\text{GTP} \xrightarrow[\text{cyclase}]{\text{guanylate}} 3',5'\text{-cGMP} + PP_i$$

Injection of cGMP into a rod cell depolarizes the cell membrane, and the effect is potentiated if an analog of cGMP that cannot be hydrolyzed is injected. Thus, in the dark the high level of cGMP acts to keep the Na^+ channels open.

Several hundred molecules of cGMP phosphodiesterase are activated by a single photon. Activation is coupled to light absorption, which generates activated opsin, by the rod protein transducin (Figure 21-50). Transducin (T), a member of the family of signal-transducing G proteins that is found only in rods, has three subunits: T_α, T_β, and T_γ. The β and γ subunits are similar to those in other G proteins. In the resting state, the α subunit has a tightly bound GDP (T_α-GDP) and is incapable of affecting cGMP phosphodiesterase. Light-activated opsin catalyzes the exchange of free GTP for a GDP on the α subunit of transducin and the subsequent dissociation of T_α-GTP from the β and γ subunits. Free T_α-GTP then activates cGMP phosphodiesterase. A single molecule of activated opsin in the disk membrane can activate 500 transducin molecules; this is the primary stage of signal amplification in the visual system.

Biochemical studies have shown that T_α-GTP activates cGMP phosphodiesterase by binding to and removing the inhibitory γ subunit, thus releasing the catalytic α and β subunits in an active form (Figure 21-50). As with other α subunits of G proteins, a GTPase activity is intrinsic to the α subunit of transducin, which slowly converts light-induced T_α-GTP back to T_α-GDP. Once re-formed, T_α-GDP combines with T_β and T_γ, thus regenerating trimeric transducin. As a result, cGMP phosphodiesterase is again



▲ FIGURE 21-50 Coupling of light absorption to activation of cGMP phosphodiesterase via light-activated opsin (opsin*) and transducin in rod cells. In dark-adapted rod cells, a high level of cGMP acts to keep Na^+ channels open and the membrane depolarized compared with the resting potential of other cell types. Light absorption leads to a decrease in cGMP by the pathway shown; as result, many Na^+ channels close and the membrane becomes transiently hyperpolarized (see Figure 21-48). [Adapted from M. Applebury, 1987, *Nature* **326**:546; and L. Stryer, 1991, *J. Biol. Chem.* **266**:10711–10714.]

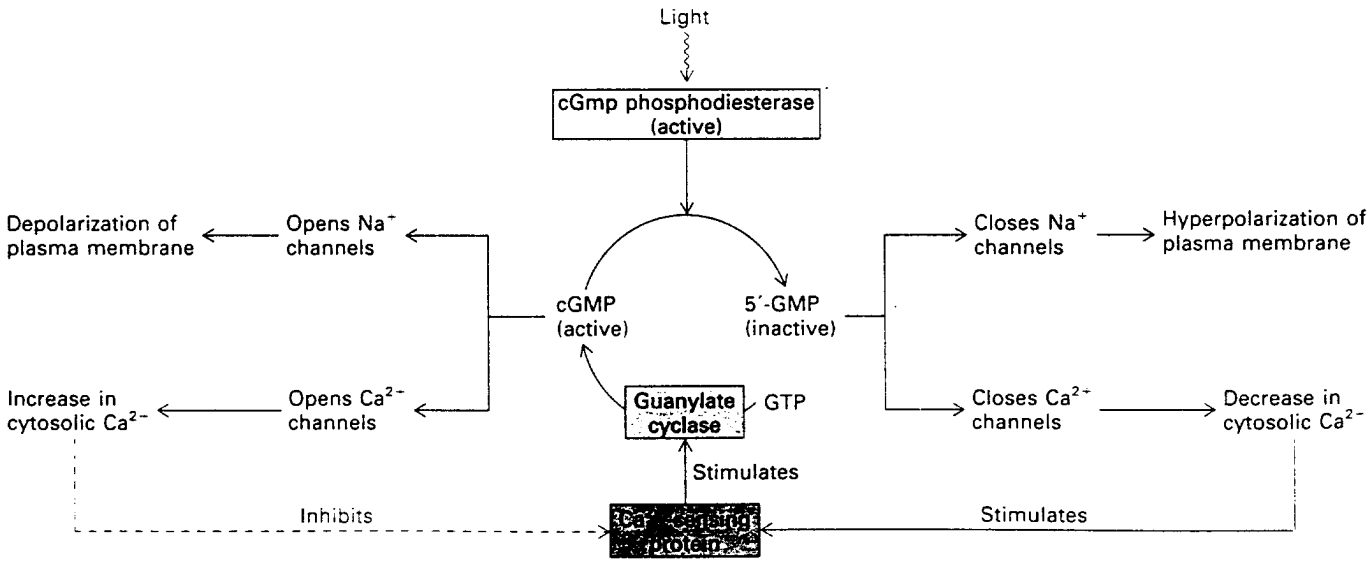
inactivated and the 3′,5′-cGMP level gradually returns to its dark-adapted level.

Direct support for the role of cGMP in rod-cell activity has been obtained in patch-clamping studies using isolated patches of rod outer segment plasma membrane, which contains abundant Na^+ channels. When cGMP is added to the cytosolic surface of these patches, there is a rapid increase in the number of open Na^+ channels. The effect occurs in the absence of protein kinases or phosphatases, and it appears that cGMP acts directly on the channels to keep them open. Three cGMP molecules must bind per channel in order to open it; this allosteric interaction makes channel opening very sensitive to changes in cGMP levels. In sequence the cGMP-gated Na^+ channel protein resembles voltage-gated K^+ channels, but contains a binding site for the cyclic nucleotide in its cytosol-facing domain (Figure 21-25). Presumably, as with voltage-gated K^+ channels, the cGMP-gated channel contains four subunits. Light closes the channels by activating the cGMP phosphodiesterase and lowering the level of cGMP.

Rod Cells Adapt to Varying Levels of Ambient Light

Cone cells are insensitive to low levels of illumination, and the activity of rod cells is inhibited at high light levels. Thus when we move from daylight into a dimly lighted room, we are initially blinded. As the rod cells slowly become sensitive to the dim light, we gradually are able to see and distinguish objects. This process, called adaptation, is mediated by negative feedback acting on the light receptor rhodopsin and on the levels of the second messenger cGMP.

A rod cell is able to adapt to a more than 100,000-fold variation in the ambient light level so that differences in light levels, rather than the absolute amount of absorbed light, are used to form visual images. One process contributing to this adaptation involves Ca^{2+} ions. As shown in Figure 21-51, the activity of guanylate cyclase, the enzyme that synthesizes cGMP, is relatively low in 0.5 μM Ca^{2+}, the concentration characteristic of resting rod cells. Cyclic GMP, besides opening Na^+ channels, also opens Ca^{2+} channels; the level of Ca^{2+} in the cell is balanced by Ca^{2+} pumps that export Ca^{2+} from the cytosol into the extracellular space. Light, as we noted, causes a reduction in cGMP levels; this leads to a closing of both Na^+ channels and Ca^{2+} channels. The resultant drop in Ca^{2+} concentration (due to the continual export of Ca^{2+} ions) causes activation of a Ca^{2+}-sensing protein that, in turn, binds to and activates guanylate cyclase, causing synthesis of more cGMP. This "resets" the system to a new baseline level, so that a greater change in light level will be necessary to hy-



▲ FIGURE 21-51 Role of a Ca^{2+}-sensing protein, guanylate cyclase, and Ca^{2+} in adaptation of rod cells to changes in ambient light levels. In dark-adapted cells, the high level of cGMP opens both Na^+ and Ca^{2-} channels; the relatively high level of cytosolic Ca^{2+} blocks the Ca^{2+}-sensing protein from activating guanylate cyclase. A reduction in cGMP, triggered by light activation of cGMP phosphodiesterase, causes a decrease in the cytosolic Ca^{2+} level as well as hyperpolarization of the plasma membrane. The reduction in the Ca^{2+} level causes activation of the Ca^{2+}-sensing protein and thus of guanylate cyclase, which catalyzes synthesis of more cGMP, restoring the cells to a new baseline state in which they are less sensitive to small changes in light level. [Adapted from E. Pugh and J. Altman, 1988, *Nature* **334**:16 and L. Stryer, 1991, *J. Biol. Chem.* **266**:10711–10714.]

