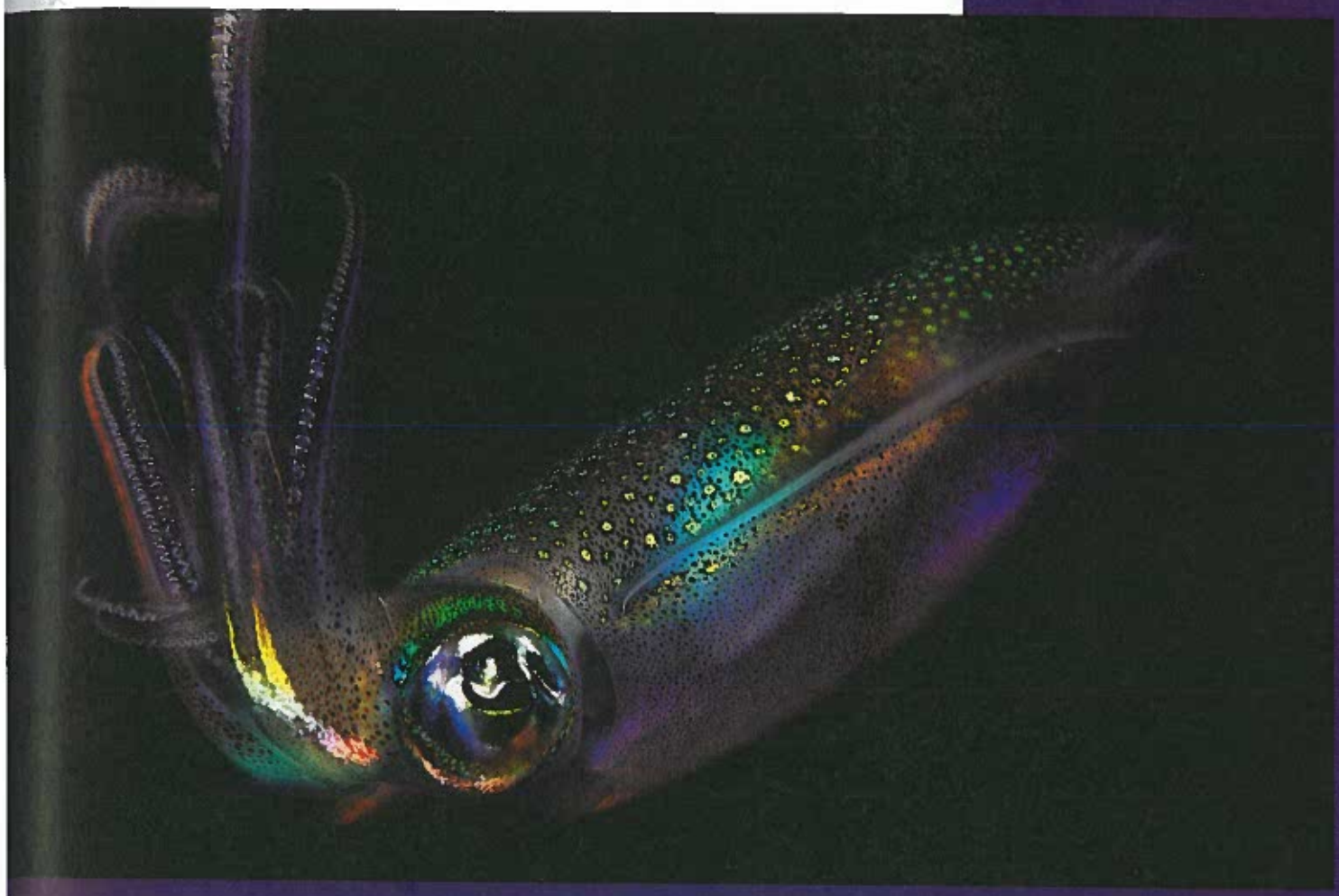
12 Neurons



Squid axons are important to physiologists—and to the squid. ●

Locomotion in a squid, whether for capturing a meal or to avoid becoming one, depends on jet propulsion: The contraction of muscles in the squid's outer mantle expels seawater through a moveable siphon, propelling the animal in the opposite direction. As is true in all animals, feeding, escape, and similar behaviors in the squid are controlled by nervous system signals, which travel rapidly in a point-to-point manner, from one specific cell to another. These signals arise from properties of nerve cells—termed *neurons*—which have long cablelike processes—termed *axons*—that convey electrical signals rapidly and faithfully from place to place in the body, even over long distances. In the squid, sensory neurons such as those in the eyes encode information about the squid's environment and convey signals to the brain. There, the signals are integrated into a decision to attack or retreat. The brain then sends commands to the mantle muscles, in part through a set of large neurons with large ("giant"), rapidly conducting axons.

As you will discover in this chapter, squid giant axons have played an important role in our understanding of neuronal functions. The diameter of these giant axons can be as large as 1 mm (1000 micrometers [μm]), and for more than half a century

investigators have taken advantage of this prodigious cellular size to perform noteworthy experiments that have revealed the mechanisms of neuronal signaling. Sir Alan Hodgkin (1914–1998), who received a Nobel Prize in 1963 for his work on squid axons, recalled that a colleague had remarked (not, Hodgkin thought, with the greatest tact) that it was the squid that really ought to be awarded the prize!

This chapter describes the electrical basis of neuronal function—the ability of neurons to generate and propagate electrical signals. The cellular mechanisms of neuronal signaling are similar in all animals, whether we examine neurons of squids, cockroaches, jellyfish, or humans. Before we turn to neuronal function, however, it is important to take a broader look at the challenges of integration and control. Doing so will clarify the range of physiological control processes and the contrasting functions of neuronal and hormonal modes of integration.

12.1 The Physiology of Control: Neurons and Endocrine Cells Compared

An animal needs to function like a coherent organism, not like a loose collection of cells. Integration is a general term that refers to processes—such as summation and coordination—that produce coherency and result in harmonious function. Cellular integration refers to processes within cells. Whole-animal integration refers to the combination and processing of sensory, endocrine, and neuronal information in ways that promote the coherent functioning of the whole organism—including all its cells, tissues, and organs—within its environment. Just as some cells are specialized to produce movements, secrete acid, or carry oxygen, nerve cells and endocrine cells are specialized for control and coordination. The integrative functions carried out by nerve and endocrine cells ensure that an animal's responses are smooth and coordinated, rather than clashing or disjointed.

The nervous system and the endocrine system both serve to control and coordinate the cells of an animal, but they do so in systematically different ways. As FIGURE 12.1A shows, a signal in a neuron travels electrically along a cell process (typically an axon) all the way to its target cell; transmission along the axon is very fast and spatially highly defined (a signal travels only along the axon in which it was initiated). When the electrical signal arrives at the end of the axon, it causes the release of a chemical substance—a **neurotransmitter**—at the **synapse**, the specialized site of communication between a neuron and its target cell.[1] In this way, signaling in the nervous system is precisely targeted—chemical signals diffuse only very short distances to act on nearby cells. In contrast to the signals of neurons in nervous systems, the signals produced by the *endocrine system* are broadly distributed throughout the animal's body. As FIGURE 12.1B shows, when an endocrine cell emits a signal, it does so by secreting a chemical substance—a **hormone**—into the general blood circulation, bathing the tissues or organs at large to act on target cells that have receptor proteins for the hormone. In the ensuing paragraphs we will discuss the broad features of neural and endocrine control. Then, in the remainder of this chapter and in Chapters 13, 14, and 15, we will consider aspects of neural control in detail. We will discuss endocrine control in detail in Chapter 16.

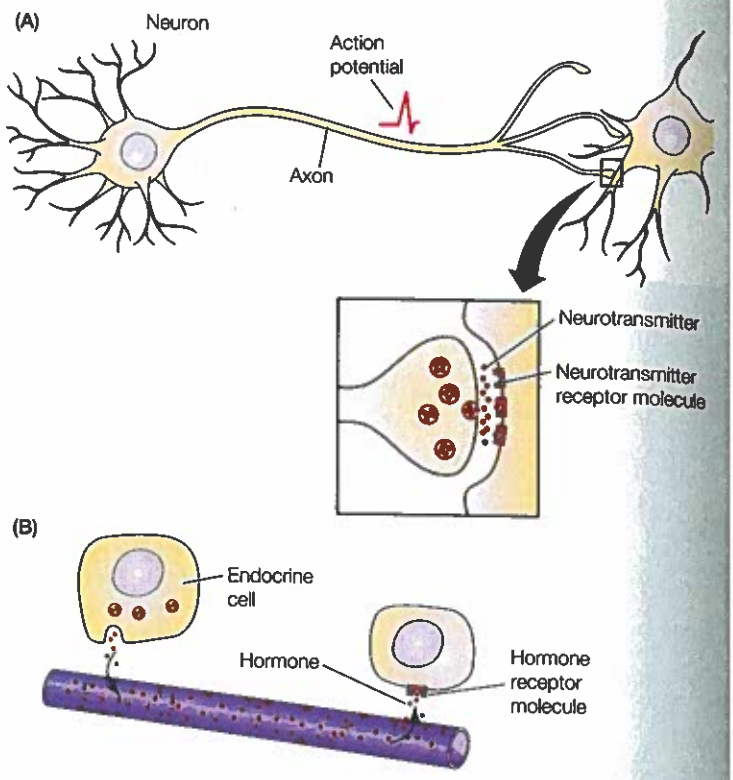


FIGURE 12.1 Neuronal and hormonal signaling (A) Neurons have long axons that rapidly propagate action potentials, and also use short-distance chemical neurotransmitter signaling to communicate from cell to cell across synapses. (B) Endocrine cells release chemical hormones into circulatory fluids that carry the hormonal message over long distances to activate hormone receptors on other cells.

Neurons transmit electrical and chemical signals to specific target cells

Because neurons are commonly likened to the wires in a telephone or computer network, most people have an intuitive understanding of what these cells do. A **neuron** is a cell that is specially adapted to generate an electrical signal, most often in the form of a brief, self-propagating impulse called an *action potential*. As FIGURE 12.2 reveals, a neuron has four parts—dendrites, cell body (also called the soma), axon, and presynaptic terminals—that generally correspond to its four functions—input, integration, conduction, and output—as a controller cell within an animal's body.

Neurons communicate with other cells at synapses. Usually the synaptic input occurs along branching processes known as **dendrites**, although synapses also commonly occur on the cell body, and more rarely on axons. Dendrite morphology varies considerably from one type of neuron to the next and can range from a single simple dendrite to complex dendritic fields

[1] As we will explain in Chapter 13, not all neurons transmit chemical signals to communicate with other cells. Some neurons are connected by specialized synapses called gap junctions that allow for direct transmission of electrical signals.

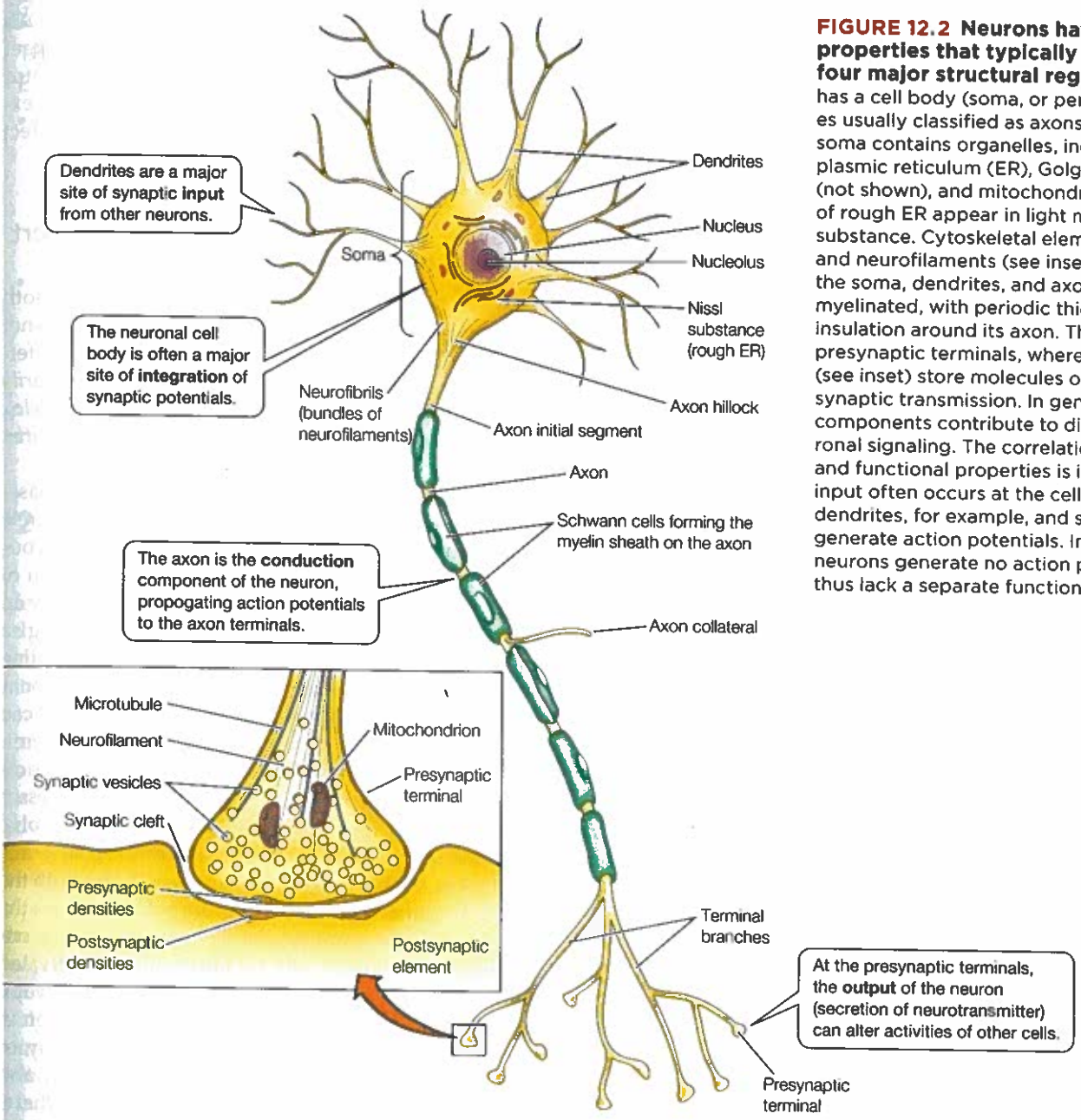


FIGURE 12.2 Neurons have four functional properties that typically correspond to their four major structural regions Every neuron has a cell body (soma, or perikaryon) and processes usually classified as axons and dendrites. The soma contains organelles, including rough endoplasmic reticulum (ER), Golgi apparatuses (not shown), and mitochondria. Stained aggregates of rough ER appear in light microscopy as Nissl substance. Cytoskeletal elements—microtubules and neurofilaments (see inset)—are present in the soma, dendrites, and axon. This neuron is myelinated, with periodic thickenings of myelin insulation around its axon. The axon ends in presynaptic terminals, where synaptic vesicles (see inset) store molecules of neurotransmitter for synaptic transmission. In general, these structural components contribute to different aspects of neuronal signaling. The correlation between structures and functional properties is imperfect: Synaptic input often occurs at the cell body as well as the dendrites, for example, and some dendrites can generate action potentials. In contrast, some local neurons generate no action potentials at all, and thus lack a separate function of active conduction.

with multiple, elaborately branched arborizations (*dendrite* is Greek for "branch"). The complexity of a neuron's dendritic morphology in part determines the information-processing capacity of the neuron, because more elaborate dendritic fields support more synaptic inputs.

A single neuron may receive thousands of synaptic contacts from other neurons. Some of these inputs excite the postsynaptic neuron (make it more likely to generate an action potential) whereas others inhibit it. In many neurons, integration of these incoming signals occurs in the **cell body**. From moment to moment, the cell membrane of the cell body combines the inhibitory and excitatory synaptic inputs, and if excitatory inputs surpass inhibitory inputs, the neuron may respond by generating one or more action potentials.

The long, slender **axon** is the conduction component of a neuron, serving to propagate action potentials along its length. The axon typically arises from the soma via a conical **axon hillock**, which leads to the **axon initial segment**, a specialized area that is commonly the site of action potential initiation. The microscopic axons from individual neurons sometimes collect together in long, macroscopically visible bundles that are called *tracts* in the central nervous system (CNS) and *nerves* in the peripheral nervous system (PNS).

Where the axon ends, it divides into several **presynaptic terminals**, which constitute the place where neuronal output occurs. The presynaptic terminals form synapses with neurons or other types of cells, such as muscle fibers. An action potential arriving at the presynaptic terminal triggers the release of neurotransmitter across the synapse to bind with specific receptor molecules on the postsynaptic cell, activating target cell responses. Neurons that form synaptic endings on a cell are said to **innervate** that cell.

The extended networks of neurons in an animal's body (along with supporting cells, described in Section 12.2) constitute

its *nervous system*. Neurons perform various roles in the nervous system. Some neurons perform sensory functions by initiating signals in response to physical or chemical stimuli, others serve to process and propagate this information throughout the brain, and yet others perform output functions by controlling effector cells. As we will discuss in Chapter 15, animals have a CNS (brain and spinal cord in vertebrates) that performs integrative and control functions, and a PNS that mediates interactions between the CNS and other parts of the body. Neurons that relay sensory signals to integrative centers of the CNS are called **afferent neurons** (*afferent*, "to bring toward"). Other neurons, called **efferent neurons** (*efferent*, "to carry off"), relay control signals (instructions) from the CNS to target cells that are under nervous control, such as muscle cells or secretory cells. Neurons that are entirely within the CNS are called **interneurons**.

Neural control has two defining features: It is fast and addressed. Neuronal signals are said to be *fast* because they travel rapidly and begin and end abruptly. A mammalian axon, for example, might conduct impulses along its length at 20–100 m/s, and it might be capable of transmitting 100 or more impulses in a second. Neuronal signals are said to be *addressed* because they provide highly discrete lines of communication. A neuron normally must make synaptic contact with another cell to exert control, and it innervates a discrete number of target cells that are determined by the neuron's morphology. Neuronal lines of communication therefore provide opportunities for fine control of other cells both *temporally* and *spatially*, sending fast, rapidly changing signals to some potential targets and not to others.

Endocrine cells broadcast hormones

In contrast to neurons, in which signaling is precisely targeted for communication across synapses, endocrine cells release hormones into the blood (or sometimes into other extracellular fluids) such that they are carried throughout the body, bathing the tissues and organs at large. Endocrine control has two defining features: It is slow and broadcast.

Hormonal signals are said to be *slow* because they operate on much longer timescales than neuronal signals. Once a hormone is released into the blood, it must circulate to target tissues and diffuse to effective concentrations within the tissues before it can elicit responses—a process that requires at least several seconds or minutes. Furthermore, a hormone may act on targets for a considerable length of time before metabolic destruction and excretion decrease its concentration to ineffective levels. In the human bloodstream, for example, the hormones vasopressin, cortisol, and thyroxine display half-lives of about 15 min, 1 h, and nearly 1 week, respectively. Thus a single release of hormone may have protracted effects on target tissues.

Unlike addressed neural control, endocrine control is said to be *broadcast*. Once a hormone is released into the blood, it can be carried to all the cells in the body. The specificity of hormone action depends on which cells have receptor molecules for the hormone, and is not restricted by anatomical location. Many types of cells may respond to the hormone, although different types may respond in different ways. Alternatively, a hormone may affect only one type of target cell, because only that type of cell has the kind of receptor to which the hormone attaches. Although hormones can exert either limited or widespread effects, they typically affect multiple tissues.

Nervous systems and endocrine systems exert control in different ways

Nervous systems are capable of much finer control—both temporal and spatial—than are endocrine systems. It is not surprising, then, that the two systems typically control different functions in the body. *Whereas the nervous system primarily controls the moment-to-moment movements of individual muscles, the endocrine system controls prolonged and widespread activities* such as metabolic changes.

Consider, for example, running to catch a fly ball in baseball. It requires split-second computation and control of discrete muscles—functions that can be mediated only by the nervous system. In contrast, metabolism requires the modulation of many tissues over a protracted period. Although in theory an animal's nervous system *could* control metabolism, doing so would require thousands of discrete axons between integrating centers and target cells, as well as propagation of impulses for as long as the modulation was required. An endocrine gland can accomplish this task by secreting a single long-lasting chemical into the blood. For this reason, control of metabolism is often under primarily hormonal control, as are other processes (growth, development, reproductive cycles, etc.) that involve many tissues and occur on timescales of days, months, or years.

Most tissues in an animal's body are controlled by both the nervous and endocrine systems. Skeletal muscle illustrates the relationship of this dual control. A typical vertebrate muscle contains thousands of muscle cells (muscle fibers) and is innervated by more than 100 **motor neurons.** Each motor neuron innervates a separate set of muscle fibers, controlling the contraction of just these fibers. By selectively activating just a few, many, or all of the motor neurons, the nervous system can rapidly and precisely control how much force the muscle generates. Whereas the nervous system controls the contractile activity of the muscle cells over the short term, the hormone insulin provides endocrine control of their metabolic activity over the long term. Insulin facilitates resting muscle fibers' uptake of glucose from the blood and their rate of glycogen synthesis. This example emphasizes the spatial and temporal distinctions between the two types of control: The nervous system controls moment-to-moment, differential contractile actions of the muscle cells in a muscle, whereas the endocrine system provides long-term metabolic control of all the muscle cells en masse.

Nervous and endocrine systems can exert control over each other, as well as over other targets. *Interaction between the nervous and endocrine systems occurs in both directions.* Nervous systems can affect the function of endocrine cells, as in innervated endocrine glands. Likewise, hormones can modulate nervous system function; for example, sex steroid hormones affect certain neurons in mammalian brains.

SUMMARY

The Physiology of Control: Neurons and Endocrine Cells Compared

- Control by the nervous system involves neurons that send axons to discrete postsynaptic cells. Neurons propagate rapidly conducting action potentials to transmit signals from point to point within the cell. They exert fast, specific control by releasing neurotransmitters at synapses.
- Endocrine cells release hormones into the bloodstream to mediate endocrine control. All body cells are potential targets of a hormone, but only those with specific receptors for the hormone actually respond. Hormonal control is slower, longer lasting, and less spatially circumscribed than neural control.

12.2 The Cellular Organization of Neural Tissue

Nervous systems are composed primarily of neural tissue, which in turn is composed of discrete cells: neurons and glial cells, as well as connective tissue cells and cells of the circulatory system. The cellular organization of nervous systems is a corollary of the **cell theory**, which states that organisms are composed of cells, that cells are the structural and functional units of organization of the organism, and that all cells come from preexisting cells as a result of cell division. Matthias Schleiden (1804–1881) and Theodor Schwann (1810–1882) formulated the cell theory in 1839.

The cell theory gained widespread and rather rapid acceptance—except as applied to nervous systems. Instead, the dominant view of nervous system organization in the latter half of the nineteenth century was the **reticular theory**, most strongly argued by Joseph von Gerlach (1820–1896) and Camillo Golgi (1843–1926). The reticular theory held that nervous systems were composed of complex, continuous meshworks of cells and processes in protoplasmic continuity with each other (i.e., the cells ran together without any boundaries).