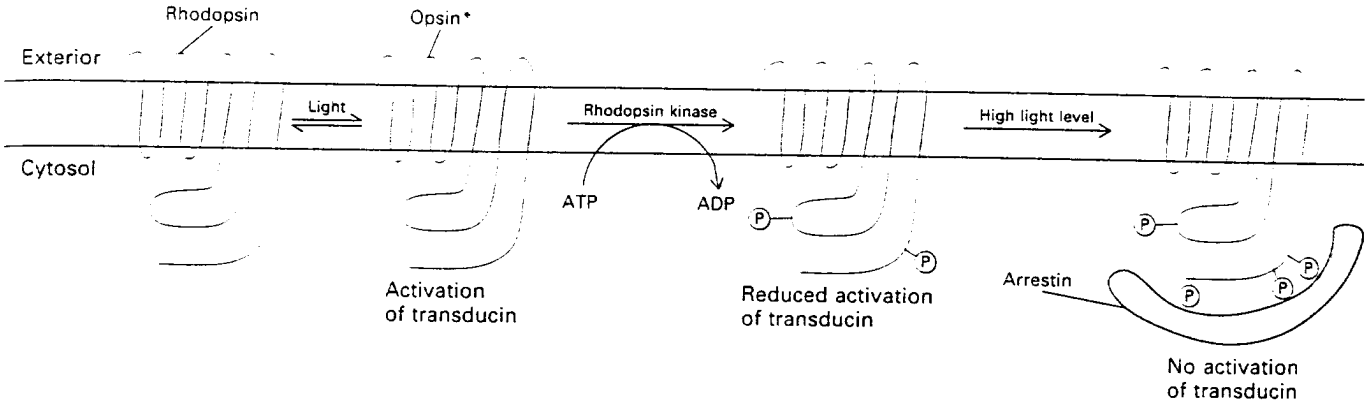


FIGURE 21-52 Role of opsin phosphorylation in adaptation of rod cells to changes in ambient light levels. Light-activated opsin (opsin*), but not dark-adapted rhodopsin, is a substrate for rhodopsin kinase. The extent of opsin* phosphorylation is directly proportional to the ambient light level, and the ability of an opsin* molecule to catalyze activation of transducin (see Figure 21-50) is inversely proportional to the number of sites phosphorylated. Thus the higher the ambient light level, the larger the increase in light level needed to activate the same number of transducin molecules. At very high light levels, arrestin binds to the completely phosphorylated opsin, forming a complex that cannot activate transducin at all. [See L. Lagnado and D. Baylor, 1992, *Neuron* **8**:995–1002.]

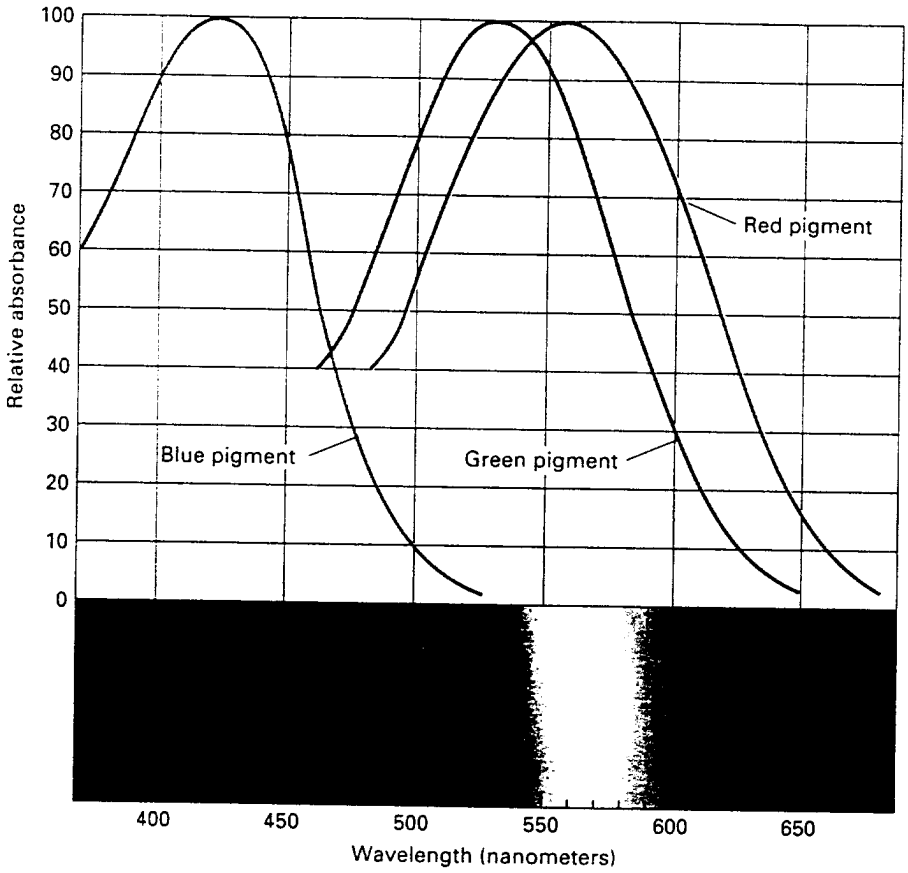
drolyze cGMP, to close the same number of Na^+ channels, and to generate the same visual signal than if the cells had not been exposed to light. In other words, the cells become less sensitive to small changes in levels of illumination.

A second process, affecting the protein opsin itself, participates in adaptation of rod cells to ambient levels of light and also prevents overstimulation of the rod cell in very high ambient light (Figure 21-52). A rod-cell enzyme, rhodopsin kinase, phosphorylates light-activated opsin (O) but not dark-adapted rhodopsin. Phosphorylated opsin is less able to activate transducin than is nonphosphorylated opsin. Each O molecule has seven phosphorylation sites; the more sites that are phosphorylated, the less able O^* is to activate transducin. Because the extent of O^* phosphorylation is proportional to the amount of time each opsin molecule spends in the light-activated form, it is a measure of the background level of light. Under high light conditions, phosphorylated opsin is abundant. Then a greater increase in light level will be necessary to generate a visual signal. When the level of ambient light is reduced, most of the opsins become dephosphorylated, and thus activation of transducin increases. Then fewer additional photons will be necessary to generate a visual signal. At high ambient light (such as noontime outdoors), the level of opsin phosphorylation is such that the protein *arrestin* binds to opsin; arrestin binds to the same site on opsin as does transducin, totally blocking activation of transducin and causing a shutdown of all rod-cell activity. The mechanism by which rod-cell activity is controlled by rhodopsin kinase is similar to adaptation of the β-adrenergic receptor to high levels of hormone (see Figure 20-50). Indeed, rhodopsin kinase is very similar to β-adrenergic receptor kinase—the enzyme that phosphorylates and inactivates only the ligand-occupied β-adrenergic receptor—and each protein can phosphorylate the other's substrate.

Color Vision Utilizes Three Opsin Pigments

There are three classes of cone cells in the human retina. Each absorbs light at a different wavelength (Figure 21-53), and each contains a different rhodopsin photopigment. One absorbs mainly blue light, one green, and one red. As in rods, the relative amount of light absorbed by each class of cones is translated into electrical signals that are transmitted to the brain. There the overall pattern of absorption of light of different wavelengths is converted into what we perceive as *color*. All cone opsins bind the same retinal as found in rods, and the three cone opsins are similar to the rod opsin and to each other. The unique absorption spectra of the three cone rhodopsins are due to different amino acid side chains that contact the retinal on the inside of rhodopsin and that affect its ability to absorb light of different wavelengths.

The most interesting results to emerge from the study of cone opsins were molecular explanations of the different types of color blindness in humans. The "blue" opsin is encoded on human chromosome 7, while the red and green opsin genes are located next to each other, head-to-tail, on the X chromosome. The red and green opsin genes are the product of an evolutionary recent gene duplication because they are 98 percent identical in sequence. Furthermore, new world monkeys have only a single opsin gene on their X chromosome, while old world monkeys, which are more closely related to humans, have two. Two adjacent and almost identical genes can be expected to recombine un-



◀ FIGURE 21-53 The absorption spectra of the three human opsins responsible for color vision. What is called the blue pigment is maximally responsive to the short-wavelength region of the visible spectrum; the green and red pigments absorb primarily in the intermediate and long-wavelength regions. Individual cone cells express one of the three opsins. The spectra were determined by measuring in a microspectrophotometer the light absorbed by individual cone cells, obtained from cadavers. [From J. Nathans, 1989, *Sci. Am.* **260**(2):44.]

equally during gamete formation rather frequently, resulting in X chromosomes with only a green or only a red opsin gene. This results in red-green color blindness, a phenotype not uncommon in males, because they have a single X chromosome, but very rare in females.

Remarkably, because of polymorphisms in the red opsin genes, even individuals with "normal" color vision see colored objects differently. Many individuals have an alanine at position 180 (in the middle of the fourth membrane-spanning α helix, a region that contacts the retinal) of the red opsin; the absorption maximum of that pigment is ≈530 nm. Others have a serine in that position, resulting in an absorbance maximum of ≈560 nm. Thus, the subjects with serine at position 180 have a higher sensitivity to red light than the others; they "see" colors differently due to the change in a single nucleotide.

More Than a Thousand Different G Protein–Coupled Receptors Detect Odors

The visual system functions efficiently with only four types of photoreceptors, three in cones and one in rods. In contrast, the olfactory system utilizes at least several hundred homologous olfactory receptors in responding to the millions of different chemicals we can smell.

Signal transduction in the olfactory system is different from that in the visual system. Each receptor cell in the olfactory epithelium in the nose probably has only one specific odorant receptor on its apical (outward-facing) plasma membrane, and "senses" only one or a few odorants. Most of these receptors are coupled to a G protein, G_{olf}, unique to olfactory epithelia; G_{olf}, like G_s, activates adenylyl cyclase, and the level of cAMP increases as a result. In olfactory cells cAMP binds to and opens a cAMP-gated Na^+ channel, unique to olfactory epithelia, that is similar in structure to the cGMP-gated Na^+ channel in the visual system (Figure 21-25). This leads to a depolarization of the cell membrane (rather than the hyperpolarization induced by activation of rhodopsin), initiating the electrical signal that is sent to the brain.

Several hundred genes encoding odorant receptors were isolated by a novel cloning strategy. First, workers identified sequences of amino acids that were conserved in many other known G protein–coupled receptors. Assuming that odorant receptors were also coupled to G proteins, the workers devised primers for the polymerase chain reaction that would allow amplification of cDNA sequences that encoded novel G protein–coupled receptors, and indeed hundreds were cloned using that approach from a cDNA library made from olfactory epithelia. The diversity

of these receptors is entirely encoded in the nuclear genome, and there is no evidence for somatic recombination, as is found in the immune system, for generation of odorant receptors. In situ hybridization showed that each of these receptor genes is expressed in only a few of the millions of olfactory epithelial cells, as might be expected for a receptor that binds a specific kind of odorant. However, it has not yet been possible to identify which of the thousands of known odorants binds to any one of the cloned receptors. It is striking that, during evolution, there was selection for so many different odorant receptors.

> Memory and Neurotransmitters

In its most general sense, learning is a process by which humans and other animals modify their behavior as a result of experience or as a result of acquisition of information about the environment. Memory is the process by which this information is stored and retrieved.

Psychologists have defined two types of memory, depending on how long it persists: short term (minutes to hours) and long term (days to years). It is generally accepted that memory results from changes in the structure or function of particular synapses, but until recently learning and memory could not be studied with the tools of cell biology or genetics. Most researchers believe that long-term memory involves the formation or elimination of specific synapses in the brain and the synthesis of new mRNAs and proteins. Because short-term memory is too rapid to be attributed to such gross alterations, some have suggested that changes in the release and function of neurotransmitters at particular synapses are the basis of short-term memory. Indeed, recent work has identified several types of proteins that function to modify synaptic activity. These proteins integrate two different signaling pathways; they respond to two (or more) different but coincident signals by generating an output that is different from that produced by either signal acting separately. We shall see how two such proteins are involved in elemental forms of learning. We begin with a molecular analysis of elemental forms of learning in the fruit fly *Drosophila* and the sea slug *Aplysia*, and then turn to long-term potentiation, a form of learning exhibited by many synapses in the mammalian brain.

Mutations in *Drosophila* Affect Learning and Memory

Remarkable as it sounds, fruit flies can be trained to avoid certain noxious stimuli. During the training period, a population of flies is exposed to two different stimuli, either two odoriferous chemicals or two colors of light. One of the two is associated with an electric shock. The flies are then removed and placed in a new apparatus, and the two stimuli are repeated but without the electric shock. The flies are tested for their avoidance of the stimulus associated with the shock. About half the flies learn to avoid the stimulus associated with the shock, and this memory persists for at least 24 h. Painstaking observation of mutagenized flies has led to the identification of six different genes in which mutations cause defects in this learning process.

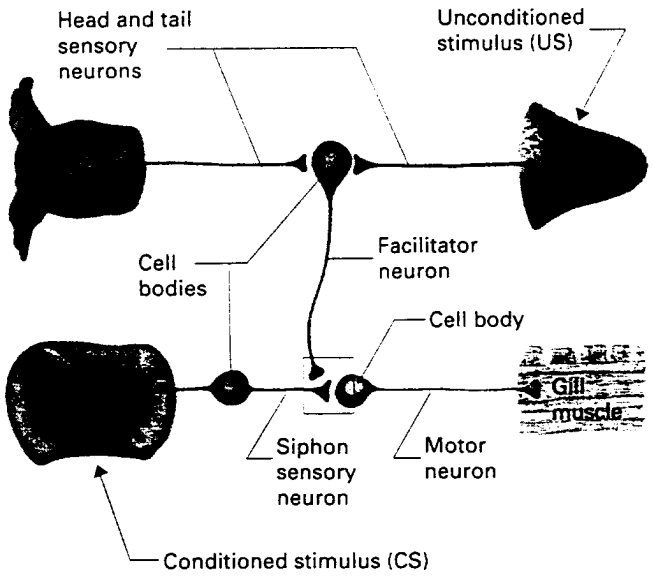
Two mutations that disrupt memory affect cAMP levels. One, *dunce*, is due to a mutation in one of two isoforms of the enzyme cAMP phosphodiesterase. A second, *rutabaga*, is caused by a mutation in the gene encoding one of four types of adenylate cyclase—the one activated by both Ca^{2+}-calmodulin and an activated $G_{s,\alpha}$ signal-transducing protein. It is not yet known which synapses are involved in the learning response or how the high levels of cAMP that result from the phosphodiesterase deficiency might affect learning. However, a dual-regulated adenylate cyclase also plays a key role in learning at a particular synapse in the sea slug *Aplysia*.