The reticular theory was supplanted only gradually, over the first third of the twentieth century, by an outgrowth of the cell theory known as the **neuron doctrine**. The neuron doctrine states that neurons are anatomically distinct and are the structural, functional, and developmental units of organization of nervous systems. Santiago Ramón y Cajal (1852–1934), the main champion of the neuron doctrine, used special staining techniques to demonstrate convincingly that neurons are contiguous (in contact with each other) but are not continuous (connected without interruption). However, the debate on contiguity versus continuity persisted until the 1950s, when electron microscopy permitted resolution of cell membranes and rigorously demonstrated the discontinuity of neurons.

Neurons are structurally adapted to transmit electrical and chemical signals

Neurons, as seen earlier, are cells that are specialized to receive and integrate inputs, generate and conduct electrical signals from place to place within the cell, and transmit output signals for communication with other cells. These functional properties of neurons arise from structural adaptations that differentiate neurons from other cells of the body. As shown in Figure 12.2, a neuron consists of a *cell body*, or *soma* (plural *somata*) (also called the *perikaryon*), which is the region that contains the nucleus, and one or more processes arising from it.

The cytology of a neuronal soma is broadly similar to that of nonneuronal cells. A neuronal soma contains a nucleus and most of the organelles and cytoskeletal elements familiar to cytologists: mitochondria, Golgi apparatus, smooth endoplasmic reticulum (ER), rough ER, microtubules, neurofilaments, and actin microfilaments. Neurons are very active in protein synthesis and thus have extensive, well-developed rough ER.

Neurons can be classified according to the number of processes emanating from the soma. Neurons may be unipolar (having one process), bipolar (two processes), or multipolar (three or more processes). Unipolar neurons predominate in the CNS of most invertebrates, multipolar neurons predominate in the vertebrate CNS, and many sensory neurons are bipolar in various taxa. The neuronal processes exhibit a bewildering geometric variety and complexity, as evidenced by the amazing diversity of neuron types (FIGURE 12.3). Early anatomists attempted to bring order to this variety by classifying

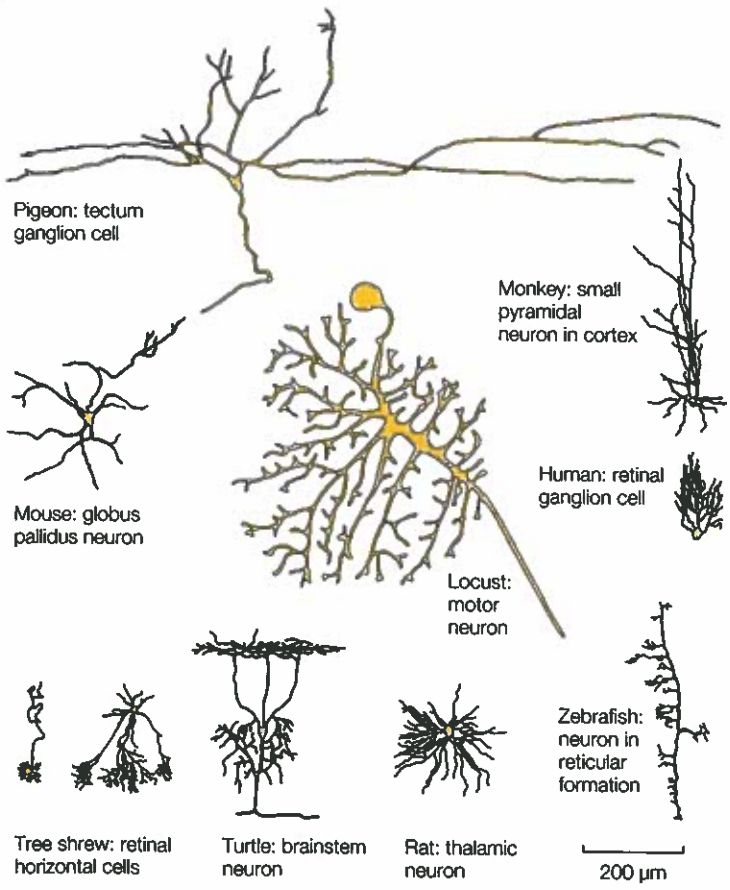


FIGURE 12.3 Neuronal morphology varies extensively
Neuron types differ across many parameters, including overall size and dendritic branching. These examples are taken from various species.

processes as *axons* and *dendrites*. Their classifications were usually based on vertebrate CNS neurons and are useful for cells resembling vertebrate CNS neurons. Definitions of dendrites and axons, however, are based on a mixture of functional and morphological criteria that do not always coincide in a single neuron. Functionally (as we noted in Section 12.1) a *dendrite* is considered to be a *receptive element* of a neuron that conveys information toward the soma. An *axon*, by contrast, is the *output element* of a neuron, carrying information away from the cell body to other cells.

Dendrites of most neurons have continuously varying diameters and lack myelin sheaths (which we'll discuss shortly). In general, the broader dendritic trunks resemble the soma in fine structure; they contain rough ER, mitochondria, microtubules, neurofilaments, and an occasional Golgi apparatus. Thinner dendritic branches may lack Golgi apparatuses and rough ER. The dendrites of many neurons bear numerous short, thin protrusions termed *dendritic spines* which, when present, are important sites of synaptic input.

A single axon typically extends from the cell body with a relatively constant diameter and few collateral branches. At the fine structural level, axons contain microtubules, neurofilaments, and elongated mitochondria (see Figure 12.2). Axons generally lack rough ER and Golgi apparatuses. The larger vertebrate axons are surrounded by **myelin** sheaths—multiple wrappings of insulating glial cell membranes (see the next paragraph) that increase the speed of action-potential transmission. Not all axons are myelinated; the smaller axons of vertebrate neurons and nearly all invertebrate axons lack myelin and are termed *unmyelinated*.

Glial cells support neurons physically and metabolically

Cells that are referred to collectively as **glial cells** or **neuroglial** ("nerve glue") **cells** (or more simply, **glia**) surround the neurons (**FIGURE 12.4**). Rudolf Virchow (1821–1902) discovered and named the neuroglial cells in 1846 and thought that their primary function was to bind the neurons together and maintain the form and structural organization of the nervous system. Glia are abundant in nervous systems and in some cases even outnumber neurons. In general, the ratio of glia to neurons increases with increasing evolutionary complexity. Thus the roundworm *Caenorhabditis elegans* has a total of 56 glial cells compared with 302 neurons (for a glia to neuron ratio of ~0.2), whereas primate nervous systems (including that of humans) have roughly equal numbers of glia and neurons. These measures suggest that glia are important in nervous system function, perhaps in ways that are not yet fully understood.

Different types of glia play diverse functional roles in nervous systems. Vertebrate nervous systems have two kinds

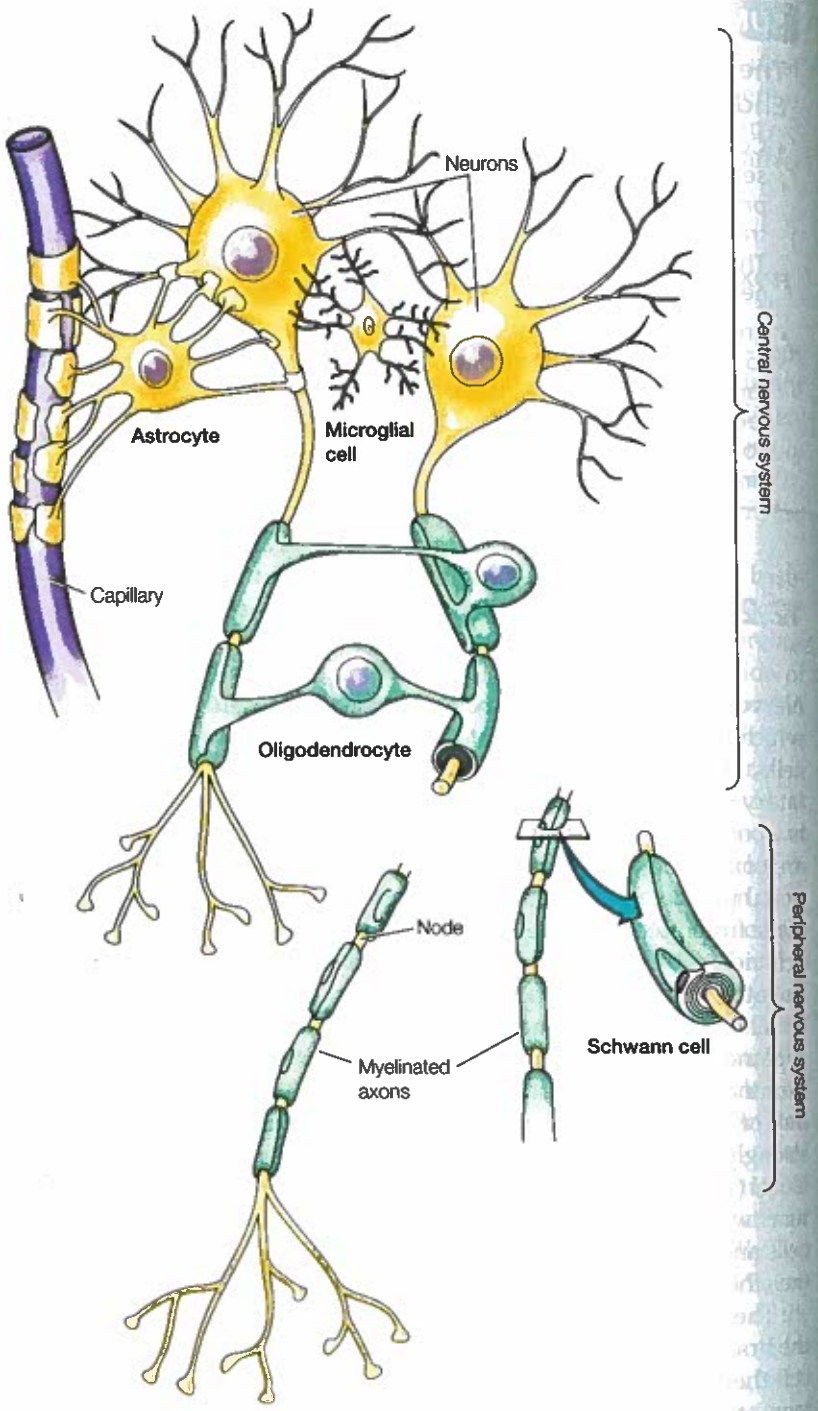


FIGURE 12.4 Glial cells There are four types of glial cells in vertebrate nervous systems. Schwann cells ensheathe axons (myelinated are shown; unmyelinated are not shown) in the PNS. Oligodendrocytes ensheathe axons in the CNS. Astrocytes are metabolic support cells in the CNS. Microglial cells are phagocytes related to cells of the immune system.

of ensheathing glia, called **Schwann cells** (in the PNS) and **oligodendrocytes** (in the CNS), that envelop the axons of neurons (see Figure 12.4). The glial sheath can be a simple encircling of an *unmyelinated* axon or a group of axons, or a *myelin sheath* consisting of multiple concentrically wrapped layers of glial membrane that insulate the axon and increase the velocity

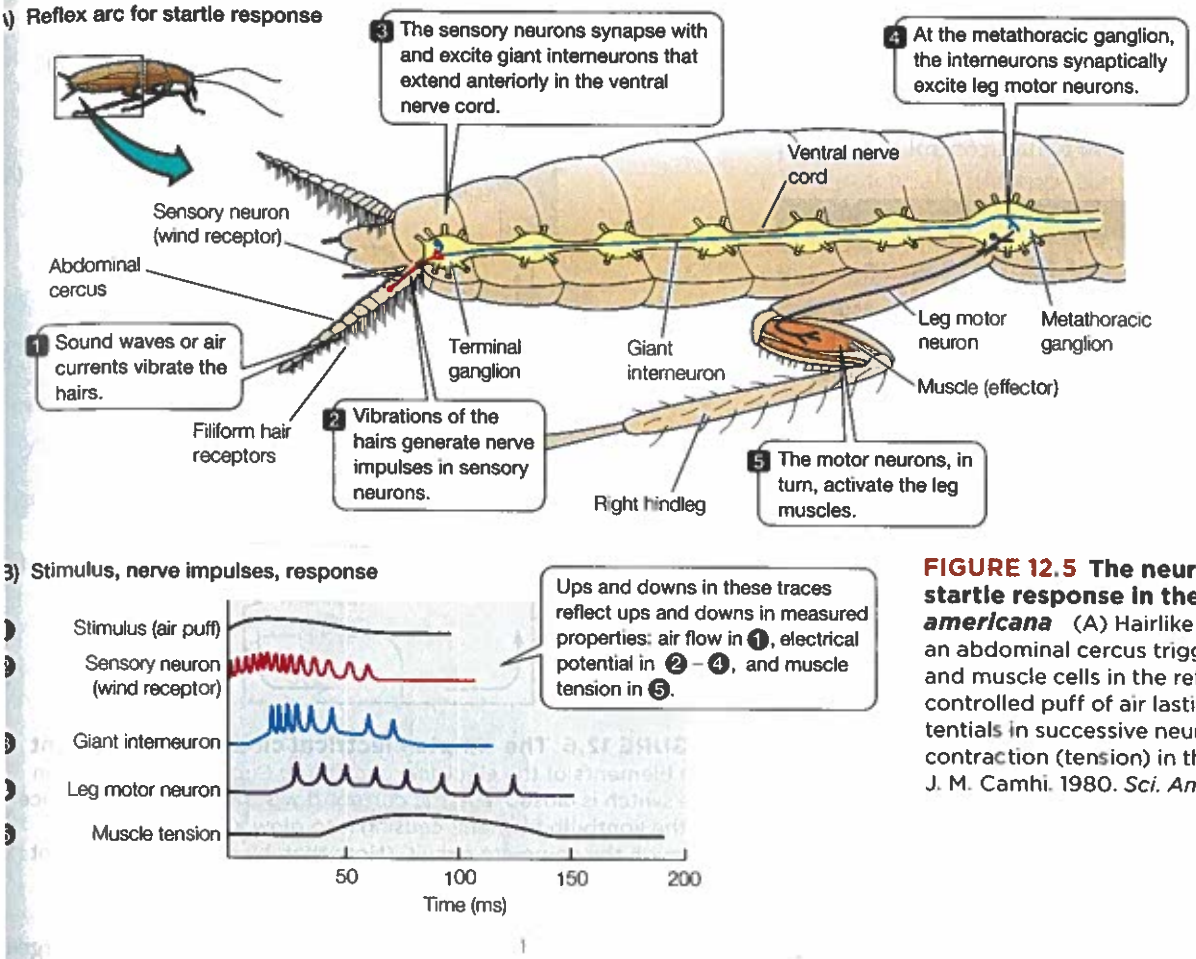


FIGURE 12.5 The neural circuit mediating the startle response in the cockroach *Periplaneta americana* (A) Hairlike wind receptors located on an abdominal cercus trigger this reflex. (B) Nerve and muscle cells in the reflex circuit respond to a controlled puff of air lasting 50 ms. The action potentials in successive neurons in the circuit lead to contraction (tension) in the muscle of the leg. (After J. M. Camhi. 1980. *Sci. Am.* 243: 158–172.)

of action potential propagation (discussed in detail in Section 2.6). Other, star-shaped glial cells called **astrocytes** interact with neurons, blood vessels, and other cells through thousands of fine processes that extend from their cell bodies. Astrocytes line the outside surfaces of capillaries in the vertebrate CNS and act as metabolic intermediaries between the capillaries and neurons. In this way they help supply metabolic substrates to neurons. Astrocytes play a particularly important role at synapses (a single astrocyte can contact thousands of individual synapses), where they modulate neurotransmission by taking up neurotransmitters from the extracellular space and regulating extracellular ion concentrations. Finally, **microglial cells**, *named for their small size*, mediate immune responses in neural tissue and may act as phagocytes, consuming pathogens and cell debris in brain injury. In addition to the functions we have already mentioned, it is becoming increasingly clear that glial cells, including astrocytes and microglia, regulate nervous system development and plasticity.

Neurons are organized into functional circuits in nervous systems

Single neurons do not operate in isolation; the functions of a nervous system depend on "wiring"—the anatomical organization by which neurons are connected into circuits. Any behavioral activity (such as swimming in the squid with which we opened the chapter) is a property of the neural circuit that mediates it. We will discuss nervous system organization in Chapter 15, but here we provide a simple illustrative example. Suppose you walk into the kitchen and surprise a cockroach. The cockroach jumps, exhibiting a *startle response* in which it turns away from the disturbance and prepares to run. This simple behavioral act is mediated by electrical signals and chemical synapses within the cockroach's nervous system.

The cockroach's jump is a **reflex**, a simple, stereotyped behavioral response to a distinct stimulus. Air currents or airborne sound waves vibrate filiform hairs that act as wind receptors at the cockroach's posterior end (FIGURE 12.5, ❶), providing the stimulus that evokes the reflex. This stimulus initiates a brief series of action potentials in sensory neurons of the PNS ❷ located at the bases of the hairs. The action potentials travel along the axons of the afferent sensory neurons toward the CNS, where the sensory neurons make synaptic contacts with a few large *interneurons*. These synapses are excitatory, so the barrage of action potentials from the sensory neurons causes the interneurons to generate their own action potentials ❸.

The interneuron axons extend anteriorly in the ventral nerve cord (part of the CNS). They in turn make synaptic contact with efferent motor neurons, whose outgoing axons exit the CNS and innervate a muscle. The interneurons synaptically excite the motor neurons ❹, which in turn excite the extensor muscles of the legs ❺ that produce the jump. At the same time, the interneurons inhibit motor neurons that excite the antagonist flexor muscles of the cockroach's legs.

As the barrage of action potentials in Figure 12.5 indicates, this startle response happens very quickly: It is less than 150 milliseconds (ms) from stimulus to jump! This rapid and selective activation of particular muscles to generate a behavioral response is the essential element of neural control.

SUMMARY

The Cellular Organization of Neural Tissue

- Neurons are the principal cells of nervous systems. They are structurally adapted to receive signals from other neurons (via dendrites) and to generate and propagate action potentials (via axons).
- Glial cells are the support cells of the nervous system. Schwann cells (in the PNS) and oligodendrocytes (in the CNS) form sheaths around neuronal axons, including insulating myelin sheaths around myelinated axons. Astrocytes surround capillaries and act as metabolic intermediaries between neurons and their circulatory supply. Microglial cells serve immune and scavenging functions.
- Neurons function as part of neuronal circuits to receive and integrate incoming sensory information and generate appropriate motor outputs.

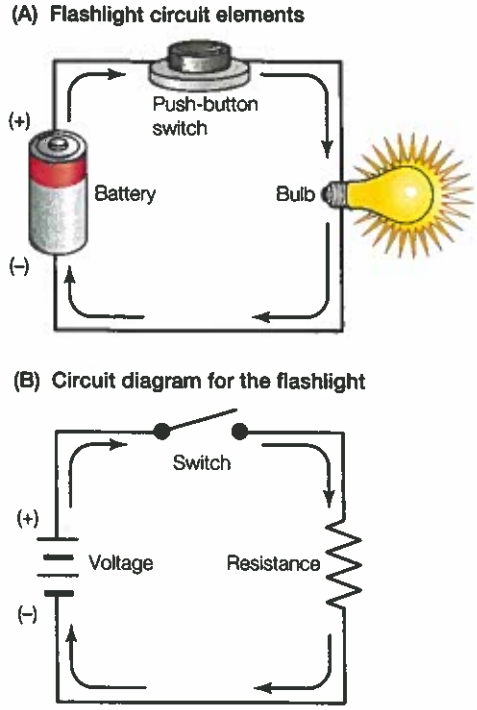


FIGURE 12.6 The simple electrical circuit of a flashlight (A) Elements of the electrical circuit. (B) Circuit diagram. When the switch is closed, electric current flows through the resistance of the lightbulb filament, causing it to glow. Current must flow through the complete circuit. (Note that, by convention, current always flows from plus to minus.)

12.3 The Ionic Basis of Membrane Potentials

What are the properties of the electrical signals of neurons, and how are these signals generated? Let's begin with a brief review of basic electrical concepts. Protons and electrons have *electrical charge*, and **ions** are atoms or molecules that bear a net charge because they have unequal numbers of protons and electrons. The net movement of charges constitutes an **electric current (I)**, which is analogous to the hydraulic current of fluids flowing in a system of pipes. The separation of positive and negative electrical charges constitutes a **voltage**, or electrical *potential difference* (*V*). This potential difference can do work when charges are allowed to flow as current. Voltage is analogous to a height difference or head of pressure in a hydraulic system, allowing water to flow downhill.

FIGURE 12.6 shows a simple electrical circuit, that of a flashlight. A battery provides voltage; closing the switch allows current to flow through the electrical circuit. The electric current in the flashlight is the flow of free electrons along metal wires. Current flows through the lightbulb filament, which acts as **resistance (R)** that limits the current flow. Consequently, the filament heats and glows, emitting light.

Electrical circuits in cells are similar to the circuit in a flashlight, but they differ in some important ways. In cells, both the inside and outside media are *aqueous solutions* in which the electrical charges are ions rather than free electrons. Furthermore, all currents in cells are carried by ions, and any voltage or potential difference results from local imbalances of ion charges.

Recall from Chapter 5 (see Figure 5.4) that fluids farther than a few nanometers from a membrane are electrically neutral, with equal numbers of positive and negative charges. Because of this *charge neutrality of bulk solutions*, the only portion of a cell that *directly* determines its electrical properties is its outer-limiting cell membrane. Any electrical activity of a nerve cell is a property of the cell membrane, and the electrical potentials observed are called *membrane* potentials. The only immediately important attribute of the rest of the cell is the concentration of ions in solution in the intracellular fluid.

Neurons maintain inside-negative membrane potentials at rest

The membrane potential of a neuron can be recorded with electrophysiological techniques, which involve the use of microelectrodes to monitor electrical activity. FIGURE 12.7 illustrates the use of electrophysiology to record the membrane potential of a squid giant axon. The largest axons of a common squid may be 2 cm long and 700–1000 μm in diameter. Because these axons are so large, it is relatively easy to cut out a length of the axon, ligate (close off) the ends, and penetrate the isolated axon with a microelectrode. The microelectrode consists of a glass capillary that has been heated and pulled to a fine tip (<1 μm in diameter) so that it can penetrate the cell membrane without causing damage. The capillary is filled with a solution of strong electrolyte, such as 3 molar (*M*) KCl, to minimize its electrical resistance.