Gill-Withdrawal Reflex in *Aplysia* Exhibits Three Elementary Forms of Learning

Sea slugs exhibit three of the most elementary forms of learning familiar in vertebrates: habituation, sensitization, and classical conditioning. Habituation is a decrease in behavioral response to a stimulus following repeated exposure to the stimulus with no adverse effect. For example, an animal that is startled by a loud noise may show decreasing responses on prolonged repetition of the noise. Sensitization, in contrast, is an increase in behavioral response to a stimulus that does have an adverse effect.

Classical conditioning is one of the simplest types of associative learning—the recognition of predictive events within an animal's environment. The animal learns that one event, termed the conditioned stimulus (CS), always precedes, by a critical and defined period, a second or reinforcing stimulus or event, the unconditioned stimulus (US). A well known example is the Pavlovian response in dogs: a bell (CS) is rung a few seconds before food (US) is presented; the dogs soon learn to associate the two stimuli and to salivate in response to the bell alone. In such a learning process, be it in mammals or sea slugs, it is essential that the conditioning stimulus always precede the unconditioned stimulus by a small and critical time interval.

Habituation When a sea slug (Figure 21-44) is touched gently on its siphon, the gill muscles contract vigorously and the gill retracts into the mantle cavity. The gill-withdrawal reflex is mediated by a simple reflex arc (Figure 21-54). Sensory neurons in the siphon synapse with motor neurons that innervate the gill muscles. However, if the siphon is touched 10–15 times in rapid sequence, the gill response decreases to only about one-third of its initial in-



▲ FIGURE 21-54 Neural circuits in the gill-withdrawal reflex of the sea slug *Aplysia*. For simplicity, certain of the interneurons are omitted. This reflex exhibits habituation, sensitization, and classical conditioning. The details of the synapses (boxed) between the sensory, facilitator, and motor neurons are shown in Figure 21-45). [See E. R. Kandel and J. H. Schwartz, 1982, *Science* **218**:433 and T. W. Abrams and E. R. Kandel, 1988, *Trends Neurosci.* **11**:128.]

tensity. By recording the electric changes in the motor neurons to the gill, researchers discovered that this habituative response is due to a progressive decrease in the amount of neurotransmitter released at the synapses between the siphon sensory neurons and the motor neurons. In other words, repeated stimulation of the siphon leads to a decrease in the magnitude of the excitatory postsynaptic potential.

We have noted that release of neurotransmitters is triggered by a rise in the intracellular Ca^{2+} concentration following opening of voltage-gated Ca^{2+} channels. Measurements of Ca^{2+} movements in the *Aplysia* siphon sensory neuron have shown that habituation results from a decrease in the number of voltage-gated Ca^{2+} channels that open in response to the arrival of the action potential at the terminal, thus reducing the amount of neurotransmitter released. Habituation does not affect the generation of action potentials in the siphon sensory neuron or the response of the receptors in the postsynaptic cells.

Sensitization If a habituated sea slug is given a strong, noxious stimulus, such as a blow on the head or tail, it will respond to the next weak stimulus to the siphon by a rapid withdrawal of the gill. The noxious stimulation is said to sensitize the animal so that it exhibits an enhanced response to touching of the siphon. *Aplysia* sensitization is mediated by interneurons called facilitator neurons (Figures 21-45 and 21-54) that are activated by shocks to the head or tail. Electron microscopy shows that the axon of a facilitator neuron synapses with the terminal of a siphon sensory neuron near the site where the siphon sensory neuron synapses with a motor neuron (Figure 21-54). Stimulation of the facilitator neuron causes the siphon sensory neuron to release more transmitter in its synapse with the motor neuron, thus increasing the magnitude of the gill-withdrawal reaction.

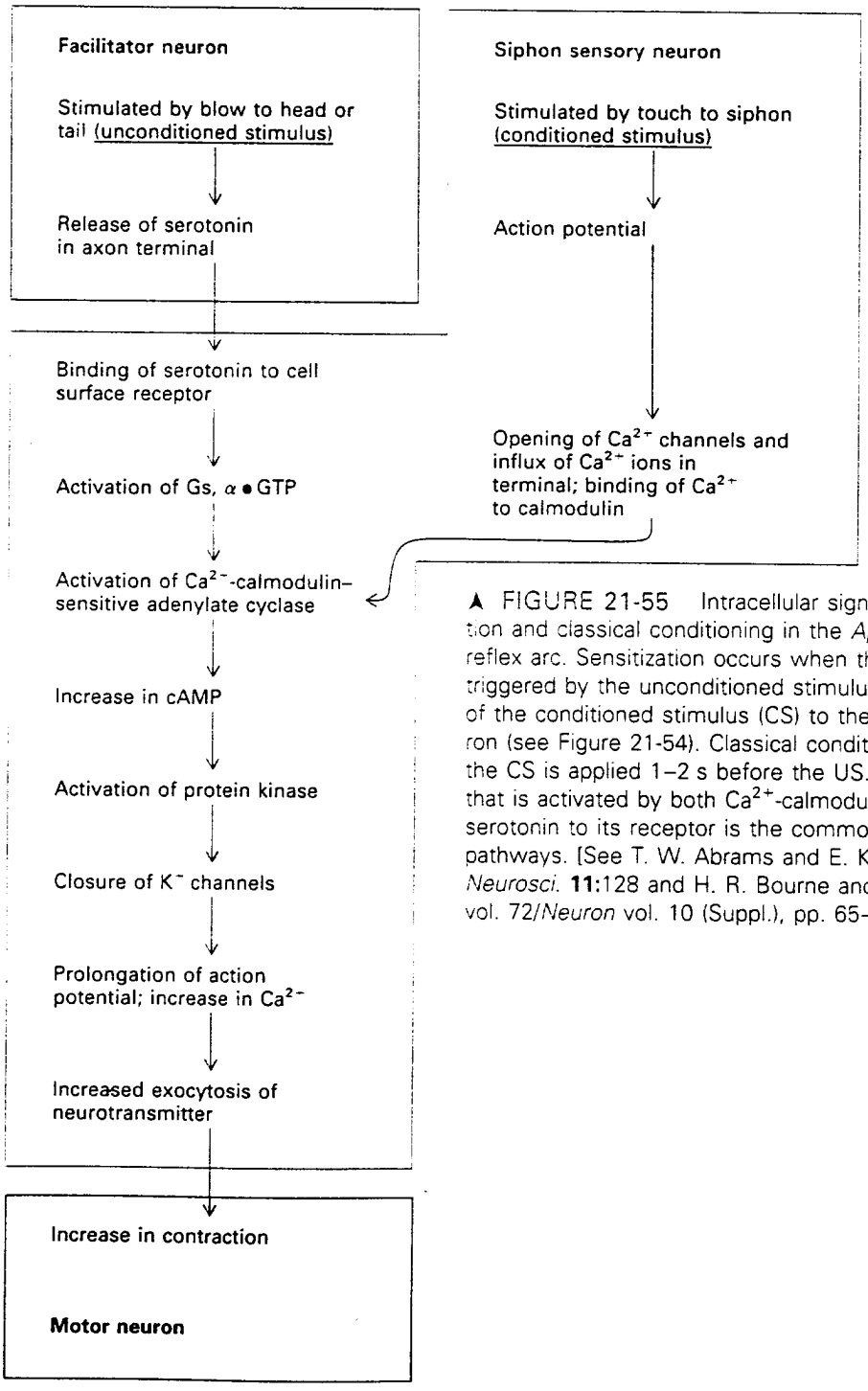
As illustrated in Figure 21-45, stimulation of the *Aplysia* facilitator neurons leads to inhibition of voltage-gated K^+ channels in the siphon sensory neurons, which normally participate in repolarizing the membrane after an action potential. As a result, action potentials reaching the nerve terminals decay more slowly. This prolonged depolarization causes a longer and larger than usual influx of Ca^{2+} ions via the voltage-gated Ca^{2+} channels. The increased cytosolic Ca^{2+} level leads to (1) more extensive exocytosis of neurotransmitter by siphon sensory neurons at their synapses with motor neurons; (2) enhanced activity of motor neurons; and (3) enhanced contraction of the gill muscle. The effect of facilitator neuron stimulation is mediated by cAMP and a cAMP-dependent protein kinase in the siphon sensory neuron terminal. Short-term sensitization persists as long as the concentration of cAMP is elevated and the kinase is activated, about 1 h after each sensitizing stimulus.

Classical Conditioning The gill-withdrawal reflex also exhibits classical conditioning. In the "training" process, a weak touch to the siphon—the conditioned stimulus (CS)—is followed immediately by a sharp blow to the tail or head—the unconditioned stimulus (US)—which, of course, evokes a marked gill-withdrawal response. After a series of such trials, the gill-withdrawal response to the CS alone is substantially enhanced, as if the animal "learns" that a weak siphon touch (CS) is followed by a noxious, sharp blow (US). As in conditioning in other animals, the CS must precede the US by a short and definite interval, in this case 1–2 s.

Sensitization occurs when the facilitator neuron is activated by the US (a blow to the head or tail) in the absence of the CS; it triggers activation of adenylate cyclase and closing of K^+ channels in the siphon sensory neuron. During conditioning, the rise in cAMP in the siphon sensory neuron terminal is much greater when the sensory neuron is triggered by the CS to fire an action potential just before the US arrives.

Figure 21-55 outlines how an adenylate cyclase activated both by $G_{s\alpha}$-GTP and by Ca^{2+}-calmodulin is the probable molecular site for convergence of the US and CS in the axon terminal of the siphon sensory neuron. The brief Ca^{2+} influx triggered by the CS action potential and resulting in the siphon sensory neuron activates this adenylate cyclase. Serotonin released by the facilitator neuron

triggered by the US also stimulates this cyclase. However, activation of the cyclase, and hence the increase in cAMP, is greatest when the cyclase is first "primed" by Ca^{2+} influx and then, within 1–2 s, activated by binding of serotonin. In this way, the enhancement of adenylate cyclase activity triggered by the unconditioned stimulus makes the sensory neuron more sensitive to a conditioning stimulus; the animal learns to associate the CS with the US and to respond to the CS alone with an enhanced response. Indeed, in isolated membranes prepared from these neurons, adenylate cyclase activity was greater if the membranes were exposed to elevated Ca^{2+} *before* exposure to $G_{s,\alpha}$•GTP than vice versa. This biochemical asymmetry mirrors the key temporal requirement for conditioning in the intact animal; conditioning is produced only when the CS precedes the US.



▲ FIGURE 21-55 Intracellular signaling during sensitization and classical conditioning in the *Aplysia* gill-withdrawal reflex arc. Sensitization occurs when the facilitator neuron is triggered by the unconditioned stimulus (US) in the absence of the conditioned stimulus (CS) to the siphon sensory neuron (see Figure 21-54). Classical conditioning occurs when the CS is applied 1–2 s before the US. An adenylate cyclase that is activated by both Ca^{2+}-calmodulin and by binding of serotonin to its receptor is the common element in both pathways. [See T. W. Abrams and E. Kandel, 1988, *Trends Neurosci.* **11**:128 and H. R. Bourne and R. Nicoll, 1993, *Cell* vol. 72/*Neuron* vol. 10 (Suppl.), pp. 65–75.]

Thus, the adenylate cyclase activated both by $G_{s,\alpha}$•GTP and by Ca^{2+}-calmodulin functions as a *molecular coincidence detector;* it responds to two different but coincident signals by generating an output that is different from that produced by either signal acting separately.

Long-Term Memory The short-term sensitization and conditioning responses in *Aplysia* can occur in the presence of inhibitors of protein synthesis, suggesting that no new proteins (or cells) are required for short-term learning responses (short-term memory). On the other hand, a series of closely spaced tail shocks (unconditioned stimulus) delivered over a few hours will produce a long-term sensitization (long-term memory), which can persist for days or even weeks. Both long-term and short-term sensitizations affect the same synapses, and even the same K^+ channels. However, protein synthesis is essential for long-term sensitization, suggesting that certain new proteins must be made in these synapses in order for long-term memory to occur. Long-term sensitization of the *Aplysia* gill-withdrawal reflex can be induced in cells in culture by the repeated application of serotonin, the hormone normally released by the facilitator neuron (Figure 21-45). Repeated application of serotonin leads to induction of synthesis of several proteins in the sensory neuron and inhibition of synthesis of others, among which are several plasma membrane proteins that function in neural cell-cell adhesion (N-CAM, or neural cell adhesion proteins—see Figure 24-32).

Sensitization and classical conditioning of the gill-withdrawal reflex of *Aplysia* are among the few cases in which short-term changes in synaptic function are understood in molecular detail. These simple forms of learning have served as models for more complex forms of behavior, such as short-term and long-term memory in vertebrates. Increasingly, neuroscientists are identifying molecules that may function in the memory of mammals.

A Novel Glutamate Receptor Is the Coincidence Detector for Long-Term Potentiation at Many Synapses in the Mammalian Brain

The hippocampus is the region of the mammalian brain associated with many types of short-term memory. Certain types of hippocampal neurons, here simply called *postsynaptic cells,* receive inputs from hundreds of presynaptic cells. In *long-term potentiation*—similar to sensitization discussed above—continual stimulation of a postsynaptic neuron makes it more responsive to subsequent stimulation by presynaptic neurons. For example, in the hippocampus stimulation of a presynaptic nerve with 100 depolarizations acting over only 200 milliseconds causes an increased sensitivity of the postsynaptic neuron that lasts hours to days.

Figure 21-56 shows how two types of glutamate receptors in the postsynaptic neuron combine to generate long-term potentiation. Both receptors are glutamate-gated cation channels that are similar in structure and amino acid sequence to the nicotinic acetylcholine receptors (Table 21-3 and Figure 21-39); both receptors depolarize the plasma membrane when activated. Because the two receptors were initially distinguished by their ability to be activated by the nonnatural amino acid N-methyl-D aspartate (NMDA), they are called NMDA glutamate receptors and non-NMDA glutamate receptors. Non-NMDA receptors are "conventional"; the ion channels open whenever glutamate, released from the presynaptic cell, binds to the receptors on the postsynaptic neuron (Figure 21-56).