When the tip of the microelectrode is outside the axon (see Figure 12.7A), a voltmeter records no potential difference

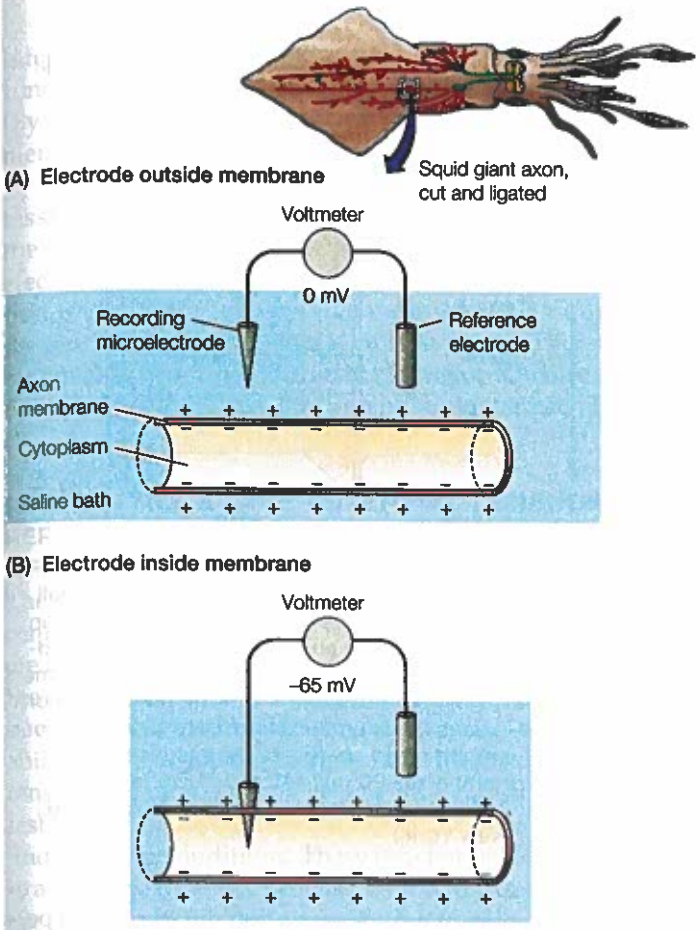


FIGURE 12.7 Recording the resting membrane potential of a squid giant axon A section of the squid axon is removed and its ends ligated to seal the axon segment. (A) A voltmeter measures the potential difference between a glass capillary microelectrode (the recording electrode) and a reference electrode in the saline bath around the axon. When the microelectrode is outside the axon, there is no potential difference between the two electrodes. (B) The recording microelectrode has been advanced through the axon membrane, and the resting membrane potential (V_m) is recorded. (C) The output of the voltmeter, recorded on a chart writer or an oscilloscope, demonstrates that the resting membrane potential is inside-negative, a condition true for all cells. (By convention, negative is down for intracellular recording.)

(voltage) between the recording microelectrode and a neutral (reference) electrode suspended in the surrounding saline bath. Both electrodes are electrically neutral because of the charge neutrality of bulk solutions.

When the recording microelectrode is advanced just past the axon membrane into the cytoplasm (see Figure 12.7B), the voltmeter records a potential difference (see Figure 12.7C). In this example we are measuring the membrane potential of the axon when it is at rest (i.e., when it is not transmitting a signal). This potential difference across the membrane is the **resting membrane potential** (V_m). For all known cells, the polarity of the resting membrane potential is *inside-negative*; that is, the inner membrane surface is negative with respect to the outer membrane surface.[2] As we will see, the resting membrane potential depends on two factors: the relative permeability of the neuron to different ion species and the concentrations of those ions inside and outside the neuron.

IONS TEND TOWARD ELECTROCHEMICAL EQUILIBRIUM: THE NERNST EQUATION Dissolved ions have charges and are surrounded by water molecules. The charged ions cannot mix with the nonpolar tails of the lipid molecules in the center of the membrane bilayer, so the ions cannot pass through the bilayer (see Chapter 5). Instead, ions must pass (if they pass at all) by way of the protein ion channels that span the bilayer. There are many kinds of ion channels, each kind *selectively permeable* to specific ions. Moreover, some ion channels can open and close, which means that *membrane permeability to specific ions is a controlled condition*.

When a neuron is permeable to a particular ion species, that ion is free to flow across the neuronal membrane (we refer to ions that are able to cross the membrane as *permeating* ions). The direction of ion flow is determined by a combination of two forces: the *diffusion (or chemical) force*, which is dictated by the concentration gradient of an ion (ions tend to move toward areas of lower concentration), and the *electrical force*, which is dictated by the membrane potential of the neuron (ions tend to flow toward areas of opposite charge).

Consider a simplified cell (FIGURE 12.8) that contains a solution of potassium ions (K^+) and nonpermeating anions (represented by A^-), such as charged proteins. (The identity of A^- is unimportant; all we're concerned with is its charge.) The cell is in a bath of nonpermeating anions (A^-) and nonpermeating sodium ions (Na^+); we stipulate that the cell membrane is permeable only to K^+. When the cell is placed in the bath, K^+ tends to diffuse out of the cell, down its concentration gradient. Because charge neutrality is always maintained in bulk solution, A^- would tend to follow the K^+ across the membrane; however, the membrane is not permeable to A^-. Therefore A^- accumulates at the inner surface of the membrane, while K^+ accumulates at the outer surface (because of charge attraction). Thus a net charge separation develops, but only at the membrane.

The net negative charge on the inner surface of the membrane and net positive charge outside tend to move K^+ ions back into the cell, by forces of charge attraction and repulsion. Eventually this system reaches *electrochemical equilibrium*, in which there is no *net* movement of ions and no work is done. In Figure 12.8, equilibrium is reached when the tendency for K^+

[2] By convention, the membrane potential is given as the inside value, with the outside considered zero.

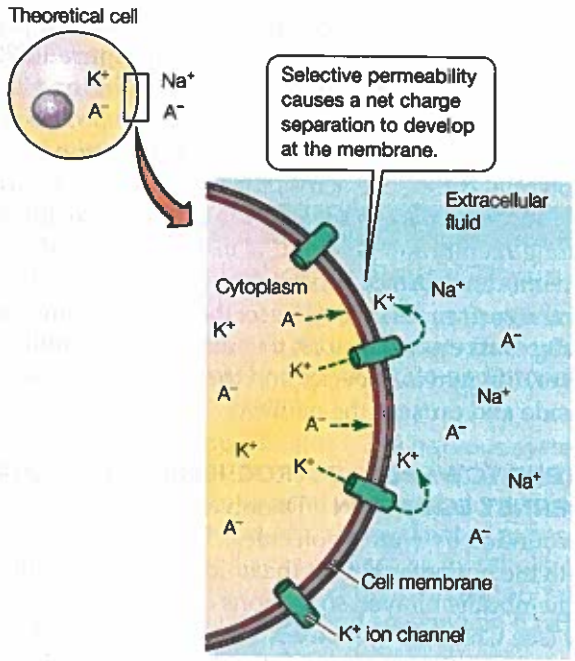


FIGURE 12.8 Selective permeability of a membrane gives rise to a membrane potential A simplified, theoretical cell containing a solution of K^+ and A^- is bathed by a solution of Na^+ and A^-. The cell membrane is permeable only to K^+. At the membrane, K^+ ions tend to diffuse out, down their concentration gradient. A^- ions attempt to follow (to maintain charge neutrality), but the membrane is not permeable to them, so they cannot pass through it. The resulting charge separation produces a membrane potential.

ions to diffuse out of the cell (down the concentration gradient) is exactly balanced by their tendency to move in (down the electrical gradient of the membrane potential). That is, when the diffusion force just equals the opposing electrical force, there is no *net* flow of K^+ ions. *For this system to come to equilibrium, there must be an electrical force (i.e., a membrane potential) across the membrane.* This simple example shows that any cell that has a transmembrane concentration difference of a permeating ion tends to generate a membrane potential. It is a good starting point for visualizing a cell's resting potential because cells at rest are more permeable to K^+ than to other ions.

It is important to grasp that the resting membrane potential results from relatively few ion charges sitting on the membrane, and to remember that the charge separation producing the membrane potential is an extraordinarily local phenomenon. Charge neutrality always prevails in the bulk solutions that make up the intracellular and extracellular fluids. For example, there are approximately 110,000 cations and 110,000 anions in a 1 μm × 1 μm × 0.001 μm "slice" of the fluid compartments on either side of the cell membrane of a mammalian muscle fiber (**FIGURE 12.9**). Of these portions, approximately six pairs of ions sit along the 1 μm × 0.001 μm area of membrane, and these six pairs are responsible for the charge imbalance that amounts to a robust –90-mV resting membrane potential! Thus the movement of only a few ions in a region can establish (or change) a membrane potential without disrupting the overall charge neutrality of the intracellular and extracellular fluids.

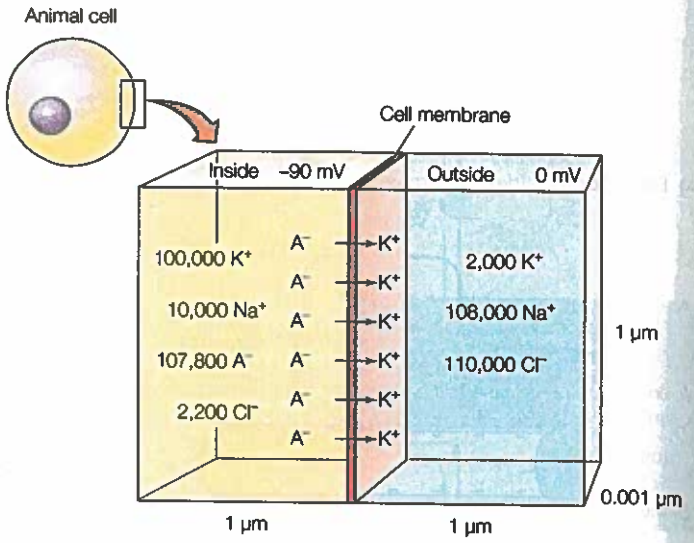


FIGURE 12.9 The membrane potential results from relatively few charges sitting on the membrane A small patch of membrane (1 μm × 0.001 μm in area) from a mammalian muscle fiber, with a small volume (1 μm × 1 μm × 0.001 μm) of adjacent cytoplasm and extracellular fluid. As in Figure 12.8, assume that the membrane is most permeable to K^+. Of the 110,000 cations and 110,000 anions in each fluid compartment, only *six* pairs of ions need to sit on the membrane and charge its capacitance to produce a membrane potential of –90 mV. (After J. F. Dudel. 1985. In R. F. Schmidt. [ed.], *Fundamentals of Neurophysiology*, 3rd ed., pp. 19–68. Springer, New York.)

The relation between the concentration difference of a permeating ion across a membrane and the membrane potential at equilibrium is given by the **Nernst equation**:

$$E = \frac{RT}{zF} \ln \frac{C_{out}}{C_{in}} \qquad (12.1)$$

in which E is the membrane potential, R is the gas constant, T is absolute temperature in Kelvin, z is the valence of the ion species (charge for the kind of ion), F is Faraday's constant (charge per mole of ions), and C_{out} and C_{in} are the ion concentrations on the two sides of the membrane. Notice that *the larger the concentration difference across the membrane, the larger the membrane potential* at which the ion species is in equilibrium. The reason for this relation is that increasing the concentration difference increases the concentration gradient of the ion species and thus increases the electrical force necessary to oppose it.

We can simplify the Nernst equation by calculating R/F, converting to $\log_{10}$, and considering an ion of a given valence at a given temperature. For K^+, a monovalent cation, at 18°C (British room temperature),

$$E(\text{in mV}) = 58 \log_{10} \frac{C_{out}}{C_{in}} \qquad (12.2)$$

For a mammal at 37°C,

$$E(\text{in mV}) = 61 \log_{10} \frac{C_{out}}{C_{in}} \qquad (12.3)$$

Thus for our simplified cell permeable only to K^+, if the internal K^+ concentration is, say, 100 m*M* and the external concentration is 5 m*M*, at 18°C, $E = 58 \log_{10} (0.05) = -75$ mV. (By convention, the minus sign means that the inside of the membrane is negative relative to the outside.)

The value of –75 mV is the **equilibrium potential** for potassium (E_K) in our system as we have defined it—that is, the value of the membrane potential at which K^+ ions are at electrochemical equilibrium and the internal K^+ concentration is 20 times the external concentration. In other words, the electrical force holding K^+ inside the cell is just balanced by the chemical (concentration) force for K^+ diffusion out of the cell. There is an equilibrium potential for each ion species (E_{Na}, E_{Cl}, etc.).

RESTING MEMBRANE POTENTIAL DEPENDS ON THE PERMEABILITIES TO AND CONCENTRATION GRADIENTS OF SEVERAL ION SPECIES: THE GOLDMAN EQUATION

The Nernst equation relates membrane potential to the concentration ratio of only one ion species; however, real neurons are permeable to multiple ion species, each attempting to push the cell toward its own equilibrium potential. The contribution of each ion to resting membrane potential is weighted by its ability to permeate the membrane, with the more-permeating ions having more effect. Permeability of the cell membrane at rest is determined by the presence of ion channels that are open under resting conditions. These ion channels are often referred to as leakage channels because they allow ions to "leak" across the cell membrane.

The resting membrane potential is largely determined by K^+ concentrations because the cell membrane is more permeable to K^+ than to other ions. If the membrane were permeable only to K^+, the membrane potential would be exactly equal to the K^+ equilibrium potential (i.e., $V_m = E_K$), as predicted by the Nernst equation employing the K^+ concentrations across the membrane. Because the membrane is somewhat permeable to other ions, however, they also contribute to the membrane potential. The value of the membrane potential produced by the contributions of several permeating ion species can be determined by the **Goldman equation**:

$$V_m = \frac{RT}{F} \ln \frac{P_K[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^-]_i}{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_o} \qquad (12.4)$$

in which P_K, P_{Na}, and P_{Cl} are relative permeability values for K^+, Na^+, and Cl^- ions, respectively.[3] (The chloride term in the equation is inverted to reflect its negative charge.)

In principle, it is necessary to add a term in the Goldman equation for every permeating ion species, but in practice it is necessary to include terms only for Na^+, K^+, and Cl^-. The contributions of other ion species can be discounted, by reason of either low permeability of the membrane to those ions (e.g., HCO_3^-) or low concentrations of those ions (e.g., $[H^+] = 10^{-7}$ *M*).[4]

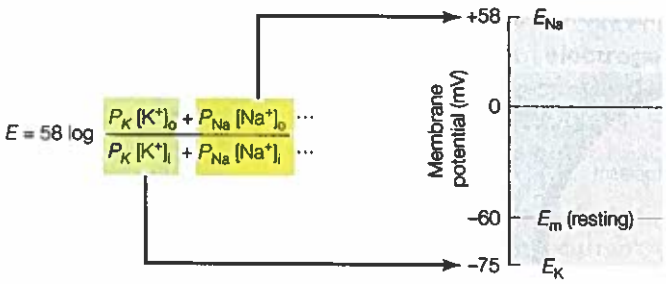


FIGURE 12.10 The Goldman equation and the "voltage thermometer" A simplified Goldman equation describes membrane potential in terms of relative permeabilities (*P*) of the membrane to K^+ and Na^+. The voltage scale graphs the membrane potential determined by these permeabilities. For the resting membrane, $P_K > P_{Na}$, so E_m is close to E_K. If P_{Na} increases to become greater than P_K, E_m will approach E_{Na}. Each arrow relates the dominant term in the equation to the value of E_m toward which it drives the membrane.

In fact, for some purposes it is useful to consider Na^+ and K^+ only, ignoring Cl^-. In such a simplification of the Goldman equation (FIGURE 12.10), we can view the membrane potential as a result of the membrane's relative permeabilities to Na^+ and K^+, visualized with a sliding voltage scale, rather like a thermometer but in units of voltage. Consider a squid axon with the following ion concentrations:

$[K^+]_{out} = 20mM$ $\quad [Na^+]_{out} = 440mM$

$[K^+]_{in} = 400mM$ $\quad [Na^+]_{in} = 44mM$

$[K^+]_{out} : [K^+]_{in} = 0.05$ $\quad [Na^+]_{out} : [Na^+]_{in} = 10$

$E_K = 58 \log 0.05 = -75mV$ (inside-negative)

$E_{Na} = 58 \log 10 = +58mV$ (inside-positive)

If the cell were permeable only to K^+, then V_m would equal E_K, or –75 mV; and if it were permeable only to Na^+, then V_m would equal E_{Na}, or +58 mV. The actual membrane potential can be anywhere between these values and is governed by the ratio of membrane permeabilities to Na^+ and K^+. The "voltage thermometer" shows that when permeability to K^+ is much greater than permeability to Na^+, the membrane potential approaches E_K (see Figure 12.10):

If $P_K = 25 \times P_{Na}$

$$V_m = 58 \log \frac{1[20] + .04[440]}{1[400] + .04[44]} = 58 \log (37.6/401.76) = -59.7mV$$

[3] In most neurons, K^+ leakage channels predominate, followed by Cl^- and Na^+ leakage channels. By convention, P_K is set to 1.0. Relative to this, P_{Cl} is typically ~0.45, and P_{Na} is ~0.04.

[4] Strictly speaking, the ion concentration values in the Nernst and Goldman equations should be those for *free* (unbound) ions rather than total concentrations. The *activity* of an ion is the concentration of the ion in its dissociated, freely diffusible form. Monovalent ions dissociate relatively completely in cytoplasm and extracellular fluids, so not much correction is needed for ion activities. Divalent ions such as Ca^{2+}, however, are predominantly bound in cytoplasm, so corrections for their activity are important.

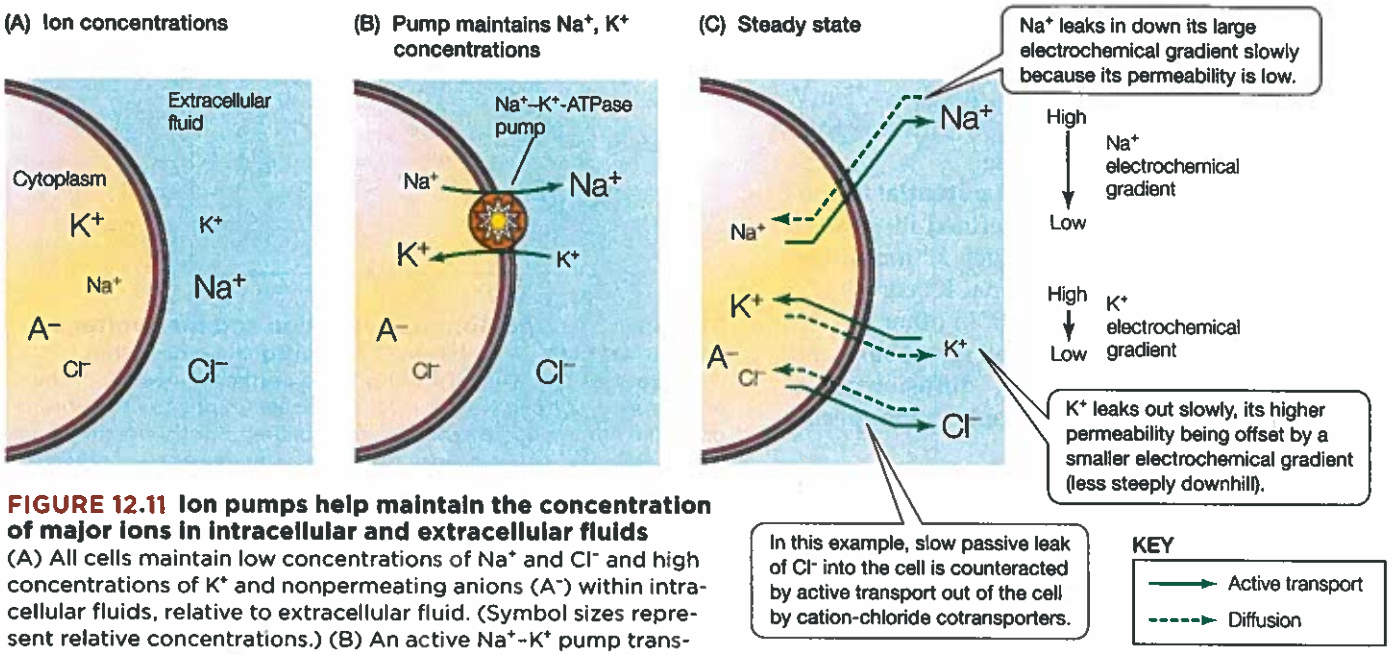


FIGURE 12.11 Ion pumps help maintain the concentration of major ions in intracellular and extracellular fluids
(A) All cells maintain low concentrations of Na^+ and Cl^- and high concentrations of K^+ and nonpermeating anions (A^-) within intracellular fluids, relative to extracellular fluid. (Symbol sizes represent relative concentrations.) (B) An active Na^+–K^+ pump transports Na^+ out and K^+ in, counteracting the tendency of Na^+ to diffuse in and K^+ to diffuse out. (C) Here the Na^+ and K^+ concentrations are maintained in a steady state across the membrane. Neither Na^+ nor K^+ is at equilibrium, so passive diffusion (across leakage channels) produces net movement of Na^+ and K^+ (dashed arrows). This passive diffusion is counteracted by active transport by the Na^+–K^+ pump (solid arrows). Chloride is also actively transported in this cell.

In contrast, when permeability to Na^+ is much higher than permeability to K^+, the membrane potential approaches E_{Na}. This visualization of membrane potential in terms of the Goldman equation will be important for our consideration of action potentials later in this chapter.

Ion concentration differences are actively maintained

All cells maintain higher concentrations of K^+ and lower concentrations of Na^+ and Cl^- in the intracellular fluids than are present in the surrounding extracellular fluid (FIGURE 12.11A). The concentrations of these ions differ from organism to organism, as shown in TABLE 12.1. Despite quantitative differences, the *concentration ratios* of ions in all cells are similar to those represented in the table. The difference in ion concentrations between the intracellular and extracellular fluids results from a combination of two processes: (1) the active transport of some ions and (2) the passive distribution of other ions.

Examine Figure 12.11A and consider the Nernst equation. It is impossible for both Na^+ and K^+ to be in passive equilibrium, because the ratios of their concentrations differ. Whereas K^+ would require an inside-negative membrane potential to be in equilibrium, Na^+ would require an inside-positive potential (to counteract inward Na^+ diffusion). In fact, *neither* ion species is in passive equilibrium in cells. At rest, Na^+ is constantly leaking into the cell to try to bring the membrane potential toward its very positive equilibrium potential. At the same time, K^+ is leaking out of the cell to try to bring membrane potential toward its very negative equilibrium potential. At some point, the inward movement of Na^+ is exactly counterbalanced by the outward flow of K^+, and the cell reaches an electrical *steady state* that maintains resting membrane potential at a constant value.

If this were to continue unabated, it would be problematic for the cell because the concentration gradients of Na^+ and K^+ would gradually run down, dissipating the membrane potential. To prevent this, neurons use *pumps* and *transporters* that maintain ionic gradients by active ion transport that requires the input of energy from hydrolysis of ATP.