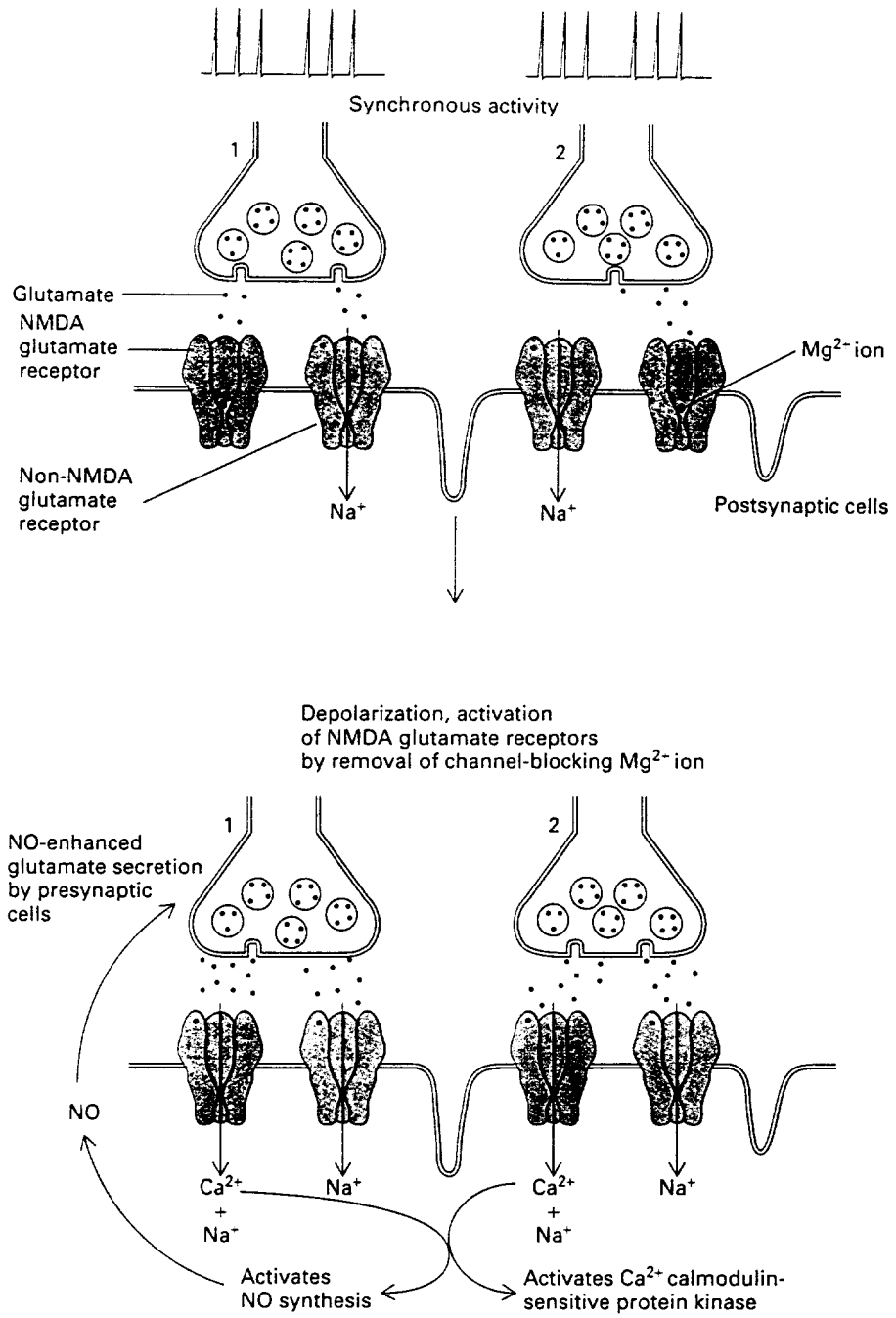
NMDA glutamate receptors are different in two key respects. First, they allow influx of Ca^{2+} as well as Na^+. Second, and more important, two conditions must be fulfilled for the ion channel to open: glutamate must be bound *and* the membrane must be partly depolarized. In this way, the NMDA receptor functions as a coincidence detector; it integrates activity of the postsynaptic cell—reflected in its depolarized plasma membrane—with release of neurotransmitter from the presynaptic cell, generating a cellular response greater than that caused by glutamate release alone.

NMDA receptors are dependent upon membrane voltage because of the voltage-sensitive blocking of the ion channel by a Mg^{2+} ion from the extracellular solution. Depolarization of the membrane causes the Mg^{2+} ion to dissociate from the receptor, making it possible for glutamate binding to open the channel. Indeed, the Mg^{2+} ion is binding in the channel itself, since mutagenesis of a single asparagine residue in the M2 helix of the NMDA receptor—the segment thought to line the pore itself (see Figure 21-39)—abolishes the effect of Mg^{2+}.

Since activation of a single synapse, even at high frequency, generally causes only a small depolarization of the membrane of the postsynaptic cell, long-term potentiation is induced only when many synapses on a single postsynaptic neuron are activated simultaneously. Thus the requirement for membrane depolarization explains a key property of long-term potentiation: *cooperativity*—a large number of synapses on a cell must be activated simultaneously.

Retrograde Signaling by the Gas Nitric Oxide May be a Part of Long-Term Potentiation

During induction of long-term potentiation the presynaptic cell is changed as well; more neurotransmitter is released during arrival of each action potential. Thus, both sides of the synapse are strengthened—more glutamate neurotransmitter is released by the presynaptic cell and the response to the neurotransmitter by the postsynaptic cell is



▲ FIGURE 21-56 Involvement of two types of glutamate receptors and nitric oxide in long-term potentiation—a type of short-term memory. This postsynaptic cell in the hippocampus region in the brain has two types of glutamate receptors, NMDA (green) and non-NMDA (pink). The ion channel in the NMDA receptor is normally blocked by a Mg^{2+} ion, and thus the glutamate released by firing of presynaptic neurons leads, at first, to opening of only the non-NMDA glutamate receptors. The resultant influx of Na^+ partially depolarizes the membrane. If many presynaptic neurons (here two are shown) fire in synchrony, the membrane of the postsynaptic cell becomes sufficiently depolarized so that the Mg^{2+} ions blocking the NMDA receptors are removed and thus the NMDA as well as the non-NMDA glutamate receptors open in response to glutamate. Ca^{2+} ions as well as Na^+ ions enter through the open NMDA receptors, causing an enhanced response in the postsynaptic cells. Among the responses induced by Ca^{2+} is induction of NO synthesis. NO diffuses to the presynaptic cell—retrograde signaling—and there activates a protein that enhances the secretion of glutamate in response to the depolarization induced by arrival of an action potential. As a result, the same response in the postsynaptic neuron can be induced by fewer action potentials in the presynaptic neurons or, in other words, the synapse "learns" to have an enhanced response to the electrical signals in the presynaptic cells. [After T. M. Jessell and E. R. Kandel (1993) *Cell* vol. 72/*Neuron* vol. 10 (Suppl.), pp. 1–30 and C. F. Stevens (1993) *Cell* vol. 72/*Neuron* vol. 10 (Suppl.), pp. 55–63.]

also increased. The increased activity of the presnaptic cell is thought to be induced by a retrograde signal sent by the postsynaptic cell; the gas nitric oxide (NO) is one such signal. Figure 21-56 traces how this happens. Opening of the NMDA glutamate receptors causes an influx of Ca^{2+} ions and an activation of many Ca^{2+}-dependent enzymes in the postsynaptic cell. Among these is NO synthase, the enzyme that synthesizes NO from the amino acid arginine. NO, unlike classical neurotransmitters, simply diffuses out of the postsynaptic cell and into neighboring cells. There it activates several enzymes, among them guanylyl cyclase, an enzyme that synthesizes cyclic GMP, and the cGMP is thought to change the presynaptic cell such that more glutamate is secreted during the arrival of each action potential.

Substantial evidence supports the role of NO in long-term potentiation. For instance, addition of inhibitors of NO synthesis to cultured brain slices blocks the induction of long-term potentiation, as does addition of hemoglobin to the brain slices, a protein that binds released NO and prevents it from reaching neighboring cells. Moreover, addition of NO to hippocampal neurons in culture causes an increase in neurotransmitter release, as expected from its role as a retrograde signaling molecule involved in the induction of long-term potentiation.

Mice Defective in the Hippocampal α-Ca^{2+}-Calmodulin-Activated Protein Kinase Are Impaired in Long-Term Potentiation and in Spatial Learning—the Beginnings of a Molecular Psychology

Opening of the NMDA receptors is, as noted, associated with an increase in cytosolic Ca^{2+} and an activation of several Ca^{2+}-dependent enzymes. Among these is the α isoform of Ca^{2+}-calmodulin-dependent protein kinase II, an enzyme found in abundance mainly in neurons in the hippocampus. For this reason, workers suspected that this enzyme was involved in induction of long-term potentiation and, perhaps, in certain types of learning. To test this hypothesis, mice were genetically engineered to have a deletion in the gene encoding this enzyme. In most respects these mice grow and behave normally; for instance, their abilities to eat and mate are normal, and they have the coordinated motor skills to swim normally in water.

However, cultured hippocampal neurons from these mice are defective in the induction of long-term potentiation. More strikingly, the mice are impaired in a particular type of learning process, supporting the notion that the α isoform of Ca^{2+}-calmodulin-dependent protein kinase II is essential for induction of long-term potentiation and that long-term potentiation, in turn, is the electrophysiological basis of a particular type of learning.

In the critical psychological test, the mice are placed in a round pool of opaque water; to escape the water the mice must swim to a submerged platform. The mutant mice can find the platform normally if it is made visible by a flag, indicating that they can learn to associate the flag with the submerged platform. In contrast, when the platform is hidden, the mice must learn to find it from integrating multiple spatial relationships between objects in the room surrounding the pool (e.g., pictures on the wall) and the position of the platform. The mice with defective Ca^{2+}-calmodulin-dependent protein kinase take longer to learn to find the platform than do their normal littermates. This demonstrates that the α isoform of Ca^{2+}-calmodulin-dependent protein kinase II plays an important role in spatial learning, and that it is not essential for some types of nonspatial learning. As more hippocampal-specific proteins are identified by molecular cloning techniques, workers may identify other enzymes or receptors essential for other types of learning processes in mice. Determining whether human learning requires these enzymes will be a difficult and controversial area of research.

SUMMARY

An electric potential exists across the plasma membrane of all eukaryotic cells because the ion compositions of the cytosol and extracellular fluid differ, as do the permeabilities of the plasma membrane to the principal cellular ions—Na^+, K^+, Cl^-, and Ca^{2+}. In most nerve and muscle cells, the resting membrane potential is about 60 mV, negative on the inside; the potential is due mainly to the relatively large number of open K^+ channels in the membrane.

Impulses are conducted along a nerve axon by action potentials. An action potential consists of a sudden (less than a millisecond) depolarization of the membrane followed by a rapid hyperpolarization and a gradual return to the resting potential. The initial depolarization in the membrane potential is caused by a sudden and transient opening of transmembrane voltage-gated Na^+ channels that admit Na^+ ions into the cytosol. Voltage-gated K^+ channels also open in response to membrane depolarization;

their opening repolarizes the membrane by permitting the efflux of K^+ ions. K^+ channels are assembled from four similar subunits; particular segments of the channel protein have been identified that line the pore, that cause channel inactivation, and that sense voltage changes. Voltage-gated Na^+ and Ca^{2+} channel proteins are single polypeptides containing four homologous domains each similar to a K^+ channel protein; they, too, have a similar positively charged "gating" helix that moves in response to a voltage change of a sufficient magnitude.

Neurons only generate action potentials when the plasma membrane in the region of the axon hillock is depolarized to the threshold value. An action potential generated at one point along an axon will lead to depolarization of the adjacent segment and thus to propagation of the action potential along its length. The speed of impulse conduction depends on the diameter of the axon and conductivity of the neuronal cytosol. Thick neurons conduct faster than thin ones, and myelinated nerves conduct faster than unmyelinated nerves of similar diameter because of insulation of the neuron by the myelin sheath.

Impulses are transmitted from neurons to other cells at specialized junctions called synapses. In electric synapses, ions pass from the presynaptic cell to the postsynaptic cell through gap junctions, and an action potential is generated in the postsynaptic cell with no time delay. In the more common chemical synapses, the arrival of an action potential in the presynaptic axon triggers the release of neurotransmitters into the synaptic cleft; from there the transmitters bind to receptors on the postsynaptic cell. Transmitters are stored in membrane-bounded vesicles, and exocytosis of these vesicles is triggered by a rise in the cytosolic Ca^{2+} level induced by the opening of voltage-gated Ca^{2+} channels.

At excitatory synapses, the neurotransmitter acts to depolarize the postsynaptic cell and generate an action potential. At the synapse of a motor neuron and striated muscle cell, binding of acetylcholine to the well-studied nicotinic acetylcholine receptor triggers a rapid increase in permeability of the membrane to both Na^+ and K^+ ions, leading to depolarization. Much information is available on the structure of the receptor protein and of the segments that line its ion channel. In other postsynaptic cells, the depolarization of the postsynaptic membrane is less extensive but longer lived, on the order of seconds. In many such synapses, receptors are coupled to G transducing proteins that directly or indirectly open or close ion channel proteins. At inhibitory synapses, the release of neurotransmitter triggers a hyperpolarization of the postsynaptic membrane, making it more difficult for the cell to generate an action potential. Depending on the specific receptor in the postsynaptic cell, the same neurotransmitter can induce either an excitatory or inhibitory response.

The inhibitory actions of GABA and glycine are mediated through ligand-gated Cl^- channels whose structures are similar to that of the nicotinic acetylcholine receptor. In some postsynaptic cells, receptors for epinephrine and serotonin modulate the activity of adenylate cyclase. The electric response of these cells is believed to be caused by phosphorylation of Na^+ or K^+ channel proteins by the cAMP-dependent protein kinase. In cardiac muscle, binding of ligand to the muscarinic acetylcholine receptor activates a transducing G protein; this in turn opens a K^+ channel and causes hyperpolarization of the membrane and a decrease in muscle contraction.

Chemical synapses allow a single postsynaptic cell to amplify, modify, and integrate signals from multiple presynaptic neurons. Especially in the central nervous system, many neurons must integrate excitatory and inhibitory stimuli from dozens or hundreds of other neurons, if not a thousand or more. Whether a threshold potential is induced at the axon hillock depends on the timing and magnitude of these stimuli, the localization and duration of the resultant local hyperpolarizations and depolarizations, and the ability of the localized changes in potential to be conducted along the plasma membrane.

Many compounds released by neurons are systemic hormones as well as neurotransmitters, affecting both distant secretory cells and adjacent neurons. Recent work suggests that small peptides such as endorphins, enkephalins, and hypothalamic releasing factors function as neurotransmitters in particular synapses in the brain and also act as hormones.

Removal of neurotransmitter from the synapse is essential for ensuring its repeated functioning. The action of acetylcholine is terminated by the enzyme acetylcholinesterase. Peptide neurotransmitters are hydrolyzed to amino acids. Other neurotransmitters are removed by reuptake into the presynaptic cell; antidepressant drugs and narcotics such as cocaine inhibit certain catecholamine transporters.

Many sensory transduction systems convert signals from the environment—light, taste, sound, touch—into electric signals. These signals are collected, integrated, and processed by the central nervous system. The sensory system understood in the most molecular detail is that of the photoreceptor rod cells. Absorption of even a single photon results in hyperpolarization of the rod-cell plasma membrane and reduces the release of chemical transmitters to adjacent nerve cells. Light causes isomerization of the 11-*cis*-retinal moiety in rhodopsin and formation of activated opsin, which then activates the signal-transducing G protein transducin (T) by catalyzing exchange of free GTP for bound GDP on the T_α subunit. Activated T_α-GTP, in turn, activates cGMP phosphodiesterase. This enzyme lowers the cGMP level, which leads to closing of the membrane Na^+ channels, hyperpolarization of the membrane, and release of less neurotransmitter. Modifications in the activity of guanylate cyclase by Ca^{2+}, and also phosphorylation of the light-activated form of opsin, result in adapta-

tion of rod-cell activity to more than a 100,000-fold range of illumination.