TABLE 12.1 Concentrations of major ions in intracellular fluid (cytoplasm) and extracellular fluid

	Squid axon			Mammalian neuron		
Ion type	**Out (m*M*)**	**In (m*M*)**	**Out/In**	**Out (m*M*)**	**In (m*M*)**	**Out/In**
Na^+	440	50	8.8	145	12	12.1
K^+	20	400	0.05	4	140	0.03
Cl^-	560	60	9.3	110	4	27.5
A^- (organic anions)	10	300	0.3	9	138	0.07

The most important pump is the Na^+–K^+ pump (see Section 5.3), which actively transports Na^+ out of the cell and K^+ into it. (See Figure 5.11 for a model of Na^+–K^+ pump function.) Recall that at rest, Na^+ ions slowly leak into the neuron and K^+ ions slowly leak out. The Na^+–K^+ pump counteracts these leaks, using ATP energy to pump Na^+ out as fast as it leaks in, and to pump K^+ in as fast as it leaks out (FIGURE 12.11B). The function of the Na^+–K^+ pump is analogous to that of a bilge pump in a boat, bailing water out as fast as it leaks in. Another good analogy for the pump is a battery charger, which can work in the background to prevent the "batteries" of Na^+ and K^+ concentration distributions from running down. For known Na^+–K^+ pumps, the ratio of Na^+ and K^+ pumped is 3:2. (We will consider a secondary consequence of the 3:2 ratio shortly.)

In some cells, Cl^- is in passive equilibrium, and Cl^- concentration gradients are maintained without the need for active transport. In most neurons, however, chloride concentration is actively regulated by molecules called cation-chloride cotransporters (CCCs). These include the Na–K–Cl (NKCC1) transporter, which uses the electrochemical driving force on Na^+ to bring Cl^- into the cell, and the K–Cl isoform 2 (KCC2) cotransporter, which extrudes Cl^- along with K^+. Interestingly, the relative prevalence of these two distinct chloride transporters is pathologically altered in neurons of some epilepsy patients, with NKCC1 expression increased and KCC2 decreased. Such a change would be expected to result in increased intracellular Cl^- levels, thereby bringing E_{Cl} to a more positive value and contributing to the extreme neuronal hyperexcitability associated with epilepsy. This example illustrates the importance of maintaining ion concentrations for proper neuronal function. In fact, it has been estimated that as much as 70% of the ATP in neurons is used to power active-transport mechanisms! FIGURE 12.11C summarizes the roles of active and passive transport in maintaining the steady-state concentrations of Na^+, K^+, and Cl^- ions in intracellular and extracellular fluids.

Electrogenic pumps have a small direct effect on V_m

Our explanation to this point of the generation of membrane potentials has been termed the ionic hypothesis. The **ionic hypothesis** argues that the concentrations of ions inside and outside a cell are maintained in a *steady state* by a mixture of active-transport processes (pumps) and passive-transport processes (diffusion). The ionic hypothesis further asserts that the concentrations of ions inside and outside the cell, and the permeability of the cell membrane to these ions, determine the resting membrane potential (V_m) as described by the Goldman equation. The ionic hypothesis is substantially accurate and provides a useful description of the factors giving rise to membrane potentials in living cells. A more complete explanation of the causes of membrane potentials, however, must include the fact that some ion pumps are electrogenic.

There are two kinds of active ion-transport mechanisms: electroneutral pumps and electrogenic pumps. An **electroneutral pump** transports equal quantities of charge inward and outward across a membrane and thus changes ion concentrations without generating an electric current. An **electrogenic pump** transports unequal quantities of charges inward and outward across the membrane. As noted already, the Na^+–K^+ pump has a 3:2 ratio, transporting 3 Na^+ ions out for each 2 K^+ ions transported into the cell.

Any ion pump that is not 1:1 generates a net current (net movement of charge) across the membrane. This current, acting across the cell's membrane resistance, directly generates a potential. The potential resulting from pump current changes V_m from the value predicted by the Goldman equation. Thus an electrogenic pump has two functional properties: It changes concentrations to offset passive leaks (its major function), and it alters V_m directly via the pump current (a smaller, secondary function).

The 3:2 Na^+–K^+ pump generates an outward ionic current (outward movement of positive charge) that hyperpolarizes the cell to a level more inside-negative than is predicted by the Goldman equation. Because Na^+–K^+ pumps can be selectively poisoned with toxins such as *ouabain*, their electrogenic contribution to resting membrane potentials can be measured as the initial change in V_m before concentrations change. (Inactivation of the pump will also [more slowly] lead to changes in ion concentrations, and thus have an additional, indirect effect on membrane potential.) In many neurons the direct contribution of an electrogenic pump accounts for only a few millivolts of the resting membrane potential, although electrogenicity can make a larger contribution in small axons and in some invertebrate neurons.

SUMMARY

The Ionic Basis of Membrane Potentials

- Neurons have inside-negative resting membrane potentials that are determined by the permeability to and concentration gradients of several ion species.
- Any ion species to which the membrane is permeable will tend to drive the membrane potential toward the equilibrium potential for that ion. The Nernst equation calculates the equilibrium potential of a single ion species in terms of its concentrations on both sides of the membrane.
- The resting membrane is dominated by permeability to K^+, so the resting membrane potential is near E_K, but other ions also contribute. The Goldman equation calculates membrane potential based on the contributions of all permeating ion species.
- All cells have higher concentrations of K^+ inside than outside, higher concentrations of Na^+ outside than inside, and higher concentrations of Cl^- outside than inside. Ion concentrations inside and outside cells are maintained by active ion pumps and transporters.
- In addition to their major role of maintaining the nonequilibrium concentrations of ions, electrogenic ion pumps generate a current that makes a small, direct contribution to V_m.

12.4 Passive Electrical Properties of Neurons

Information in the nervous system is carried by changes away from resting membrane potential as a result of current flow across the membrane. In the previous section we investigated the factors that establish and maintain the resting membrane potential of the neuron. In the following sections we investigate the electrical properties of neurons during active signaling, when membrane potential is dynamically regulated.

Stimulation of a neuron results in a local change in membrane potential at the site of stimulation. Depending on the nature of the stimulus, the response can be either a membrane depolarization or hyperporalization. With **depolarization**, membrane potential becomes less negative (the inside positivity of the cell membrane increases); with **hyperpolarization**, the cell membrane becomes more inside-negative. This signal is then propagated throughout the neuron and integrated with other inputs, potentially resulting in the generation of an action potential at the axon hillock.

Cell membranes have passive electrical properties: Resistance and capacitance

What determines the rate at which and the distance that neuronal signals are propagated? All cells respond to electric currents, but not all cells generate action potentials. The universal responses are *passive* responses (meaning that the cell's electrical properties do not change), but action potentials are active responses in which the properties do change. A cell's passive responses depend on the **passive electrical properties** of the membrane, principally its resistance and capacitance. A membrane exhibits *resistance* (measured in ohms, Ω) resulting from the fact that ions must flow through restrictive ion channels because the membrane's lipid bilayer is impermeable to ions. A membrane exhibits **capacitance** (measured in farads, F) because of the insulating properties of the bilayer. In electrical circuits, a capacitor has two conducting plates separated by an insulating layer; in cells, the conducting fluids on either side of the membrane act as plates, and the lipid bilayer acts as the insulator that separates and stores oppositely charged ions.

The resistance and capacitance of a cell's membrane depend on membrane area; specific membrane resistance and capacitance are measured per unit of area (e.g., R_m = 1000 Ω × cm^2; C_m = 1 μF/cm^2).[5] Whereas the specific membrane capacitance does not change, resistance may or may not change (depending on the behavior of ion channels). *When we speak of a cell's passive electrical properties, we mean those conditions in which membrane resistance does not change.* A cell's passive electrical properties govern how voltages change over space and time along neuronal processes. Passive electrical properties do not explain the generation of action potentials (in which resistances change), but they are important for understanding how neurons generate and propagate action potentials.

[5] The resistance of a whole cell's membrane is usually called its *input resistance*.

Passive electrical properties retard membrane voltage changes

To understand the passive electrical properties of neurons, we again turn to the squid giant axon. What would happen to the resting membrane potential if we inserted a second microelectrode into the squid axon and generated a pulse of electric current between it and an extracellular electrode (**FIGURE 12.12**)? The current pulse would either depolarize or hyperpolarize the membrane, depending on the direction of the current. In the example of Figure 12.12, we apply a depolarizing current that brings the membrane potential to a slightly more positive value, perhaps from –65 to –55 mV. (Remember that we are not considering action potentials yet, so assume that this depolarization is too small to trigger an action potential.) According to **Ohm's law**, the current should change the membrane potential by an amount proportional to the resistance to current flow:

$$\Delta V = IR \qquad (12.5)$$

where ΔV is the change in potential (termed a **graded potential** because the magnitude of the response is dictated by the size of the stimulus), I is the current (in amperes), and R is the resistance (in ohms). If the membrane exhibited only resistance, the change in membrane potential would occur instantaneously, as shown by the "theoretical" line in Figure 12.12B. However, the actual change in membrane potential occurs more gradually, reaching a plateau after a short delay, as shown by the "observed" line in Figure 12.12B.

The delay in membrane depolarization (or hyperpolarization) occurs because the membrane behaves electrically like a resistor and a capacitor in parallel (see Figure 12.12C). On the one hand, the lipid bilayer of a cell membrane behaves like a *capacitor*: The bilayer blocks the exchange of ions between the extracellular fluid and the intracellular fluid, and its insulative properties enable oppositely charged ions to accumulate along the inner and outer surfaces of the membrane. On the other hand, the membrane-spanning ion channels behave like *resistors*: They allow ions to flow across the membrane at a rate governed by the structure of the channels and the potential difference between the inside and outside of the membrane. Current first redistributes the charges on the membrane capacitance (capacitive current) and then flows through the membrane resistance (resistive or ionic current). This redistribution of charges slows (retards) the change in voltage on the membrane, by a factor that increases if the resistance or capacitance is increased.[6]

The exponential time course of the voltage change shown in Figure 12.12B is described by the **time constant**, τ (tau), the time it takes the voltage change to reach 63% of its final value. The time constant of a cell depends on the resistance and capacitance of its membrane:

$$\tau = RC \qquad (12.6)$$

where R is the cell's resistance (input resistance) and C is capacitance. For many cells, τ is in the range of 2–20 ms.

[6] To understand this, it is helpful to realize that *capacitance* (expressed in farads) is the amount of charge stored per unit of voltage ($C = Q/V$). The greater the capacitance, the more ions the membrane can separate and store for a given potential difference, and therefore the more time it takes for ions to be redistributed in response to a pulse in current.

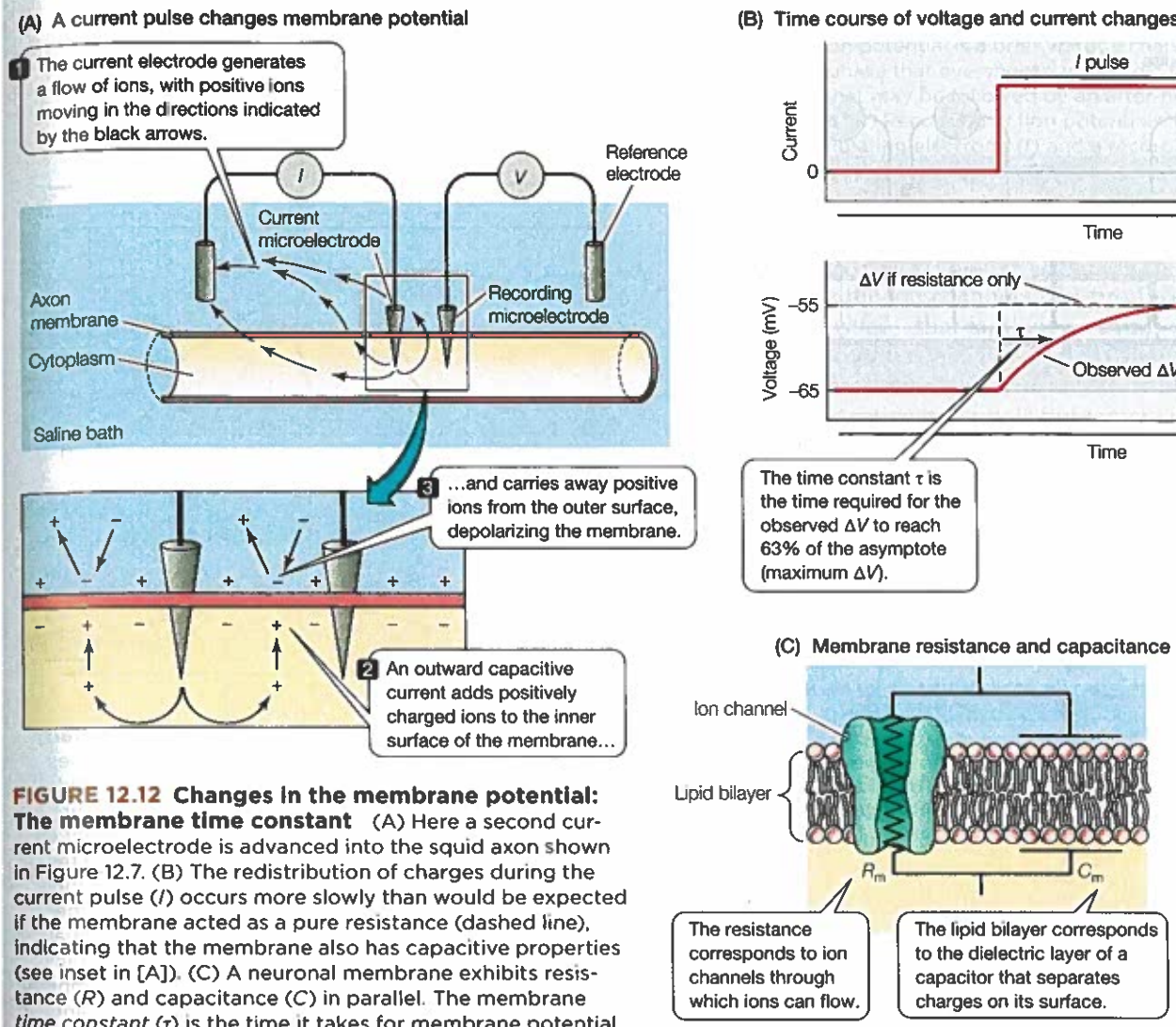


FIGURE 12.12 Changes in the membrane potential: The membrane time constant (A) Here a second current microelectrode is advanced into the squid axon shown in Figure 12.7. (B) The redistribution of charges during the current pulse (*I*) occurs more slowly than would be expected if the membrane acted as a pure resistance (dashed line), indicating that the membrane also has capacitive properties (see inset in [A]). (C) A neuronal membrane exhibits resistance (*R*) and capacitance (*C*) in parallel. The membrane *time constant* (τ) is the time it takes for membrane potential to reach 63% of its final value. In many neurons, τ is 2–20 ms.

Passive electrical properties limit the spread of graded potentials

How does a change in voltage spread over *distance* in a membrane? (Remember that we are considering only the *passive* electrical properties of a membrane.) Suppose we insert yet another electrode into the squid axon, this one farther away from the other two (FIGURE 12.13A). The V_2 electrode will record a smaller voltage displacement in response to a current pulse than the nearer V_1 electrode (FIGURE 12.13B). The voltage change (ΔV) will decrease exponentially with distance from the source producing it, a property called **passive spread** (**decremental spread**) or **electrotonic conduction**. The steepness of this decrease with distance is described by the membrane **length constant**, λ (lambda), which represents the distance at which the decaying voltage change (ΔV_m) is 37% of its value at the origin (FIGURE 12.13C).

The reason for this decrease with distance is that as current flows along the inside of the axon, some of it leaks out through ion channels. For simplicity we ignore the slowing effects of membrane capacitance, and we lump the resistive pathways as R_m (the resistance to current flow out across the membrane ion channels in a segment) and R_i (the resistance to current continuing down the axon to the next segment). An axon (or dendrite) with a high R_m value and a low R_i value will have a large λ value. We will consider the membrane length constant again in Section 12.6, when we discuss action-potential propagation.

The electrical properties of membrane resistance (R_m), membrane capacitance (C_m), and resting membrane potential (V_m), and the related time constant (τ) and length constant (λ), adequately describe the passive electrical properties of a neuron or any other cell. Because the passive electrical properties of axons are similar to those of underwater telephone cables, these passive electrical properties are often called *cable properties*. For the same reason, the equations describing the length constant and time constant of neuronal membranes are called *cable equations*.

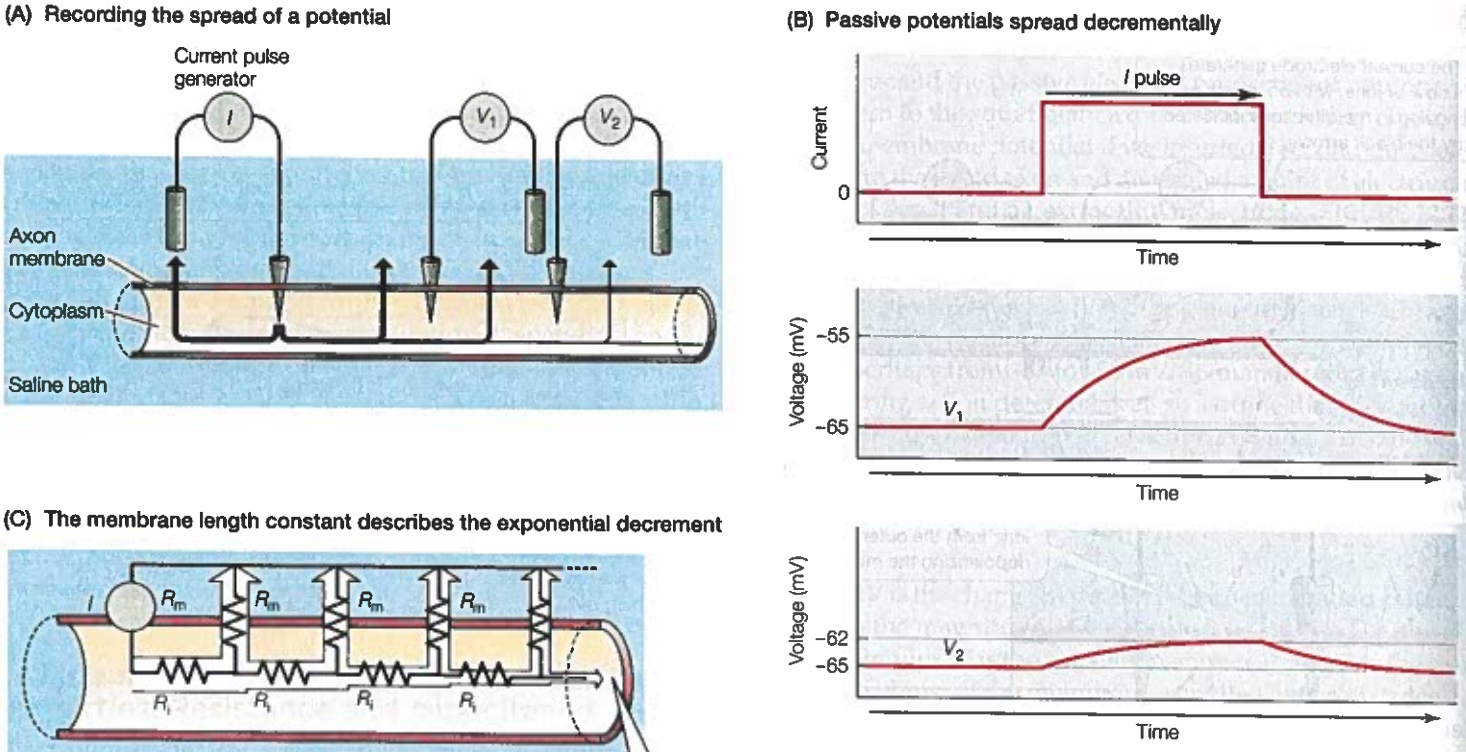


FIGURE 12.13 Graded potentials decrease exponentially with distance The amplitude of a voltage change decreases with distance along the axon. (A) A third electrode (V_2) is added to the setup diagrammed in Figure 12.7 and is used to measure the potential change at some distance from the source of current (I). Arrows show the local paths of current flow that depolarize the membrane. (Capacitances are ignored.) (B) The voltage change measured at V_2 is smaller than that at V_1, which is closer to the source of current. This decremental spread of graded potentials is referred to as *electrotonic conduction*. (C) The decrement in voltage change is exponential with distance from I. The membrane *length constant* (λ) describes the exponential decay of a change in voltage (ΔV) with distance.

SUMMARY

Passive Electrical Properties of Neurons

- Neurons are excited by depolarization, which makes the membrane potential more inside-positive, and are inhibited by hyperpolarization, which makes the membrane potential more inside-negative.
- Graded potentials, which include hyperpolarizations and subthreshold depolarizations, are passively propagated. The magnitude of a graded potential is correlated with the size of the stimulus that produced it.
- Cell membranes have properties of electrical resistance and capacitance, which regulate current flow across the membrane. The passive electrical properties of membranes determine how membrane potentials change with time (the time constant, τ) and with distance (the length constant, λ).

12.5 The Action Potential

Excitable cells such as neurons and muscle fibers have the ability to generate electrical signals. The hallmark electrical signal of an excitable cell is the *action potential*. Action potentials (which in neurons may also be called *nerve impulses*) are one of the most important kinds of electrical signals underlying the integrative activity of nervous systems. Some kinds of neurons do not generate action potentials, however, so the association of neurons with action potentials is not universal.