Modifications in the activity of certain synapses are associated with short-term memory, at least in some invertebrate and mammalian systems. Certain *Drosophila* mutants that cannot learn are defective in cAMP metabolism, most notably in a Ca^{2+}-calmodulin-activated adenylate cyclase. In the sea slug *Aplysia,* the gill-withdrawal reflex exhibits habituation, sensitization, and classical conditioning—three forms of simple learning. Habituation is linked to the closing of Ca^{2+} channels in the presynaptic axon terminals of siphon sensory neurons originating in the siphon; this alters the flux of Ca^{2+} in the terminals and the amount of transmitter released to the motor neurons. Sensitization and classical conditioning are mediated by facilitator neurons that synapse with the siphon sensory neurons. Serotonin released by stimulation of the facilitator neurons causes an increase in activity of a Ca^{2+}-calmodulin-activated adenylate cyclase in the siphon sensory neurons, which in turn leads to an elevation in the level of cAMP and to closure of voltage-gated K^+ channels. This prolongs depolarization and increases exocytosis of neurotransmitter. In classical conditioning, a conditioned stimulus, triggering the siphon sensory neurons and increasing Ca^{2+} levels, and an unconditioned stimulus, triggering the facilitator neurons, converge on activation of adenylate cyclase in the terminals of the siphon sensory neurons.

Long-term potentiation in the hippocampus region of the brain is a form of synaptic plasticity associated with learning. Activation of the NMDA glutamate receptors in these cells requires both glutamate and depolarization of the postsynaptic membrane; thus continuous activation of the postsynaptic cell makes it more sensitive to additional stimulation. Activation of the NMDA receptors causes an influx of Ca^{2+}; among the responses induced by Ca^{2+} is induction of NO synthesis. NO diffuses to the presynaptic cell—retrograde signaling—and there activates a protein that enhances glutamate secretion in response to the depolarization induced by arrival of an action potential. As a result, fewer action potentials in the presynaptic neurons can induce the same response in the postsynaptic neuron; the synapse "learns" to have an enhanced response to the electric signals in the presynaptic cells.

REVIEW QUESTIONS

1. The vast majority of cells have a resting voltage potential across their membranes, where the cytoplasm is negative with regard to the surrounding milieu. What is the origin of the resting membrane potential, and how is it maintained?

Neurons are the specialized cells within the nervous system that conduct electrical impulses. Describe the basic structure of a neuron. What is meant by orthograde and retrograde transport? Recall that each neuron has only one axon. In which direction along an axon does an action potential move? How is an impulse conducted down an axon? What guarantees the unidirectionality of the impulse? Which conducts action potentials faster, an axon with a small diameter or an axon with a large diameter? Why?

What happens to the membrane potential of a neuron when the permeability of the plasma membrane to K^+ is increased? What happens when the permeability to Na^+ is increased? Likewise, what happens when the permeability to Cl^- is increased?

For an impulse to be transmitted between two neurons, it must cross a synapse. What are the differences between a chemical and an electrical synapse? Electrical synapses conduct action potentials with little delay. Why, then, are chemical synapses much more common than electrical synapses if they transmit action potentials relatively slowly?

The nervous system is often divided into two principal components, the central nervous system and the peripheral nervous system. What comprises each of these systems, and what are their functions?

a. Refer to Figure 21-15 which shows the values of the membrane potential over time following excitation of a nerve cell. Of great importance is the relationship of the action potential to the membrane permeabilities of the ions involved.

When an action potential occurs, is the change in the internal concentration of Na^+ ions significant, or is it negligible? Explain your answer.

b. Refer to equation 21-2 on page 934, which presents a complex version of the Nernst equation that approximates the membrane potential based on the concentrations of relevant ions and their corresponding membrane permeabilities. These values are summarized in the table.

	Concentration (mM)		
Ion	Cytosol	Medium	Permeability (cm/s)
K^+	140	4	10^{-7}
Na^+	12	150	10^{-8}
Cl^-	4	120	10^{-8}

At the peak of an action potential, the $E_{membrane}$ can reach +50 mV. The opening of the voltage-dependent sodium channels is the initial occurrence that permits the formation of an action potential; the changes in K^+ and Cl^- permeabilities are negligible at the onset of the event. Assuming that the depolarization during an action potential peaks at −50 mV, and assuming that the initial permeabilities of K^+ and Cl^- do not change, what is the membrane permeability to Na^+ at the peak of an action potential? How much larger or smaller is this than the permeability of the membrane to Na^+ when the cell is at rest?

2. The rate of conductance of nerve impulses along long neurons is enhanced by myelination. How does this happen?

Review the technique of patch clamping. How is it used to investigate the flow of ions through ion channels?

What is meant by the term threshold potential? How does this contribute to the all-or-none phenomenon as it relates to voltage-gated sodium channels?

What is known about the comparative structures of voltage-gated ion channels? Be certain you are aware of how the *Drosophila shaker* mutant was used to isolate the voltage dependent K^+ channels.

a. You are using the technique of patch clamping to study the characteristics of a voltage-dependent Na^+ channel from human cells. How would you set up the patch clamp to ensure that you measure sodium current alone?
b. In experiment I, you maintain the membrane potential at −55 mV and determine the ability of Na^+ to move through the channel. You do the same in experiments II, III, and IV, but you maintain the membrane potentials at −40 mV, −20 mV, and +20 mV, respectively. The results in terms of Na^+ permeability are presented in the following table.

Experiment	Clamped Membrane Potential (mV)	Na^+ Permeability
I	−55	Absent
II	−40	Absent
III	−20	Present
IV	+20	Present

Explain these results. Can you state anything about the approximate magnitude of the threshold potential for these cells?

c. If you performed the same experiments in the presence of tetrodotoxin, what would occur?

3. Where are neurotransmitters stored at chemical synapses? What causes secretion of a neurotransmitter into the synaptic cleft?

What are the differences between excitatory and inhibitory synapses? What are ligand-gated ion channels? How do neurotransmitter receptors coupled to G proteins function? What is signal computation?

What are cholinergic synapses? How is acetylcholine made and stored in the presynaptic vesicles? What is the underlying mechanism governing the fusion of these vesicles with the presynaptic membrane? What is the difference between a nicotinic and a muscarinic receptor, and what types of channels are controlled by each?

What are the ways of inactivating a neurotransmitter?

a. You are investigating two neuronal cell lines derived from two strains of mice that exhibit neurological defects. In cells from the strain marked A, you have discovered that the class V ATPase in endosomes is defective, resulting in an internal pH of 7.0 in the organelle. Where would the neurotransmitter be found in the cell, and how would this affect the ability of the cell to transmit an impulse across the synapse?

b. In cells obtained from the strain marked B, you have determined that the K_D for the binding of the neurotransmitter for the H^+/neurotransmitter antiport is approximately one order of magnitude higher than that found in the normal mouse. How would this affect the storage of the neurotransmitter and its subsequent function at the synapse?

4. The visual system involving rods and cones presents a good illustration of a sensory system. How do rod cells respond to increased levels of light? Rhodopsin is the photoreceptor in rod cells. What are the components of rhodopsin? What happens to rhodopsin following absorption of a photon? What happens to internal cGMP levels following the absorption of light? How do rod cells adjust to increased or decreased levels of light? What is rhodopsin kinase? What is arrestin? Suppose that a patient had a visual defect resulting from partial lack of the phosphatase that removes phosphate residues from phosphorylated opsin. How would this affect the ability of the patient to adapt to dim light after being out in the sun?

What are the three classes of cone cells in humans? How do we achieve a sense of color from these cells? Why is red/green color blindness more prevalent in males? Why is loss of the red pigment gene more common than the loss of the blue pigment gene?

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