Action potentials are voltage-dependent, all-or-none electrical signals

Action potentials have characteristic features. An **action potential** is a momentary reversal of membrane potential from about –65 mV (inside-negative) to about +40 mV (inside-positive)—a voltage change of about 100 mV, lasting about 1 ms, followed by restoration of the original membrane potential (FIGURE 12.14A). Action potentials result from

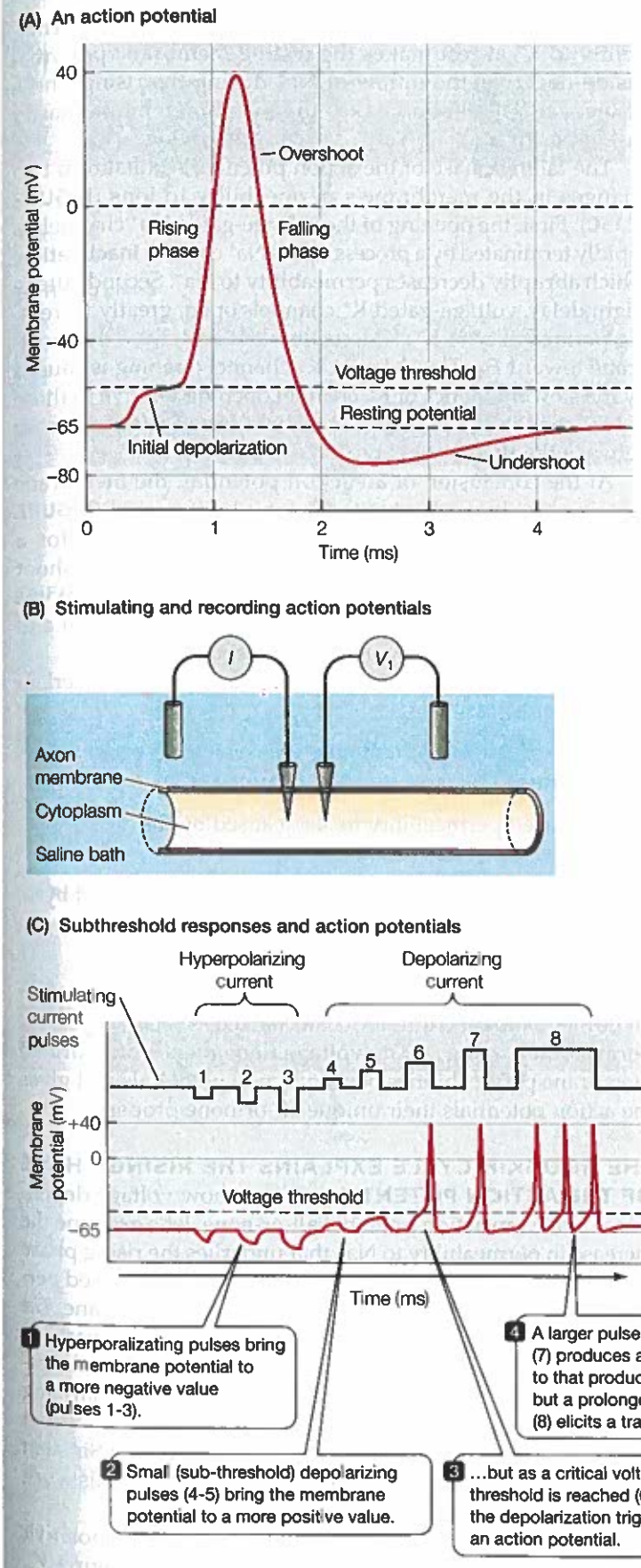


FIGURE 12.14 General features of action potentials
(A) An action potential is a brief voltage change characterized by a rising phase that overshoots zero and a falling phase (repolarization) that may be followed by an after-hyperpolarization, or undershoot. (B) Recording action potentials in a squid giant axon, using a stimulating electrode (I) and a recording electrode (V_1). (C) Responses of the axon to stimulating current pulses.

voltage-dependent changes in membrane permeabilities to ions because the ion channels that produce action potentials are *voltage-gated*—that is, their opening depends on the membrane potential (see Figure 5.5). The voltage dependence of ion permeabilities is a critical feature of action potentials, and it makes action potentials fundamentally different from resting potentials or from graded potentials.

The action potential is triggered by any depolarization of the membrane that reaches a critical value of depolarization, the **voltage threshold**. After the suprathreshold (above threshold) depolarization, the action potential has a rapid *rising phase* that reaches a peak more positive than zero potential (*overshoot*) followed by a rapid repolarization (the *falling phase*). In the squid axon and in many other neurons, the action potential is followed by an *undershoot*, a transient after-hyperpolarization lasting a few milliseconds.

To illustrate the voltage-dependent properties of action potentials, let's perform a hypothetical experiment using a squid giant axon (FIGURE 12.14B). As we did in Figure 12.12, we penetrate the axon with two glass capillary microelectrodes—one to apply current pulses and one to record voltage. The first three current pulses hyperpolarize the membrane in the vicinity of V_1; the amount of hyperpolarization is proportional to the strength of each current pulse (FIGURE 12.14C). This relation follows from Ohm's law (ignoring the time constant) and indicates that with hyperpolarization the membrane resistance does not change. Thus hyperpolarization can't induce action potentials, because it doesn't change the permeabilities of membrane ion channels. Weak injections of positive current (pulses 4 and 5 in Figure 12.14C) elicit small depolarizations that approximately mirror the preceding hyperpolarizations, again indicating no significant change in membrane resistance.

Stronger depolarizing currents (pulses 6–8 in Figure 12.14C) that exceed the voltage threshold produce action potentials. However, a stronger depolarizing current (beyond threshold) does not produce a larger action potential (compare the responses to pulses 6 and 7). Instead, action potentials are **all-or-none** phenomena; that is, a depolarization below threshold elicits no impulse, but all suprathreshold depolarizations produce complete impulses substantially alike in amplitude and duration.

Immediately following an action potential, another action potential cannot be generated for at least 1 ms (the **absolute refractory period**) and is harder to generate for a few milliseconds longer (the **relative refractory period**). We discuss the membrane properties

that impose these refractory periods in Section 12.6. Because of the all-or-none property of the action potential and the succeeding refractory period, impulses cannot summate. Instead, a prolonged suprathreshold depolarizing current (pulse 8 in Figure 12.14C) can elicit a *train* of discrete action potentials. For many neurons, the frequency of impulses in a train increases with increasing strength of depolarizing current (within limits).

An action potential, once initiated, *propagates* along the axon without a decrease in amplitude and at a constant velocity that depends on the diameter of the axon (among other factors). If in Figure 12.14B,C a remote electrode measured voltage at the end of the axon (not shown), it would record each action potential that the local electrode (V_1) records, with no decrease in amplitude. Each impulse recorded remotely follows the impulse at V_1 by a short latency that represents the time required for the impulse to propagate along the axon between the two electrodes. The distant V_2 electrode would not record the subthreshold depolarizations and hyperpolarizations because these graded potentials are not actively propagated; instead they spread decrementally and so are weakened before reaching V_2 (compare Figure 12.13).

In summary, action potentials are all-or-none electrical signals that propagate rapidly and without degradation over long distances. This ability to send signals over long distances rapidly and without distortion was presumably an important factor allowing the evolution of large animals whose complex physiology and behavior require extensive neural coordination.

Action potentials result from changes in membrane permeabilities to ions

The permeability terms in the Goldman equation (see Equation 12.4) show that any factor that changes the permeability of the membrane to one or more ion species will change the value of the membrane potential. *An action potential results from intense, localized increases in permeabilities to specific ions*—increases that are both voltage- and time-dependent. What's more, the permeability increases are selective for specific ions: first Na^+ and then K^+.

PERMEABILITIES AND ION CHANNELS Let's follow the rise and fall of one action potential to see when and how these changes in the membrane's permeability to Na^+ and K^+ ions occur. As discussed earlier, neurons contain some ion channels that are normally open and are not voltage-gated. These *leakage channels* allow ions to diffuse across the membrane following the electrochemical gradient, and determine the resting membrane potential of the neuron (**FIGURE 12.15A**). The leakage channels remain open throughout an action potential, but the more numerous voltage-gated channels swamp their effects.

The rising phase of the action potential (depolarization and polarity reversal) begins when a stimulus depolarizes the membrane past threshold. Voltage-gated Na^+ channels open in response to the depolarization, vastly increasing the membrane's permeability to Na^+ ions (**FIGURE 12.15B**). Na^+ rushes in, driving the membrane potential toward E_{Na} (which is inside-positive because of the much higher concentration of Na^+ outside the cell). Thus just as a dominant permeability to K^+ at rest makes the resting membrane potential inside-negative, the inflow of Na^+ during the rising phase of the action potential makes the membrane momentarily inside-positive.

The falling phase of the action potential results from two changes in the membrane's permeability to ions (**FIGURE 12.15C**). First, the opening of the voltage-gated Na^+ channels is rapidly terminated by a process called Na^+ channel **inactivation**, which abruptly decreases permeability to Na^+. Second, after a slight delay, voltage-gated K^+ channels open, greatly increasing permeability to K^+. K^+ ions flow out and drive the membrane toward E_K. The delay in K^+ channel opening is caused by the slower kinetics of K^+ channel opening relative to those of Na^+ channels (the voltage threshold for opening is similar between the two channel types.)

At the conclusion of an action potential, the membrane remains highly permeable to K^+ for a brief period (**FIGURE 12.15D**). Voltage-gated K^+ channels remain open for a few milliseconds, producing a characteristic undershoot (after-hyperpolarization) in many neurons. At this point, the voltage-gated Na^+ channels recover from inactivation and again become ready to be opened by depolarization.

In summary, the action potential results from three overlapping permeability changes:

1. Increased permeability to Na^+, caused by the rapid opening of voltage-gated Na^+ channels
2. Decreased permeability to Na^+, caused by the inactivation of Na^+ channels
3. Increased and prolonged permeability to K^+, caused by the slower opening and closing of voltage-gated K^+ channels

All three permeability changes are initiated by depolarization of the membrane and thus are characterized as *voltage-dependent* permeability changes. The voltage dependence of neuronal membrane permeabilities permits action potentials and gives the action potentials their unique all-or-none property.

THE HODGKIN CYCLE EXPLAINS THE RISING PHASE OF THE ACTION POTENTIAL To see how voltage dependence makes an action potential all-or-none, let's examine the increase in permeability to Na^+ that underlies the rising phase of the action potential. We have discussed how increased permeability to and inflow of Na^+ depolarizes the membrane. *The critical feature of action-potential generation is that the permeability to Na^+ that produces depolarization itself depends on depolarization.* The **Hodgkin cycle** describes the effects of depolarizing an excitable membrane in which the permeability to sodium (P_{Na}) is voltage-dependent. (The cycle is named after Sir Alan Hodgkin, who was a corecipient of a Nobel Prize for his work clarifying the ionic mechanism of action potentials.)

The Hodgkin cycle (**FIGURE 12.16**) describes a positive feedback loop that starts with depolarization: Changing V_m changes P_{Na}, and (as predicted by the Goldman equation) changing P_{Na} changes V_m. At rest, the membrane is 20–50 times

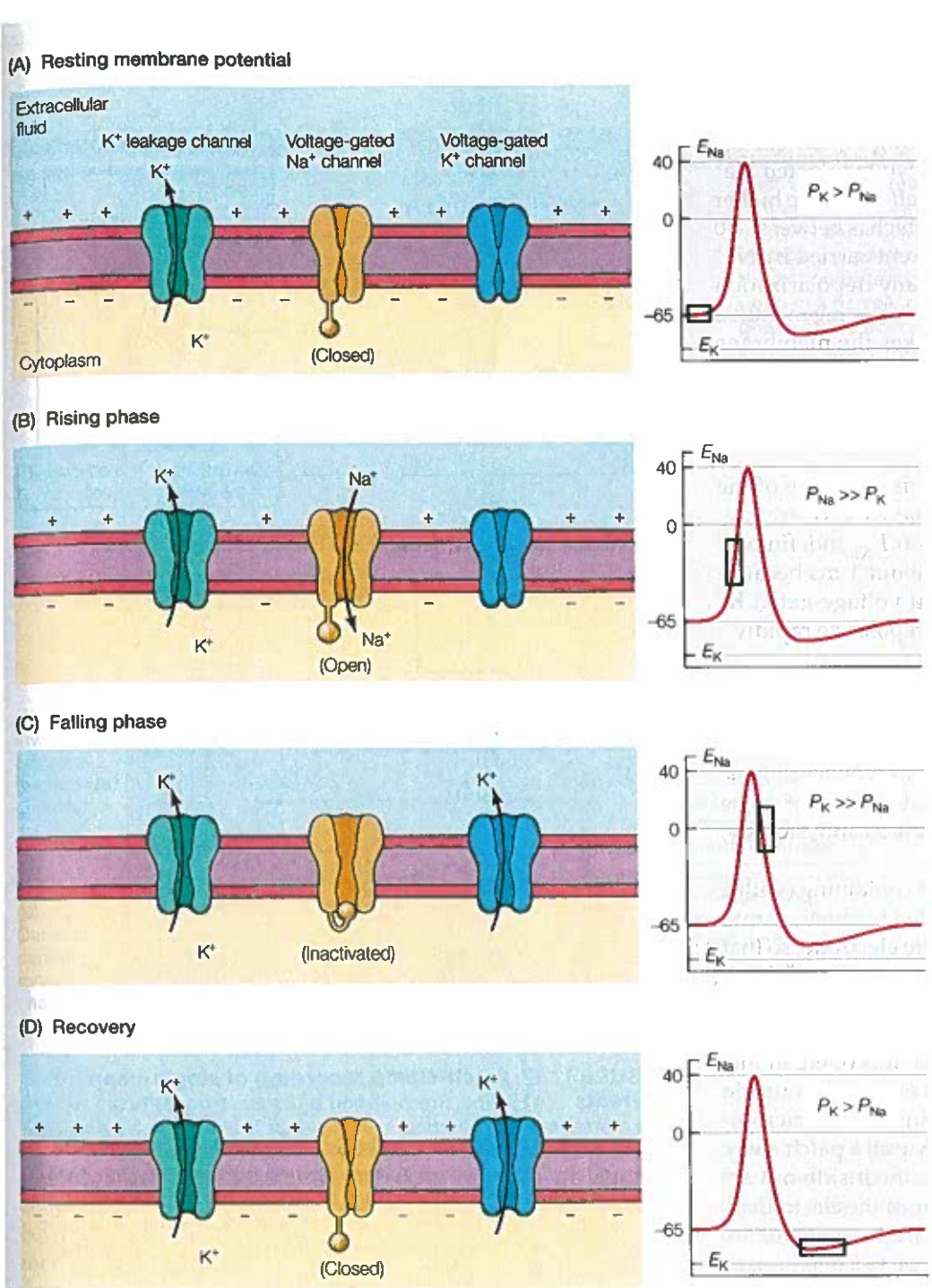


FIGURE 12.15 Membrane permeability changes that produce an action potential (A) At rest the membrane is most permeable to K^+, as represented here by leakage channels that are always open. The box in the graph on the right indicates the membrane potential at this stage, described by the "voltage thermometer" (see Figure 12.10). (B) During the rising phase of the action potential, voltage-gated Na^+ channels open, and the high permeability to Na^+ dominates, driving the membrane potential toward E_{Na}. (C) Na^+ channels are inactivated soon after they open, and voltage-gated K^+ channels begin to open. Thus, during the falling phase permeability to K^+ again dominates, driving the membrane toward E_K. (D) K^+ channels remain open for a short time after an action potential, producing an undershoot in some cells. Na^+ channels recover from inactivation and again become ready to be opened by depolarization. (Leakage channels remain open throughout, but their effects are swamped by those of the more numerous voltage-gated channels.) (After M. F. Bear et al. 2001. *Neuroscience: Exploring the Brain*, 2nd ed. Lippincott, Williams & Wilkins, Baltimore, MD.)

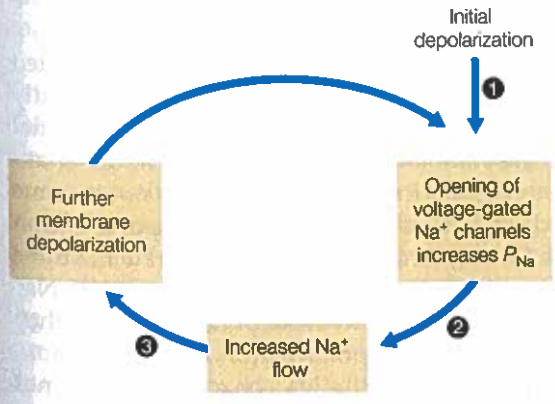


FIGURE 12.16 The Hodgkin cycle produces the rising phase of the action potential The critical feature of the cycle is that permeability to Na^+ is *voltage-dependent*. ❶ An initial depolarization increases P_{Na} by opening voltage-gated Na^+ channels. ❷ The increased permeability to Na^+ allows inflow of Na^+ down its electrochemical gradient, which further depolarizes the membrane. ❸ The cycle intensifies as each depolarization step opens additional Na^+ channels.

as permeable to K^+ as to Na^+, so the resting V_m is near E_K. Subthreshold depolarizations open some voltage-gated Na^+ channels, but not enough to overcome the effects of the higher resting permeability to K^+. At threshold, which is between –40 mV and –60 mV in most neurons, the current carried by Na^+ inflow just equals the K^+ current, and at any depolarization above threshold the Hodgkin cycle "wins." The regenerative increase in P_{Na} in the Hodgkin cycle makes the membrane transiently much more permeable to Na^+ than to K^+ (during the rising phase of the action potential, the membrane is ~600 times as permeable to Na^+ as to K^+), so V_m approaches E_{Na} (+40 to +55 mV inside-positive).

The Hodgkin cycle explains only the rising phase of the action potential, because if the cycle alone were operating, the membrane potential would remain near E_{Na} indefinitely. Instead, the polarity reversal lasts only about 1 ms because the Na^+ channels become inactivated and voltage-gated K^+ channels open, causing the membrane to repolarize rapidly.

SINGLE-CHANNEL CURRENT RECORDING FROM ION CHANNELS The changes in membrane permeability that cause action potentials can be visualized as the actions of individual ion channels. (They can also be seen as whole-cell ionic currents, which we discuss next.) Evidence at the level of single ion channels comes from **single-channel electrophysiology**, also termed **patch-clamp recording**.

In this procedure, a patch of membrane containing (with a little luck) a single Na^+ ion channel is sealed by suction onto the smoothed tip of a fine glass micropipette electrode, so that any current must flow through a channel in the isolated patch (FIGURE 12.17A). The electrode records the opening and closing of the membrane ion channel by recording the ionic current that flows through the single channel when it is open. In this configuration, the experimenter has access only to the outside of the patch in the extracellular medium inside the micropipette. It is also possible, however, to rapidly pull a patch away from a cell and maintain the tight seal. In the inside-out arrangement of this detached patch, the inside of the electrode is the equivalent of the outside of the cell and the bathing fluid is equivalent to the inside of the cell. In response to a depolarization (caused by setting the voltage across the membrane patch to a less negative value) (FIGURE 12.17B), the channel opens, allowing Na^+ ions to flow out of the electrode and into the bathing medium (remember, the bathing medium is now acting as the *inside* of the cell). This inward-flowing current lasts for about 1 ms before the channel closes again (conventionally, an inward-flowing current is shown downward and an outward-flowing current is upward). By providing data about the opening and closing of single channels, patch-clamp recording allows direct visualization of the permeability changes underlying action potentials. This technique has resulted in such major advances in our understanding of single channel function in neurons (as well as other cell types) that its developers were awarded a Nobel Prize, in 1991.

Patch-clamp recordings of voltage-gated Na^+ and K^+ channels reveal conspicuous differences in latency and action of each type of channel (FIGURE 12.18). The channels are normally closed at resting potential, and depolarization

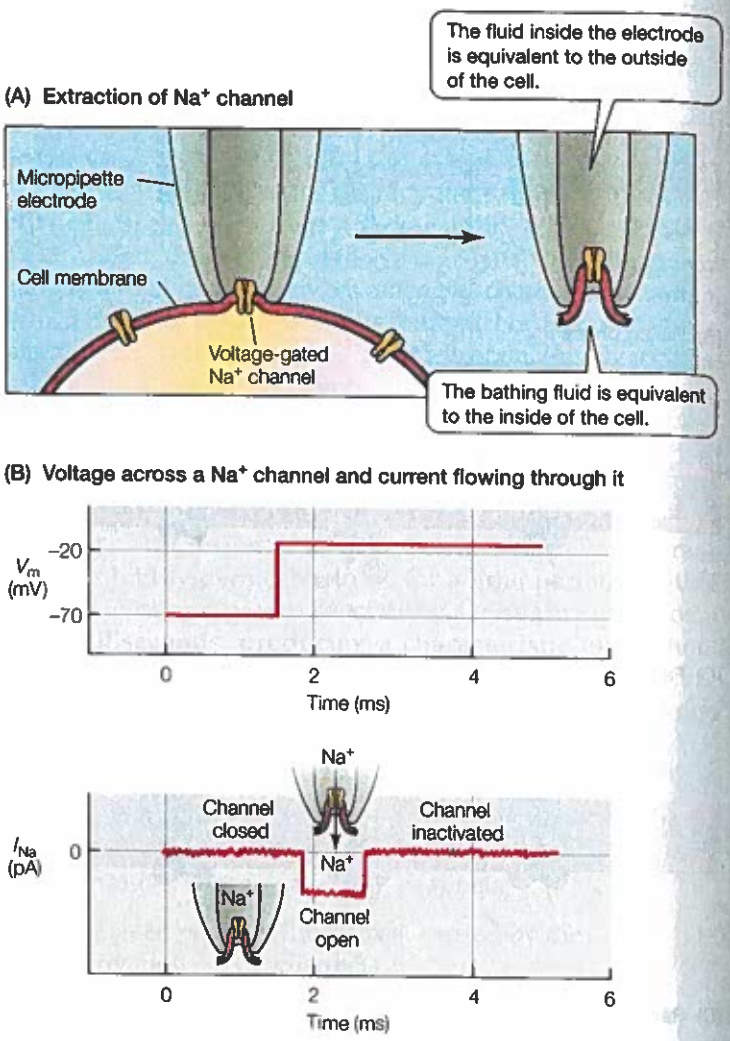


FIGURE 12.17 Patch-clamp recording of single-channel currents (A) A fine, fire-polished glass electrode is fused to the membrane with suction, making what is known as a *gigaohm seal*, and the patch of membrane is pulled away from the cell. The electrode will then record current flowing through the channel when it opens. (B) A voltage-gated Na^+ channel is closed at resting potential (–70 mV), and no current flows through it. When the membrane patch is depolarized, the channel opens transiently, allowing an inward current carried by Na^+ ions. (Note that "inward" is toward the cytoplasmic side, not relative to the pipette, and that ion concentrations of the solutions in the bath and inside the electrode on either side of the patch are similar to the respective concentrations inside and outside the cell before detachment.) pA = picoampere (a measure of electric current).

increases the *probability* that they will open. The voltage-gated Na^+ channels have a short latency and open first, but rapidly become inactivated and remain so until membrane potential returns to near baseline (see Figure 12.18A). The K^+ channels open with a slightly longer latency but do not become inactivated, and tend to stay open until the depolarization ends (see Figure 12.18B). These recordings illustrate the three effects of depolarization on Na^+ and K^+ channels: (1) Na^+ channels open first in response to the depolarization, (2) they are then inactivated during depolarization, and (3) K^+ channels open slightly later than the Na^+ channels but do not become inactivated.

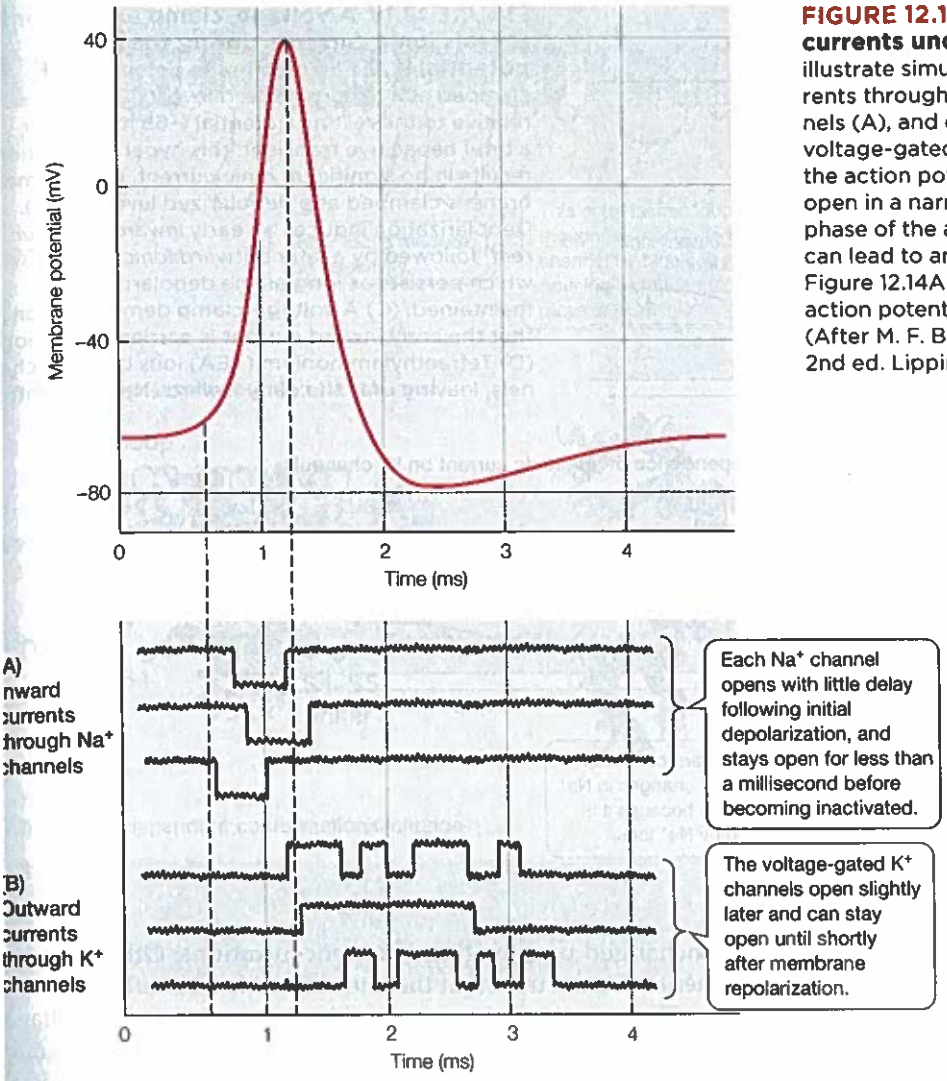


FIGURE 12.18 Patch-clamp recording of single-channel currents underlying an action potential These diagrams illustrate simulated patch-clamp recordings of inward currents through three representative voltage-gated Na^+ channels (A), and outward currents through three representative voltage-gated K^+ channels (B) of the hundreds that produce the action potential. Note that the voltage-gated Na^+ channels open in a narrow time window that corresponds to the rising phase of the action potential. The extended permeability to K^+ can lead to an after-hyperpolarization of the membrane. (See Figure 12.14A for a description of the different phases of the action potential diagrammed at the top of this illustration.) (After M. F. Bear et al. 2001. *Neuroscience: Exploring the Brain*, 2nd ed. Lippincott, Williams & Wilkins, Baltimore, MD.)

VOLTAGE-CLAMP EXPERIMENTS SHOW WHOLE-CELL IONIC CURRENTS Before the development of single-channel electrophysiology, researchers used a whole-cell current-measuring technique called a *voltage clamp* in experiments to study action-potential generation. These experiments remain a cornerstone of the physiological investigation of action potentials. A **voltage clamp** is an electronic device that allows the experimenter to measure whole-cell ionic currents, by setting membrane potential very rapidly to a predetermined value, delivering whatever current is necessary to keep it there, and measuring the imposed current.

Recall that we described the Hodgkin cycle as a positive feedback loop in which a change in membrane potential changes the permeability to Na^+, and vice versa. As the Hodgkin cycle exemplifies, any ion flow through the membrane constitutes an ionic current that tends to change the membrane potential. Clamping the membrane potential uncouples the feedback loop of the Hodgkin cycle. To keep the potential constant, the clamp circuit must generate an opposing (bucking) current that is exactly opposite to the net ionic current (current carried by ion flows through ion channels). *By measuring the bucking current, the experimenter has an accurate measure of the amplitude and time course of the net ionic current, because the two must be equal and opposite to each other.* Hence a voltage clamp uncouples the feedback loop of the Hodgkin cycle (at step ❸ in Figure 12.16) so that ionic currents resulting from permeability changes are prevented from changing the membrane potential.

In 1952 Alan Hodgkin and Andrew Huxley published a series of landmark papers in which they used voltage-clamped squid axons to demonstrate and quantify the voltage-dependent permeability changes underlying the action potential. FIGURE 12.19 shows the most fundamental result of such a voltage-clamp experiment. When the membrane potential is clamped to a hyperpolarized value (see Figure 12.19A), the current-measuring circuit shows only a brief blip of capacitive current required to set the membrane potential to a new level (changing the charge stored by the membrane capacitance). Following the capacitive transient there is only a slight leakage current resulting from holding the membrane at a hyperpolarized level (too small to appear within the scale of the current record shown in Figure 12.19A). Hyperpolarization thus leads to no significant flow of ionic current because it doesn't increase permeability to any ions.

In contrast, clamping the membrane potential to a value more *depolarized* than action-potential threshold produces quite different effects (see Figure 12.19B). Following the initial capacitive transient, bucking current is required to hold the membrane at the set value. The bucking current (which is not shown) flows first outward and then inward. Because the bucking current is equal and opposite to the net ionic current, this pattern shows that there is an *early inward ionic current* that is reversed in 1–2 ms to a *later outward ionic current*. Depolarization of the membrane thus induces permeability changes that (if the currents are carried by cations) result in first an inward movement of cations and then an outward movement of cations. If the membrane were not clamped, these ionic currents would produce first a depolarization and then a repolarization of the membrane, as in an action potential.

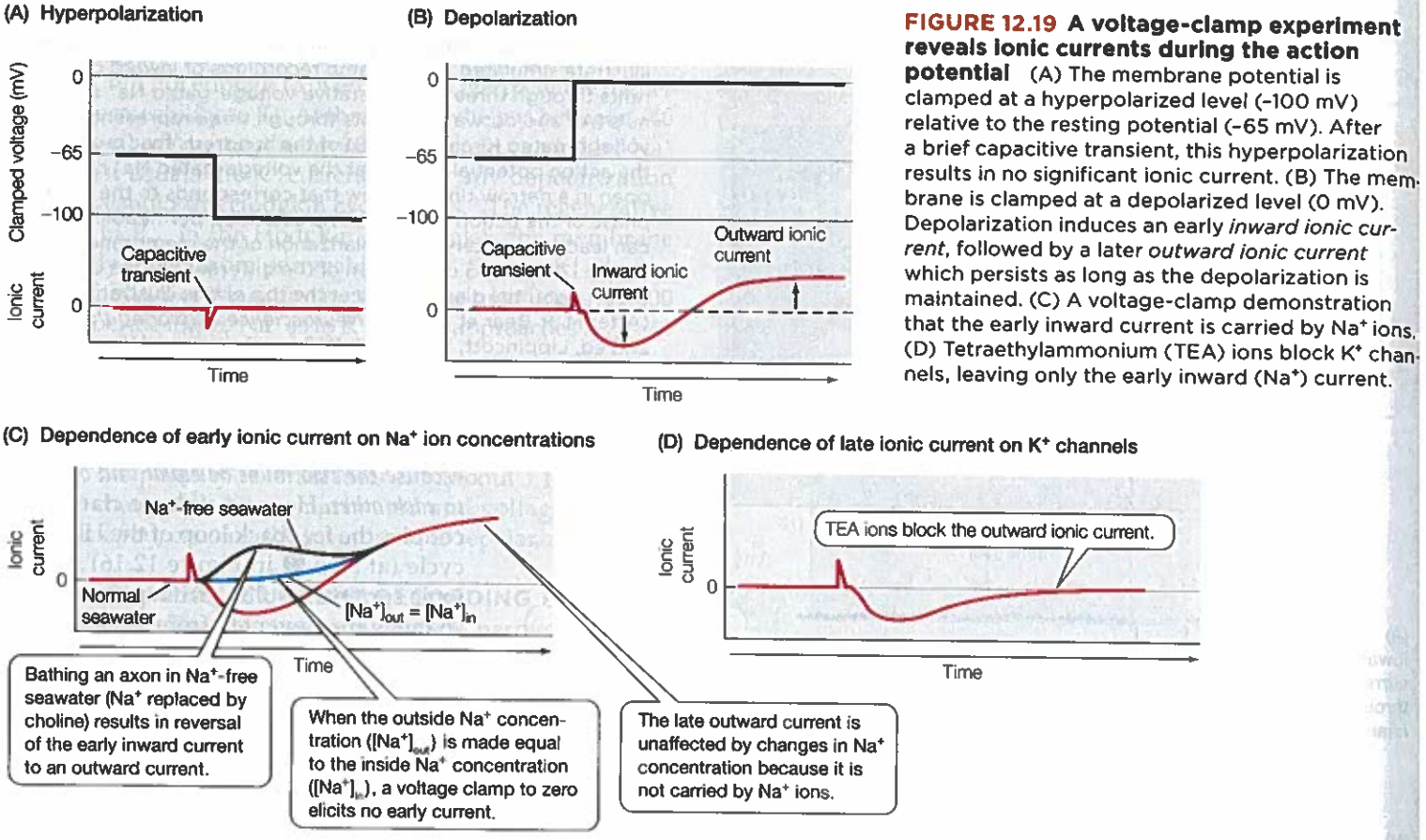


FIGURE 12.19 A voltage-clamp experiment reveals ionic currents during the action potential (A) The membrane potential is clamped at a hyperpolarized level (−100 mV) relative to the resting potential (−65 mV). After a brief capacitive transient, this hyperpolarization results in no significant ionic current. (B) The membrane is clamped at a depolarized level (0 mV). Depolarization induces an early *inward ionic current*, followed by a later *outward ionic current* which persists as long as the depolarization is maintained. (C) A voltage-clamp demonstration that the early inward current is carried by Na^+ ions. (D) Tetraethylammonium (TEA) ions block K^+ channels, leaving only the early inward (Na^+) current.

Hodgkin and Huxley proposed that the early inward ionic current (which generates the rising phase of the action potential in unclamped axons) is an influx of Na^+ ions. How could this prediction be tested? Hodgkin and Huxley replaced the Na^+ in the seawater with which they bathed the axon with choline, a nonpermeating cation. In the absence of extracellular Na^+, the early inward current was replaced by an early outward current (see Figure 12.19C). That is, depolarization induced an increase in permeability to Na^+, which, in the absence of extracellular Na^+, resulted in Na^+ diffusion *outward* down its concentration gradient.

This interpretation predicts that if the Na^+ concentration is equal on both sides of the membrane, there will be no Na^+ concentration gradient and no early Na^+ current in either direction. Hodgkin and Huxley replaced about 90% of the extracellular Na^+ with nonpermeating ions so that $[Na^+]_{in} = [Na^+]_{out}$. When the membrane was clamped to 0 mV (so that there was no voltage gradient), there was no early current (see Figure 12.19C).

Further evidence that the early inward current is carried by Na^+ was provided by experiments in which a squid axon in normal artificial seawater was clamped to the sodium equilibrium potential (E_{Na} = +58 mV). There was no resultant early current because there was no driving force on Na^+ ions at E_{Na}. Clamping the membrane at a level beyond E_{Na} (more inside-positive than E_{Na}) resulted in an early outward current, representing Na^+ efflux toward E_{Na}. These experiments demonstrate that Na^+ ions carry the early inward current during a voltage clamp, but not the later outward current, which is unchanged by changing Na^+ concentrations. Other experiments demonstrate that the late current is a K^+ efflux.

Pharmacological agents used in conjunction with a voltage clamp confirm that Na^+ and K^+ currents flow through separate ion channels. Certain drugs can selectively block Na^+ and K^+ channels when they are applied to the membrane. For example, tetrodotoxin (TTX), an extremely poisonous substance found in puffer fish, selectively blocks voltage-gated Na^+ channels. If a squid axon is bathed in seawater containing TTX and is voltage-clamped to a depolarized level such as 0 mV, the early inward Na^+ current is blocked. The delayed outward (K^+) current, however, is completely unaffected. However, tetraethylammonium (TEA) ions, which block K^+ channels, selectively eliminate the delayed outward current (see Figure 12.19D). TEA ions have no effect on the early inward current flowing through Na^+ channels.

From their voltage-clamp experiments, Hodgkin and Huxley were able to quantify the voltage dependence and time course of the changes in permeability to Na^+ and K^+.[7] They developed a set of equations by which they showed that these three voltage-dependent processes are sufficient to describe the behavior of action potentials in unclamped squid giant axons. These studies remain critical for our understanding of the physiology of excitable membranes.

[7] Permeabilities are often measured in electrical units of **conductance**, the inverse of resistance ($g = 1/R$). *Conductance* and *permeability* are not synonymous, because increasing ion concentrations increases conductance but not permeability.

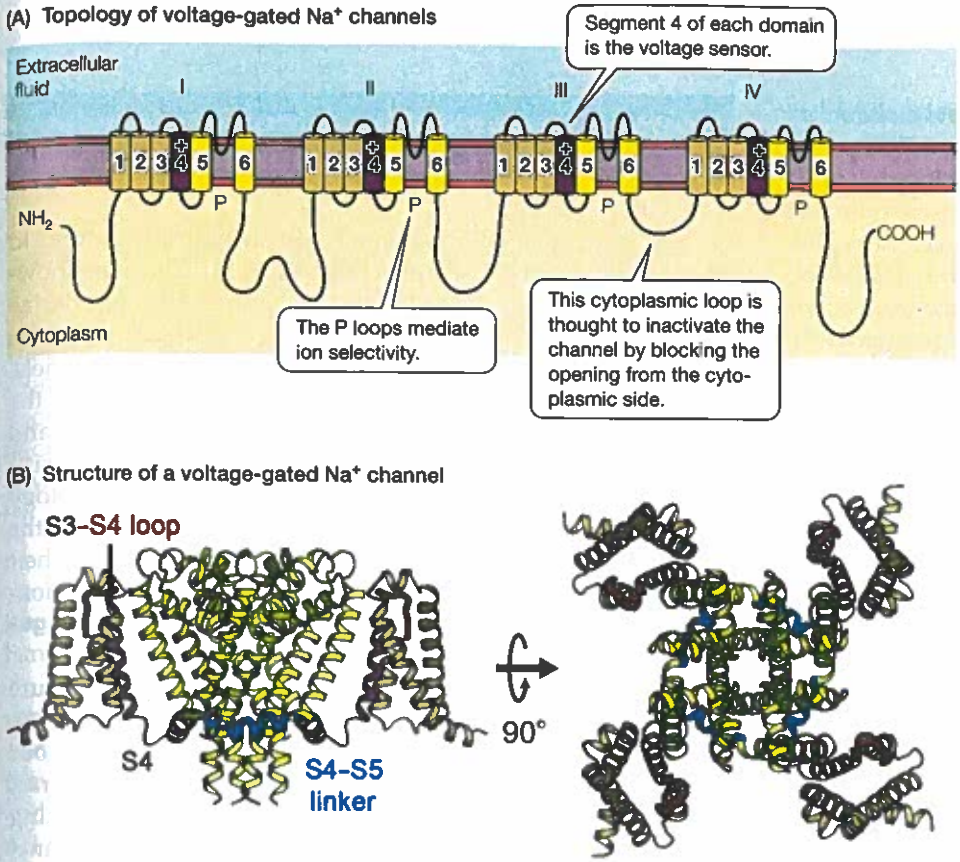


FIGURE 12.20 The molecular structure of voltage-gated Na^+ channels (A) The predicted structure of the principal (α) subunit of the voltage-gated Na^+ channel. This subunit makes up the pore-forming channel itself and is a single polypeptide chain, with four homologous domains labeled I, II, III, and IV. Each domain has six membrane-spanning α-helical segments (labeled 1 through 6). The P (for pore) loops between segments 5 and 6 of each domain are thought to line the pore and determine ion selectivity. (B) Side (left) and surface (right) views of a voltage-gated Na^+ channel as determined by cryo-electron microscopy. The numbered segments (S) on the side view correspond to those in the domains shown in (A). Segments 5 and 6 of each domain (shown in yellow) are near the central pore, whereas segments 1 to 4 are farther away. Segment 4 (shown in magenta) constitutes the voltage sensor. (C) A model of the Na^+ channel, showing closed and open conformations. The segment-4 voltage sensors are thought to rotate and slide upward in response to depolarization, leading to channel opening. (B from G. Wisedchaisri et al. 2019. *Cell* 178: 993–1003.)

ION MOVEMENTS IN ACTION POTENTIALS DO NOT SIGNIFICANTLY CHANGE BULK ION CONCENTRATIONS

In the generation of an action potential, a neuron gains a small amount of Na^+ and loses a small amount of K^+. These amounts have been calculated to be 3×10^{-12} to 4×10^{-12} molecules/cm^2 of membrane per impulse. As with the slow passive leaks of Na^+ in and K^+ out across the resting membrane, the ions crossing the membrane during an impulse must be pumped back again by the Na^+–K^+ pump. It is important to realize that the pumping process is *slow* relative to the time course of the action potential, and serves only to keep the ion concentrations constant over minutes, hours, and days. The Na^+–K^+ pump does not contribute directly to the generation of action potentials, and the ion movements underlying impulse generation are very small relative to the quantities of ions inside and outside the axon.

If the Na^+–K^+ pump of a squid giant axon is poisoned, the axon can still generate about 100,000 impulses before the internal Na^+ concentration is increased by 10%! Smaller axons, however, have a greater ratio of membrane surface to internal volume, so the concentration changes produced by impulses are greater. Therefore the smallest axons (0.1 μm in diameter) presumably cannot generate impulses at a rate that greatly exceeds the moment-to-moment ability of the Na^+–K^+ pump to maintain normal ion concentrations.

The molecular structure of the voltage-gated ion channels reveals their functional properties

The voltage-gated Na^+ channel protein changes its tertiary structure in response to membrane depolarization to achieve three conformations: closed, open, and inactivated. Molecular analysis of the voltage-gated Na^+ channel suggests which parts of the protein mediate specific aspects of its function.

The major (α) protein of the voltage-gated Na^+ channel forms the channel itself. It consists of a single polypeptide chain (FIGURE 12.20; see also Figure 2.11B). (There are two smaller, variable β-peptide subunits that interact with and modify the single α protein. However, the α protein alone is sufficient to produce voltage-gated Na^+ currents, and we consider it only.) The Na^+ channel α polypeptide chain has four *domains*, or regions, each domain consisting of amino acid sequences very similar to the other three. Thus there is said to be extensive *sequence homology* among the four domains. Each of the four domains contains six *membrane-spanning segments*, regions of the polypeptide that contain predominantly hydrophobic amino acid side chains that can form α-helices and cross the lipid bilayer of the membrane. The four domains of

the voltage-gated Na^+ channel α protein surround an aqueous channel pore, through which Na^+ ions can diffuse in response to depolarization.

Particular structural regions of the channel protein impart to it particular functional properties. For example, the region of the protein that responds to voltage is membrane-spanning segment 4 of each domain (see Figure 12.20). The voltage-sensor region of the channel must be charged, but it must also be in or close to the membrane in order to detect changes in the transmembrane electric field, and segment 4 has a collection of positively charged amino acids appropriate to act as the voltage-sensor region. Moreover, mutations affecting segment 4 selectively alter the voltage sensitivity of the channel. The channel's voltage-sensor segments are thought to move outward in response to depolarization, leading to an overall conformational change in the channel from closed to open (see Figure 12.20C).

Another structural correlate of a critical channel function is the P loop connecting segments 5 and 6 of each domain. This loop lines the pore of the ion channel and helps mediate ion *selectivity*. Mutations in the P-loop region alter ion selectivity in ways consistent with this idea. Finally, the cytoplasmic loop between domains III and IV appears to mediate inactivation of the Na^+ channel; it is thought to act like a "ball on a string" that can block the (open) channel from the cytoplasmic side (see Figure 12.15C).

Other voltage-gated channels are structurally similar to the voltage-gated Na^+ channel (**FIGURE 12.21**). Channels showing such similarity include the K^+ channels that repolarize the membrane in an action potential, as well as Ca^{2+} channels involved in neurotransmitter release. (Like the Na^+ channels, these channels have modulatory polypeptides, but only the principal α protein of each is considered here.) Na^+, Ca^{2+}, and K^+ channels are similar in overall structure and have extensive homology in amino acid sequence. The sequence homology is greatest in certain regions (conserved regions), such as the voltage-sensor region of membrane-spanning segment 4. Their homology suggests that the various ion channels are evolutionarily related, and therefore they are referred to as the **voltage-gated channel superfamily** of membrane proteins.

The Na^+ and Ca^{2+} channels are most similar in structure (see Figure 12.21A,B). The K^+ channels (see Figure 12.21C) consist of four identical subunits that resemble one of the four domains of a Na^+ channel. Four K^+ channel proteins interact as subunits to form a channel, aligning like the four domains of a Na^+ channel protein around a central pore. There are several subtypes of each kind of ion channel, with an especially large number of K^+ channel subtypes. **BOX 12.1** and **BOX EXTENSION 12.1** discuss the evolution of the voltage-gated channel superfamily and the molecular bases of their actions.

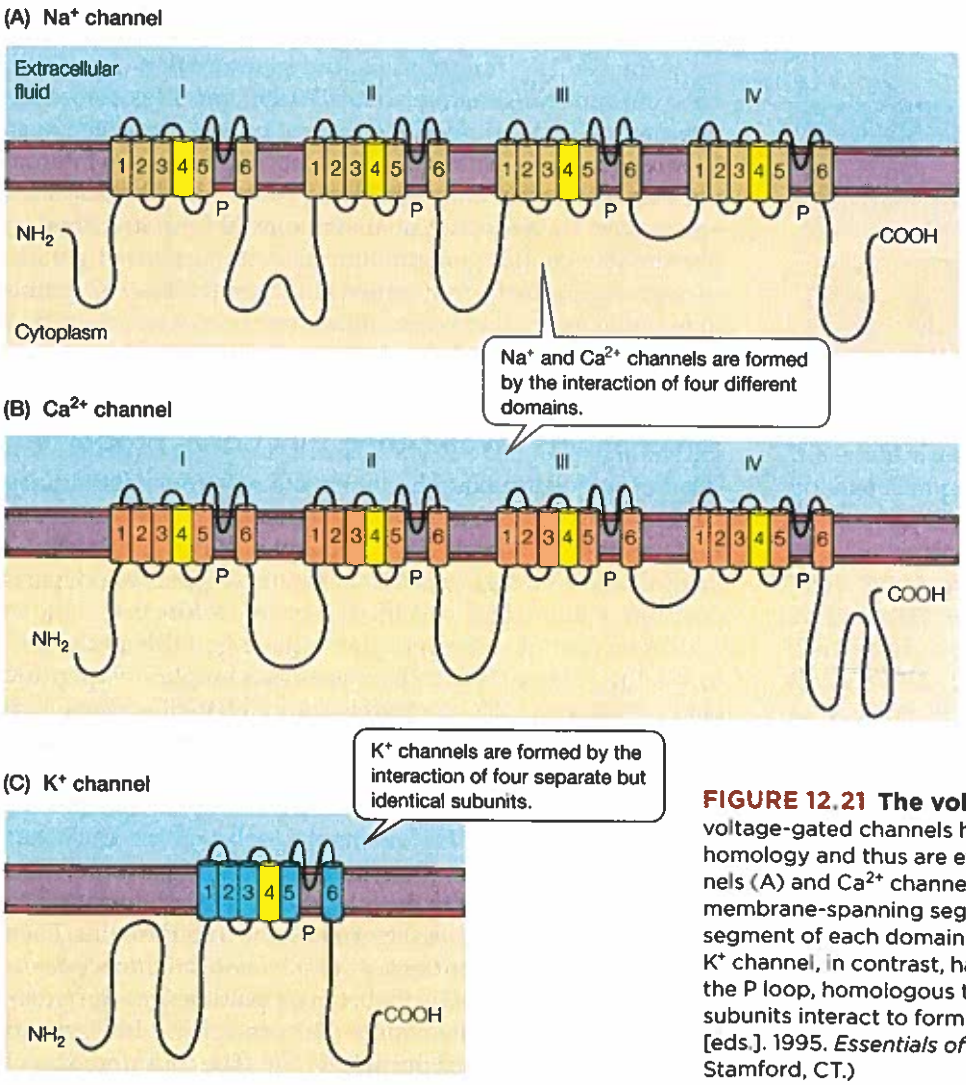


FIGURE 12.21 The voltage-gated channel superfamily All the voltage-gated channels have principal subunits with extensive sequence homology and thus are evolutionarily related. Voltage-gated Na^+ channels (A) and Ca^{2+} channels (B) have four domains, each with six α-helical membrane-spanning segments and a P loop (P). The voltage-sensing segment of each domain is highlighted in yellow. (C) The voltage-gated K^+ channel, in contrast, has only a single domain of six α-helices and the P loop, homologous to one domain of a Na^+ channel. Four separate subunits interact to form a complete K^+ channel. (After E. R. Kandel et al. [eds.]. 1995. *Essentials of Neural Science and Behavior.* Appleton & Lange, Stamford, CT.)

There are variations in the ionic mechanisms of excitable cells

How universal are the ionic mechanisms of action potentials? Studies have shown that the basic aspects of impulse generation elucidated in squid axons apply to most excitable cells. The action potentials of vertebrate and invertebrate unmyelinated axons, amphibian myelinated axons, and vertebrate skeletal twitch-muscle fibers have ionic mechanisms qualitatively similar to those of squid axons. In fact, even ion channels

BOX 12.1 Evolution and Molecular Function of Voltage-Gated Channels

Voltage-gated channels are amazing molecules that make possible the functions of nervous systems. Recent molecular studies have suggested a sequence of steps in the evolution of voltage-gated channels, and have largely clarified the structural basis of their action. BOX EXTENSION 12.1 shows how voltage-gated channels are thought to have evolved, and how their critical features—ion selectivity and voltage gating—work at the molecular level.

from algae have been shown to be able to function in mammalian neurons (BOX 12.2 and BOX EXTENSION 12.2).

There are, however, variations on the theme: Some neurons may lack some voltage-gated channels or may possess additional channels. Genomic and other studies reveal entire families of ion channels related to the voltage-gated Na^+, K^+, and Ca^{2+} channels we have described. The members of a channel family may differ in gating kinetics, voltage-activation range, or binding by modulators. The resultant variations in action potential–generating mechanisms may endow the cell with special features, such as the ability to generate spontaneous action potentials or bursts of action potentials. Here we consider three examples of variations on the usual action-potential theme.

GRADED POTENTIALS IN NONSPIKING NEURONS

Not all nerve cells generate action potentials. Researchers report increasingly numerous examples of **nonspiking neurons** (neurons that do not generate the sharp "spikes" of action potentials). Nonspiking neurons produce only *graded* membrane-potential changes in response to a stimulus or synaptic input because they substantially lack voltage-gated Na^+ channels.

Signal transmission differs in spiking and nonspiking neurons. The input of both neurons (sensory stimulation or synaptic input) is graded in amplitude, and so is their output (neurotransmitter release). Spiking neurons encode the graded input signals into trains of action potentials for long-range transmission; the action potentials are recoded at the terminal to control graded release of neurotransmitter. Nonspiking neurons, in contrast, are typically compact cells with short axons or no axons, so a graded potential change at one part of the cell can spread passively (electrotonically) to the terminal without major decrement.

The inputs and outputs of spiking and nonspiking neurons are the same, but the short-axon nonspiking neuron does not require spike encoding to carry the signal over large distances.

BOX 12.2 Optogenetics: Controlling Cells with Light

Matthew S. Kayser

The human brain is a remarkably complex organ, with billions of neurons and trillions of synapses communicating through precisely timed electrical signals. A major limitation in understanding how our brains work is that researchers have been unable to manipulate the system on the same millisecond timescale on which it normally operates. To learn how every movement, thought, and experience we have results from groups of neurons talking to each other, don't we need a way to speak to neurons on the same timescale they use when communicating with each other? The field of optogenetics has begun to accomplish just this by combining optics (the use of light) with manipulation of genes (thus *opto* + *genetics*). Specifically, scientists have figured out how to put genes into cells that make those cells responsive to pulses of light. Unexpectedly, this technology is possible because of light-sensitive transporter proteins and ion-channel proteins first discovered in microorganisms a few decades ago. **Optogenetics** involves taking the genes encoding these light-sensitive transporter and channel proteins, inserting them into target cells, and then delivering light to those cells as a way of controlling their functions. For example, neurons in the mammalian brain can be targeted to express the light-sensitive channels. Then, by delivering light to those neurons (see the figure), investigators are able to exert millisecond control over neuronal firing patterns, shedding light—literally—on mysteries of neuroscience in the process. This essay continues on the web at BOX EXTENSION 12.2, which describes how optogenetics was developed and its many potential applications.

Courtesy of Mike J. F. Robinson

A mouse prepared for an optogenetic experiment

Examples of nonspiking neurons include the photoreceptors, bipolar cells, and horizontal cells of the vertebrate retina (see Section 14.6), granule cells of the olfactory bulb, and many arthropod interneurons.

PACEMAKER POTENTIALS OF SPONTANEOUSLY ACTIVE CELLS Many neurons are spontaneously active, generating action potentials at rather regular intervals without an external source of depolarization. The somata of some molluscan neurons, for example, generate action potentials in regular trains, or even in repetitive bursts, in the absence of synaptic input. Vertebrate cardiac muscle fibers and some other excitable cells are also spontaneously active.

The membrane potential of a spontaneously active cell, instead of maintaining a fixed resting value, undergoes a continuous upslope of depolarization between action potentials, until it reaches threshold for the generation of the next action potential. The repolarizing phase of an action potential restores the membrane to a relatively hyperpolarized level, from which the next ramp of depolarization begins. These ramp depolarizations are termed **pacemaker potentials** because they determine the rate of impulse generation by the cell. For example, in a cardiac muscle cell in the pacemaker region of a vertebrate heart, the greater the rate of depolarization during the ramp phase, the sooner the cell reaches threshold for the next action potential and thus the faster the heart rate. For vertebrate cardiac muscle fibers, norepinephrine increases the rate of depolarization during the ramp phase, whereas acetylcholine decreases it. (See Chapter 25 for discussion of heart rate control.)

The ionic basis of pacemaker potentials can be complex and may vary somewhat among cells. Spontaneously active cardiac muscle fibers have a slow inward cationic current (termed I_h) that is activated by hyperpolarization rather than by depolarization. I_h is activated at potentials more negative than –40 mV. The unique features of this current—that it is inward and activated by hyperpolarization—give it pacemaker properties. I_h produces a ramp depolarization that triggers an action potential and then is inactivated at –40 mV. After the action potential, the repolarizing potassium current (I_K, flowing through voltage-gated K^+ channels) restores the membrane to a value near E_K. The hyperpolarization turns off I_K and turns on I_h for the next ramp depolarization.

CARDIAC MUSCLE ACTION POTENTIALS The action potentials of heart muscle fibers differ from action potential generation in squid axons in another way—their duration. In contrast to most action potentials, which last about 0.4–3 ms, the action potentials of vertebrate cardiac muscle fibers have typical durations of 100–500 ms (FIGURE 12.22A). The long duration of cardiac muscle action potentials is functionally important because action potential depolarization is the necessary stimulus for myocardial contraction, and because cardiac muscle must contract for about 100 ms to pump blood effectively. A cardiac muscle fiber action potential has a rapid upstroke and a rapid initial recovery to near 0 mV, but it remains depolarized near zero for many milliseconds. This prolonged depolarization, the *plateau* of the action potential, gradually decreases and is followed by a relatively slow repolarization.

Two separate inward currents underlie the cardiac action potential. The first is a fast current resulting from increased permeability to Na^+ (P_{Na}) (FIGURE 12.22B), very similar to the squid axon's permeability to Na^+. The fast Na^+ current produces the rapid upstroke of the cardiac action potential and is inactivated within a few milliseconds. A second, slow inward current results mainly from increased permeability to Ca^{2+} (P_{Ca}) and helps produce the plateau. The Ca^{2+} channels take at least 20 ms to open, and their slow inward current is much weaker than the fast Na^+ current.

The other factor sustaining the plateau is a *decrease* during the plateau in permeability to K^+ (P_K) from the resting level (in contrast to the increased permeability to K^+ that occurs in axons). Thus the plateau represents a balance between two small currents, a slow inward Ca^{2+} current and a diminishing

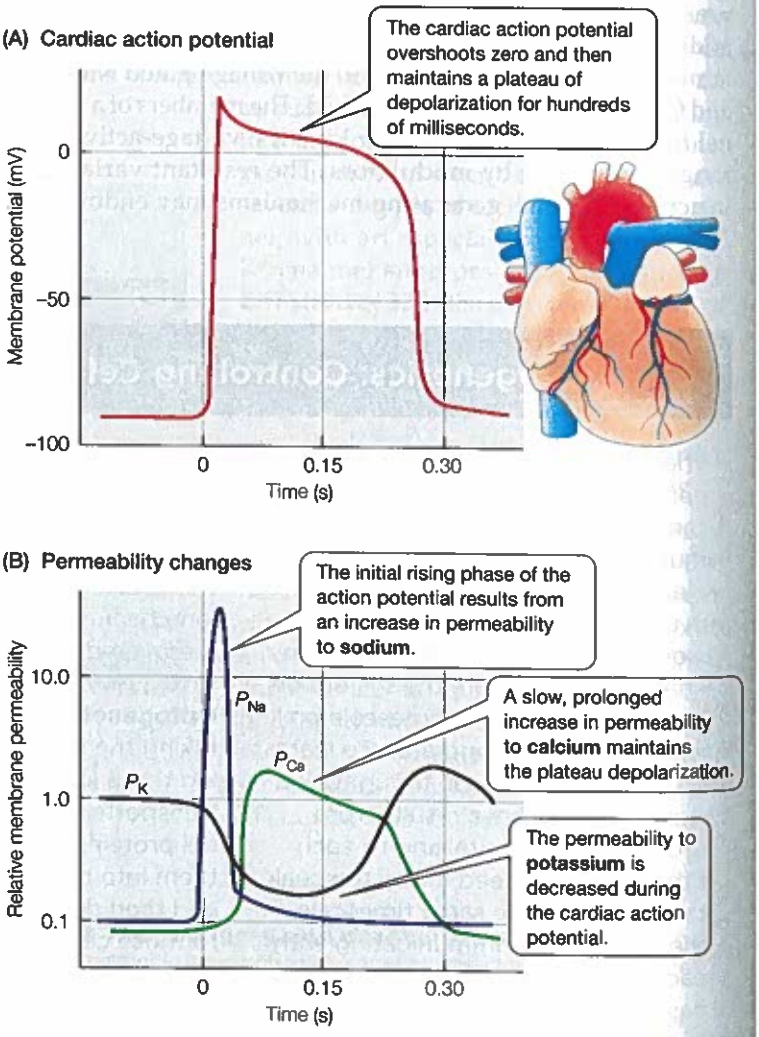


FIGURE 12.22 The cardiac muscle fiber action potential (A) An action potential in a vertebrate cardiac muscle fiber has a long duration. (B) Permeability changes underlie the cardiac action potential. Eventually, increased $[Ca^{2+}]$ opens Ca^{2+}-activated K^+ channels, leading to repolarization.

outward K^+ current. Repolarization depends on two factors: (1) The Ca^{2+} channels gradually become inactivated during the plateau depolarization, and (2) permeability to K^+ gradually increases. Some of the K^+ channels are activated by intracellular Ca^{2+} ions (see Box Extension 12.1), which accumulate during the plateau and eventually open the K^+ channels.

The complex ionic basis of cardiac action potentials increases their energetic efficiency. Recall that in a 1-ms action potential of a typical neuron, only the in-rushing fast Na^+ current sustains the brief depolarization. A cardiac muscle cell would be flooded with Na^+ if the fast current alone had to sustain a 100-ms action potential, and metabolically expensive ion-exchange pumps would be needed to clear the Na^+ ions from the cytoplasm. During a cardiac action potential, however, the duration of the fast Na^+ current is about the same as that of a standard action potential. Instead, *depolarization is sustained by the slow Ca^{2+} current and by the decreased membrane permeability to K^+*. Thus a cardiac muscle fiber can generate a protracted action potential with only a relatively modest exchange of ions across its membrane.

SUMMARY

The Action Potential

- An action potential is a voltage change—a brief, transient reversal of membrane potential from inside-negative to inside-positive. Action potentials are all-or-none responses to any depolarization beyond a voltage threshold and are each followed by a brief refractory period.
- Action potentials result from voltage-dependent changes in membrane permeability to ions. Depolarization first opens voltage-gated Na^+ channels, allowing Na^+ ions to flow in and further depolarize the membrane toward E_{Na}. The voltage-gated Na^+ channels rapidly become inactivated to terminate the rising phase of the action potential; then voltage-gated K^+ channels open to repolarize the membrane.
- The effects of depolarization on membrane permeability to ions can be studied at the level of single channels by patch clamp, and at the whole-cell level by voltage clamp.
- Ongoing investigations are clarifying the molecular structures of voltage-gated channels. The principal protein subunit of a K^+ channel is a single chain with six transmembrane regions; a K^+ channel consists of four of these protein subunits around a central pore. Na^+ and Ca^{2+} channels consist of a single polypeptide chain with four similar domains; each domain corresponds to one of the four subunits of the K^+ channel. Functional attributes of the channels can be localized to particular regions of the proteins.
- Nonspiking neurons do not generate action potentials, and the ionic mechanisms of action potentials in excitable cells can vary. Calcium ions can make substantial contributions to action potentials in cardiac muscle cells and in some neurons. Other varieties of voltage-gated channels modify the excitable properties of neurons.

12.6 The Propagation of Action Potentials

Consider that in large animals, single axons—such as those that control wiggling of your toes—can be several meters long. Now recall that an electrotonic voltage change at one point on a membrane decreases exponentially with distance. Indeed, with passive or electrotonic spread, the amplitude of a voltage change typically decreases to a third of its starting value in a fraction of a millimeter! Because of this decrement, passive electrotonic spread of a voltage change cannot serve as an electrical signal over long distances. Instead, there must be a mechanism to amplify or refresh the electrical signal.

We have seen that an action potential is all-or-none because the voltage-dependent, regenerative permeability increases bring the membrane potential toward a limiting value, which is the sodium equilibrium potential (E_{Na}). *Nondecremental propagation of the action potential is possible because the action potential at one location on an axon can itself initiate an action potential at a neighboring location, and the induced action potential will have the same all-or-none amplitude as the original action potential.* By repeating this process, a signal can travel 1 m along an axon without any decrease in amplitude.

Local circuits of current propagate an action potential

An action potential at one locus on an axon depolarizes an adjacent locus by setting up local circuits of current flow (**FIGURE 12.23**). A complete local circuit is composed of a series of ionic and capacitive currents, which can be broken down into four components, as shown in Figure 12.23B:

❶ At the locus of the action potential, an ionic current begins with the inflow of Na^+ ions through open Na^+ channels into the intracellular fluid (cytosol).

❷ Ions diffuse in intracellular fluid, carrying the current to more distant parts of the membrane (see Figure 12.13).

❸ At the membrane, the ion movements change the distribution of charges on the membrane capacitance (see Figure 12.23C): Cations accumulate along the membrane interior, displacing negative charges and repelling an equivalent number of cations from the membrane exterior. Although ions do not physically cross the membrane at this point, the movements of ions onto and off the membrane surface constitute a capacitive current.

❹ An (extracellular) ionic current completes the local circuit as cations move toward the locus of the action potential and anions move away.

During an action potential, local circuits of current such as the one described here spread the depolarization passively (electrotonically) along the surface of the membrane. *The action potential propagates to an adjacent portion of the axon because the capacitive depolarization produced by the local current drives the*

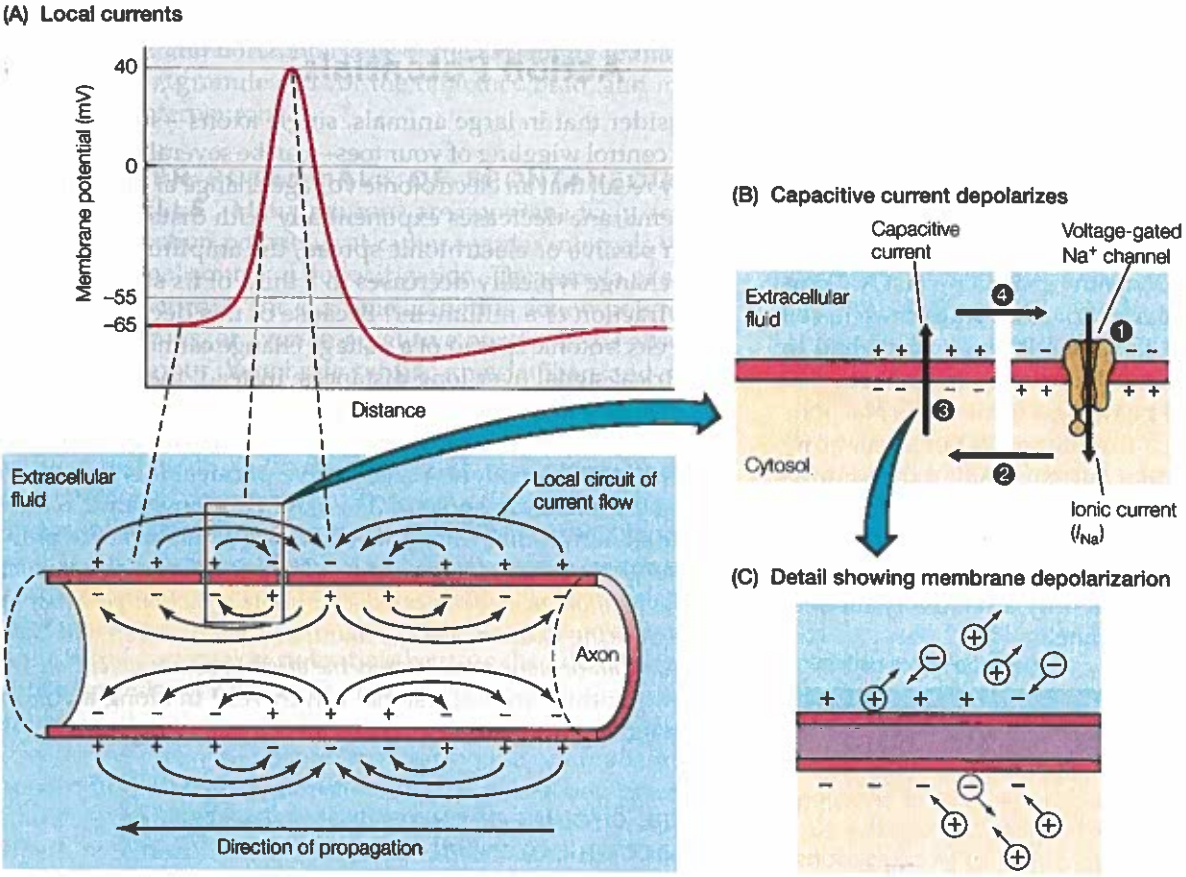


FIGURE 12.23 Propagation of an action potential An action potential is diagrammed here at an instant in time, while propagating from right to left. (A) The action potential induces local circuits of current flow along the axon, ahead of the advancing action potential and behind it. (B) The local circuit ahead of the action potential can be divided into four components, as shown. These components are described in the text. (C) This diagram illustrates how the capacitive current in (B) depolarizes the membrane ahead of the advancing action potential, by adding cations and removing anions on the inner side of the membrane, and adding anions and removing cations on the extracellular side. These changes in the distribution of charges on the membrane depolarize it, without any ions crossing the membrane. The depolarization resulting from the local currents opens voltage-gated Na^+ channels, leading (via the Hodgkin cycle) to an action potential at the new location.

membrane potential to threshold. When this happens, the Hodgkin cycle takes over; the region of the membrane that reaches threshold undergoes a regenerative increase in permeability to Na^+ and generates its own action potential. In this way the nerve impulse passes along the entire length of the axon without any decrease in amplitude.

Membrane refractory periods prevent bidirectional propagation

An axon can conduct impulses equally well in either direction. For example, an action potential that is triggered via an electrode placed in the middle of an axon will be propagated in both directions from that point. Normally, however, impulses start at or near one end of an axon and travel along the axon in one direction. As an impulse is propagated, its local currents depolarize the membrane behind it, as well as the membrane ahead of it. Why don't these local currents initiate reverse-traveling impulses going the other way? The membrane behind a traveling impulse is not reexcited by the local currents because the membrane is still in its refractory period (FIGURE 12.24).

Two aspects of the ionic mechanisms of action potentials produce the absolute and relative refractory periods following an impulse, and thereby prevent reexcitation and bidirectional propagation:

1. The inactivation of Na^+ channels (which turns off the voltage-dependent increase in permeability to Na^+) persists until the membrane potential returns to near its negative resting state, which means that inactivation lasts for at least 1 ms after an impulse passes a region of the membrane. Na^+ channel inactivation prevents the channels from entering the Hodgkin cycle until the action potential is far enough away to minimize local depolarization. During this time the membrane's voltage threshold is infinite because no amount of depolarization can open the inactivated Na^+ channels. This is the primary basis for the absolute refractory period.

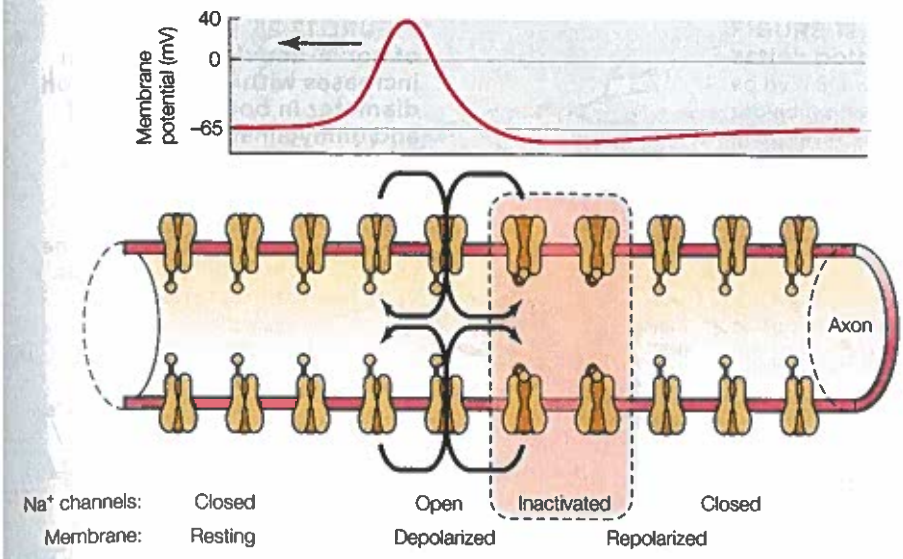


FIGURE 12.24 Inactivation of voltage-gated Na^+ channels prevents reverse propagation of an action potential An action potential is shown propagating from right to left; local currents depolarize the axon membrane ahead of the advancing action potential and behind it. The axon membrane behind the advancing action potential (shaded region) is refractory because its Na^+ channels are still inactivated. This refractory period prevents self-reexcitation by the trailing local currents.

2. The increased permeability to K^+ (the slowest of the three voltage-dependent processes in onset) does not decrease to resting levels until after repolarization. The lingering P_K increase after an impulse may hyperpolarize the membrane toward E_K for a few milliseconds after the impulse. Thus (for those neurons whose action potentials have an undershoot) a region of membrane that has just generated an impulse is hyperpolarized *away* from its voltage threshold for new impulse generation. The increase in P_K also renders a membrane refractory because it represents a decreased membrane resistance. The decreased resistance means that by Ohm's law ($V = IR$), local currents will cause a smaller voltage change, so more current is needed to depolarize the membrane to threshold. The effects of residual P_K increase are largely responsible for the relative refractory period.

During the relative refractory period the voltage threshold, membrane potential, and membrane resistance gradually return to resting levels within a few milliseconds. The refractory periods outlast the backward spread of local currents, thus preventing reverse propagation of the action potential.

The conduction velocity of an action potential depends on axon diameter, myelination, and temperature

Several factors can affect the velocity of propagation of an action potential along an axon. In general, these factors affect either or both of two conduction parameters: (1) the spatial parameter and (2) the temporal parameter. Spatially, the farther that local currents can spread along an axon, the farther they can (directly) depolarize the membrane to threshold. Therefore any factor that increases the spread of local currents (i.e., increases the membrane length constant, λ) tends to increase the conduction velocity of an action potential. With respect to the temporal parameter, the less time it takes the membrane to reach threshold, the faster the conduction velocity. The three major evolutionary variables that influence conduction velocities are axon diameter, myelination, and temperature.

AXON DIAMETER AND CONDUCTION VELOCITY

Large-diameter axons tend to conduct action potentials more rapidly than small-diameter axons. Many animal groups have evolved rapidly conducting neuronal giant axons (**BOX 12.3** and **BOX EXTENSION 12.3**). Conduction velocity increases with axon diameter because larger-diameter axons have longer length constants and thus more distant spread of local currents. The length constant depends principally on two types of resistance (see Figure 12.13C): the resistance across the membrane (R_m) and the axoplasmic resistance (R_i) to current flow

BOX 12.3 Giant Axons

Because an increase in axon diameter increases the conduction velocity of an action potential and because animals often face circumstances in which a rapid response is advantageous for survival, **giant axons** have evolved in several animal groups. No particular diameter qualifies an axon as *giant*. Rather, the term is relative: A giant axon is of exceptional diameter compared with other axons in the same animal. Some axons are truly giant in cellular dimensions, such as the third-order giant axons in the squid, which may be 1 mm (1000 μm) in diameter. At the other extreme, the giant axons in the fruit fly *Drosophila* are only about 4 μm in diameter, but they are still an order of magnitude larger than other nearby axons. This essay continues on the web at **BOX EXTENSION 12.3**, which describes the structure and function of giant axons in squid and in some other invertebrates.

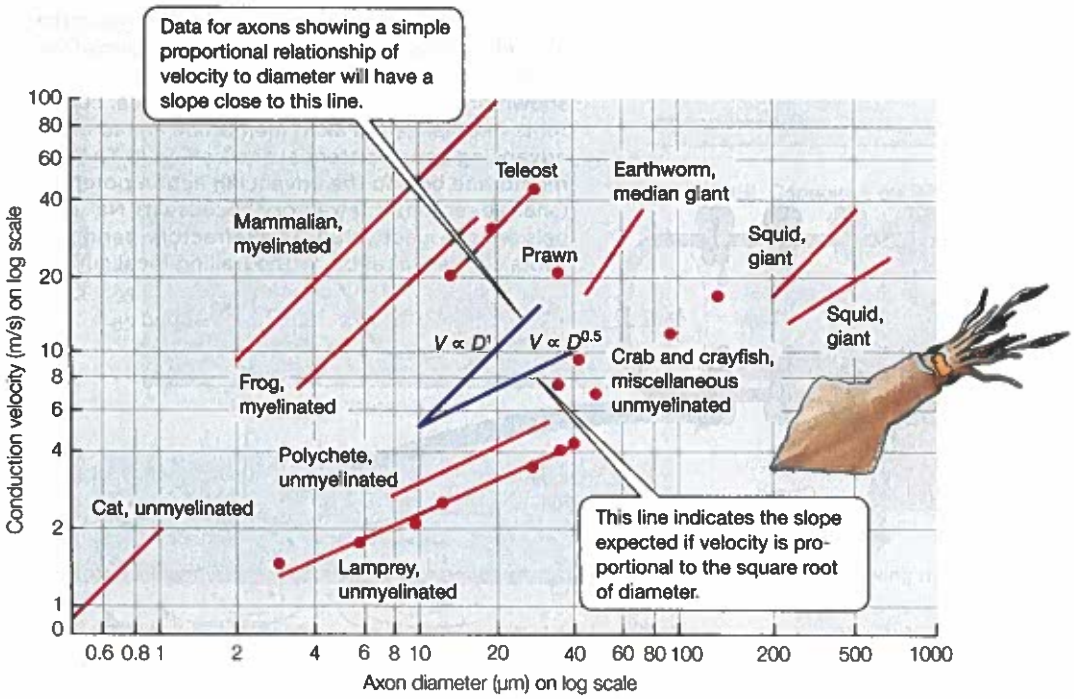


FIGURE 12.25 The velocity of nerve-impulse conduction increases with increasing axon diameter in both myelinated and unmyelinated axons
Points not connected by lines are axons of different types. (After T. H. Bullock and G. A. Horridge. 1965. *Structure and Function of the Nervous Systems of Invertebrates.* W. H. Freeman, San Francisco.)

along the length of the axon (the cytoplasm inside the axon is called *axoplasm*). (The external longitudinal resistance is usually small and is ignored.) Because ions tend to flow along the path of least resistance, increasing R_m or decreasing R_i causes more flow down the axon and less flow across the membrane. A slightly simplified equation for the length constant (λ) is

$$\lambda = K\left[\frac{R_m}{R_i}\right]^{1/2} \qquad (12.7)$$

where K is a constant.

The membrane surface area increases proportionally with increasing axon diameter, which lowers R_m by adding resistances in parallel. However, R_i decreases in proportion to an increase in cross-sectional area of the axoplasm—that is, in proportion to the square of the diameter. The net effect is that the ratio R_m/R_i increases linearly with increasing diameter. If other factors are equal, the length constant and the conduction velocity should increase with the square root of the diameter. This would make the conduction velocity an allometric function of diameter: $V \propto D^b$, with $b = 0.5$ (see Appendix E).

FIGURE 12.25 shows that conduction velocity empirically increases with increasing axon diameters. The data are plotted on log–log coordinates. On these coordinates, the allometric relation $V \propto D^b$ will plot as a straight line regardless of the value of b (see Appendix E). The two lines forming a V at the center show the slopes for $V \propto D^1$ (simple proportionality) and $V \propto D^{0.5}$. As you can see, the empirical plots (red lines) have slopes between these extremes. For some unmyelinated axons, V and D follow the square-root relationship ($V \propto D^{0.5}$), but many myelinated axons have a more nearly proportional relationship of velocity to diameter ($V \propto D^1$). That the relation of velocity and diameter differs for different kinds of axons implies that other factors, including intrinsic membrane differences, are also involved.

MYELINATION INCREASES CONDUCTION VELOCITY

Myelinated axons of vertebrates represent a tremendous evolutionary advance because they allow very high conduction velocities with relatively small axon diameters. A **myelinated axon** (FIGURE 12.26) is wrapped with 200 or more concentric layers of glial membrane (the membrane of Schwann cells in PNSs and of oligodendrocytes in CNSs; see Figure 12.4). The glial cytoplasm is extruded from between the glial membrane layers so that the whole wrapping serves as an insulating layer. This multiply-wrapped insulating layer, termed *myelin*, stops at intervals of 1 mm or so along the length of the axon. The gaps at which the glial wrappings are absent (Nodes A, B, and C in Figure 12.26) are called the **nodes of Ranvier**.

Myelin electrically insulates the major part of the axon (the regions between nodes, or **internodes**) nearly completely, leaving only the nodes of Ranvier as loci of ion flow across the axon membrane (see Figure 12.26). *In myelinated axons, action potentials occur only at the nodes of Ranvier*, in contrast to the continuous sweep of action potentials over an unmyelinated axon. Myelinated axons are therefore said to exhibit **saltatory conduction**, in which the action potential jumps (saltates) from node to node without active propagation in the internode. Action potentials are typically initiated at the axon initial segment, and then saltate from node to node because these are the only regions with high concentrations of voltage-gated Na^+ channels (FIGURE 12.27).

The principal effect of myelin is to increase the membrane resistance of myelinated axon regions by 1000- to 10,000-fold over the resistance at the nodes of Ranvier. When a node of Ranvier undergoes an action potential, the local currents

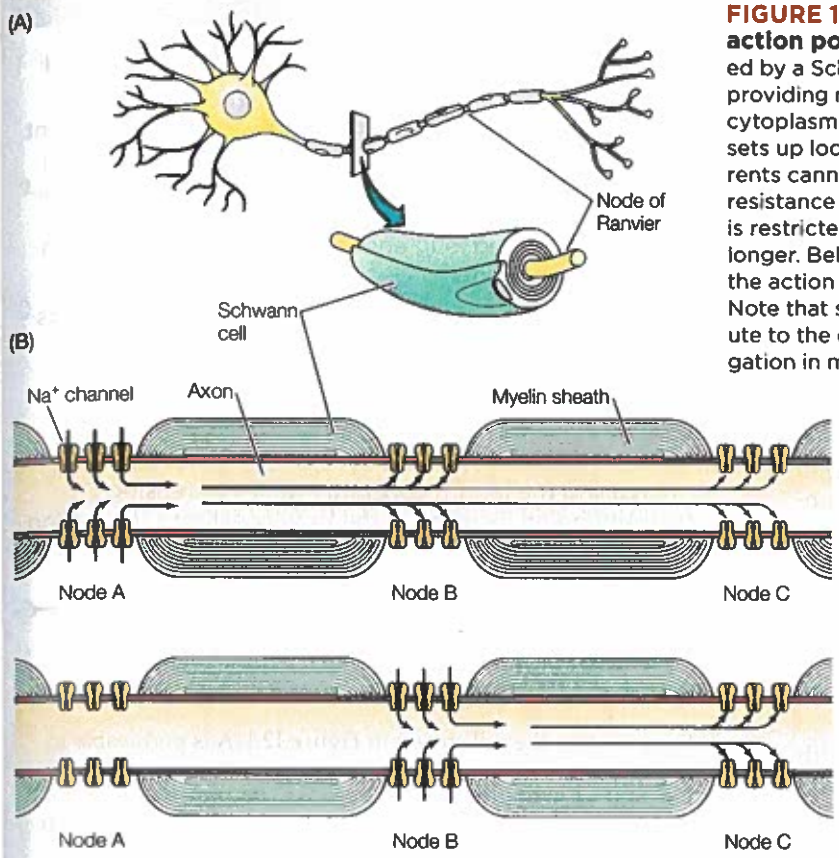
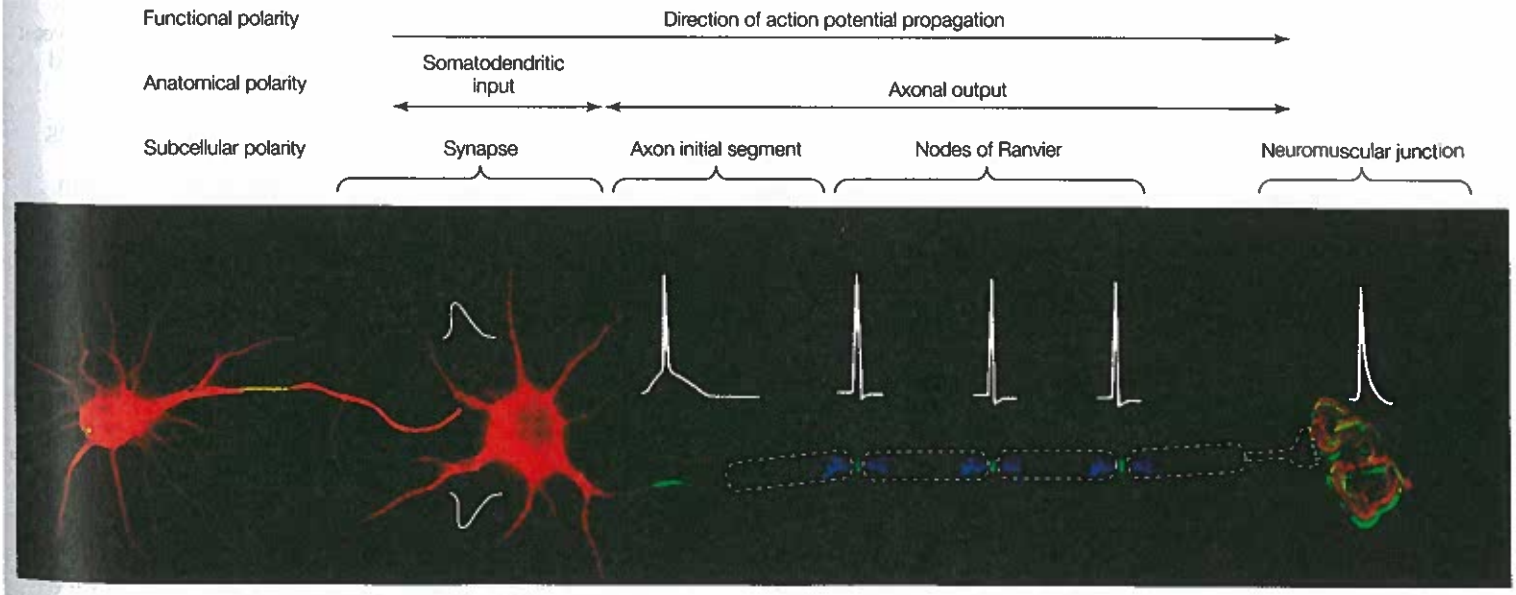


FIGURE 12.26 Myelinated axons speed the propagation of an action potential (A) Each segment of an axon in the PNS is myelinated by a Schwann cell. The Schwann cell wraps around the axon segment, providing many layers of Schwann cell membrane without intervening cytoplasm. (B) An action potential at one node of Ranvier (Node A) sets up local currents that flow along the axon, as in Figure 12.24. Currents cannot cross the membrane in the internode, which has very high resistance and low capacitance. Therefore transmembrane current flow is restricted to the nodes, and the membrane length constant is much longer. Below, depolarization of Node B opens Na^+ channels, generating the action potential and setting up local currents that spread to Node C. Note that some local current can flow past the nearest node to contribute to the depolarization of more distant nodes. Action potential propagation in myelinated axons is saltatory, jumping from node to node.

cannot leak out through the high membrane resistance of the adjacent internode, but instead must flow farther to the next node of Ranvier (see Figure 12.26). Thus myelination greatly increases the spatial spread of local currents (i.e., the axon length constant) by this resistance effect.

A second, equally important function of myelin is to decrease membrane capacitance. If myelin only increased membrane resistance (without decreasing capacitance), its effect on the axon length constant would be largely offset by an increase in the membrane time constant, τ. (Recall that the time constant is equal to the product of membrane resistance and membrane capacitance, $R_m C_m$.) An increase in the time constant would tend to *slow* conduction velocity because it would take more time for a current to depolarize a patch of membrane to threshold. Capacitance is inversely proportional to the distance separating the charges on the "plates" of a capacitor,



From M. Rasband. 2010. *Nat. Rev. Neurosci.* 11: 552–562

FIGURE 12.27 Spatial distribution of voltage-gated channels at the surface of a myelinated neuron This composite fluorescence micrograph depicts the locations of high concentrations of voltage-gated Na^+ channels (green, yellow) and K^+ channels (blue). The Na^+ channels are localized at the axon initial segment (site of action potential initiation) and at the nodes of Ranvier. K^+ channels are localized at paranodal regions just next to the nodes.

which in this case is the distance between the axoplasm and the extracellular fluid. Myelin increases this distance in proportion to the number of glial membrane wrappings, so that capacitance is decreased about 1000-fold. Thus the increase in R_m of myelinated regions is offset by a decrease in C_m, and the membrane time constant is nearly unaffected.

Myelination, then, greatly increases conduction velocity by increasing the axon length constant without increasing the time constant. Currents from an action potential at one node must travel to the next node before crossing the membrane, and the currents are not slowed by having to displace much charge in the intervening myelinated internode.

Myelinated axons permit vertebrates to have neural coordinating and control systems with small-diameter axons that nonetheless conduct rapidly. A frog myelinated axon 12 μm in diameter has a conduction velocity of 25 m/s at 20°C. An unmyelinated squid giant axon must be about 500 μm in diameter to achieve the same 25-m/s velocity at 20°C! Thus myelination allows the same velocity to be achieved with a 40-fold reduction in diameter and a 1600-fold reduction in axon cross-sectional area and volume. *With a reduction in axon dimensions, many more axons can be incorporated into a nervous system of a given size.*

Although myelin is usually considered to have evolved exclusively in vertebrates, some crustaceans have axons with analogous sheaths of glial wrappings that exhibit increased conduction velocities similar to those for vertebrate compact myelin. The 30-μm myelinated axons of a shrimp conduct at 20 m/s at 17°C, a velocity comparable to that of a 350-μm squid axon. Other myelinated shrimp axons 100–120 μm in diameter conduct at velocities exceeding 90 m/s at 20°C, rivaling the fastest mammalian myelinated axons. In contrast, unmyelinated lobster axons of the same 100- to 120-μm diameter have conduction velocities of only 8 m/s. Vertebrates, however, remain the only group with substantial numbers of myelinated neurons. Crustacean myelinated neurons are rare, specialized adaptations in high-velocity escape systems, but myelinated neurons are ubiquitous features of vertebrate nervous systems.

TEMPERATURE The gating kinetics of voltage-gated ion channels are temperature-dependent. Thus the time course of membrane depolarization to threshold shortens with increased temperature. For both myelinated and unmyelinated axons, an increase of 10°C tends to nearly double conduction velocity (the value of Q_{10} is approximately 1.8; see Section 10.3). A frog myelinated axon 12–14 μm in diameter conducts at 25 m/s at 20°C, but a cat myelinated axon of only 3.5–4 μm conducts at the same 25 m/s at 37°C. Thus the evolution of homeothermy in birds and mammals—along with myelination—has allowed further axon miniaturization and higher conduction velocities.

Finally, the propagation of action potentials merely ensures that a neuron's electrical signals reach the end of the axon undiminished. For a neuron's signals to affect other cells, another process—synaptic transmission (the topic of Chapter 13)—is required.

SUMMARY

The Propagation of Action Potentials

- Action potentials propagate because the membrane's underlying permeabilities to ions are voltage-dependent. Local circuits of current flow spread the depolarization along the axon, depolarizing a new region to threshold. Behind an advancing action potential, Na^+ channels remain inactivated long enough to prevent reexcitation by the local currents.
- The conduction velocity of an action potential depends on axon diameter, myelination, and temperature. Larger-diameter axons have higher conduction velocities because their length constants are longer, so local currents spread farther along the axon. Myelin greatly increases conduction velocity by increasing R_m (increasing the length constant) while decreasing C_m (preventing an increase in the time constant). Increasing temperature speeds the gating of channels so that the membrane responds faster to the local currents.

STUDY QUESTIONS

1. Suppose that the cell shown in Figure 12.11A is permeable to Na^+, K^+, and Cl^- but not to A^-. In the absence of ion pumps, Na^+ and Cl^- will diffuse in, the cell will become out of osmotic balance, and water will enter. Explain why a cell cannot be in thermodynamic equilibrium and also be in osmotic balance, unless there is a nonpermeating ion in the extracellular fluid (as would be the case if Na^+ were nonpermeating). What does this suggest about the evolutionary origin of the Na^+–K^+ pump?
2. Unmyelinated axons conduct action potentials without decrement, but when myelinated axons lose myelin in demyelinating diseases such as multiple sclerosis, conduction of action potentials is blocked. Why?
3. Using Figure 12.25, compare and explain the difference (a) between the velocity of action potential propagation in lamprey axons and frog myelinated axons of equal diameter, (b) between squid axons and frog myelinated axons of equal velocity, and (c) between frog and mammalian myelinated axons.
4. The ion flows across neuronal membranes at rest and during an action potential do not significantly change bulk ion concentrations, except for that of Ca^{2+} ions. Resting Ca^{2+} ion concentrations in cells are usually about 10^{-7} *M*, and Ca^{2+} ions exert physiological effects at concentrations of perhaps 10^{-5} *M*. Explain why relative changes of intracellular $[Ca^{2+}]$ are much greater than for, say, $[Na^+]$ (12–50 m*M*).
5. For the ion concentrations in Table 12.1, calculate the equilibrium potentials for each ion species in squid axons and in mammalian neurons.
6. Using the values in question 5, determine what ions are in passive equilibrium at a membrane potential of –60 mV in the squid at 18°C, and at –88 mV in mammalian neurons at 37°C.
7. Suppose you voltage-clamp a squid axon from a resting membrane potential of –60 mV to a clamped value of +55 mV. Describe the early ionic current (say, at 0.5 ms after clamping). Use ion concentrations from Table 12.1.

8. Suppose a squid axon at rest is 20 times as permeable to K^+ as to Na^+. Using the simplified version of the Goldman equation in Figure 12.10, calculate the resting membrane potential. If during the rising phase of an action potential the permeability to Na^+ increases to 100 times the permeability to K^+, at what value will the action potential peak?

9. Suppose you stimulate an axon so that you generate an action potential at both ends at the same instant. Describe the propagation of these action potentials. What happens when they meet?

10. With increased neuronal electrical activity in a brain area, the rates of glucose uptake, O_2 consumption, and blood flow increase. (These changes are the basis of activity imaging such as functional magnetic resonance imaging [fMRI].) Why does neuronal activity increase local metabolic rate?

11. Toxins such as tetrodotoxin (from puffer fish and newts) and saxitoxin (from red-tide dinoflagellates) block voltage-gated Na^+ channels selectively. What effect would they have on currents in a voltage clamp to 0 mV? How do you suppose such toxins evolved?

12. Part of the evidence for a "ball-and-string" model of Na^+ channel inactivation is that the proteolytic enzyme pronase can selectively disable channel inactivation when perfused into the axoplasm of a squid axon. Where would you expect pronase to cleave the Na^+ channel protein?

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See also Additional References.