CHAPTER

12

Membrane Potential and Action Potential

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The communication of information between neurons and between neurons and muscles or peripheral organs requires that signals travel over considerable distances. A number of notable scientists have contemplated the nature of this communication through the ages. In the second century AD, the great Greek physician Claudius Galen proposed that "humors" flowed from the brain to the muscles along hollow nerves. A true electrophysiological understanding of nerve and muscle, however, depended on the discovery and understanding of electricity itself. The precise nature of nerve and muscle action became clearer with the advent of new experimental techniques by a number of European scientists, including Luigi Galvani, Emil Du Bois-Reymond, Carlo Matteucci, and Hermann von Helmholtz, to name a few (Brazier, 1988; McComas, 2011). Through the application of electrical stimulation to nerves and muscles, these early electrophysiologists demonstrated that the conduction of commands from the brain to muscle for the generation of movement was mediated by the flow of electricity along nerve fibers.

With the advancement of electrophysiological techniques, electrical activity recorded from nerves revealed that the conduction of information along the axon was mediated by the active generation of an electrical potential, called the action potential. But what precisely was the nature of these action potentials? To know this in detail required not only a preparation from which to obtain intracellular recordings but also one that could survive *in vitro*. The squid giant axon provided precisely such a preparation, as was first demonstrated by J. Z. Young in 1936 (Young, 1936). Many invertebrates contain unusually large axons for the generation of escape reflexes; large axons conduct more quickly than small ones and so the response time for escape is reduced (see Chapter 17). The squid possesses an axon approximately 0.5 mm in diameter, large enough to be impaled by even a coarse micropipette (Fig. 12.1). By inserting a glass micropipette filled with a salt solution into the squid giant axon, Alan Hodgkin and Andrew Huxley demonstrated in 1939 that axons at rest are electrically polarized, exhibiting a resting membrane potential of approximately −60 mV inside versus outside (Hodgkin and Huxley, 1939; Hodgkin, 1976). In the generation of an action potential, the polarization of the membrane is removed (referred to as depolarization) and exhibits a rapid swing toward, and even past, 0 mV (Fig. 12.1B). This depolarization is followed by a rapid swing in the membrane potential to more negative values, a process referred to as *hyperpolarization*. The membrane potential following an action potential typically becomes even more negative than the original value of approximately −60 mV. This period of increased polarization is referred to as the *afterhyperpolarization* or the undershoot.

The development of electrophysiological techniques to the point that intracellular recordings could be obtained from the small cells of the mammalian nervous system revealed that action potentials in these neurons are generated through mechanisms similar to that of the squid giant axon (Brock et al., 1952; Buser and Albe-Fessard, 1953; Tasaki et al., 1954; Phillips, 1956).

It is now known that action potential generation in nearly all types of neurons and muscle cells is accomplished through mechanisms similar to those first detailed in the squid giant axon by Hodgkin and Huxley. In this chapter, we consider the cellular mechanisms by which neurons and axons generate a resting membrane potential and how this membrane potential is briefly disrupted for the purpose of propagation of an electrical signal, the action potential. The following chapters describe in more detail the molecular properties of ion channels (Chapters 11 and 13) and

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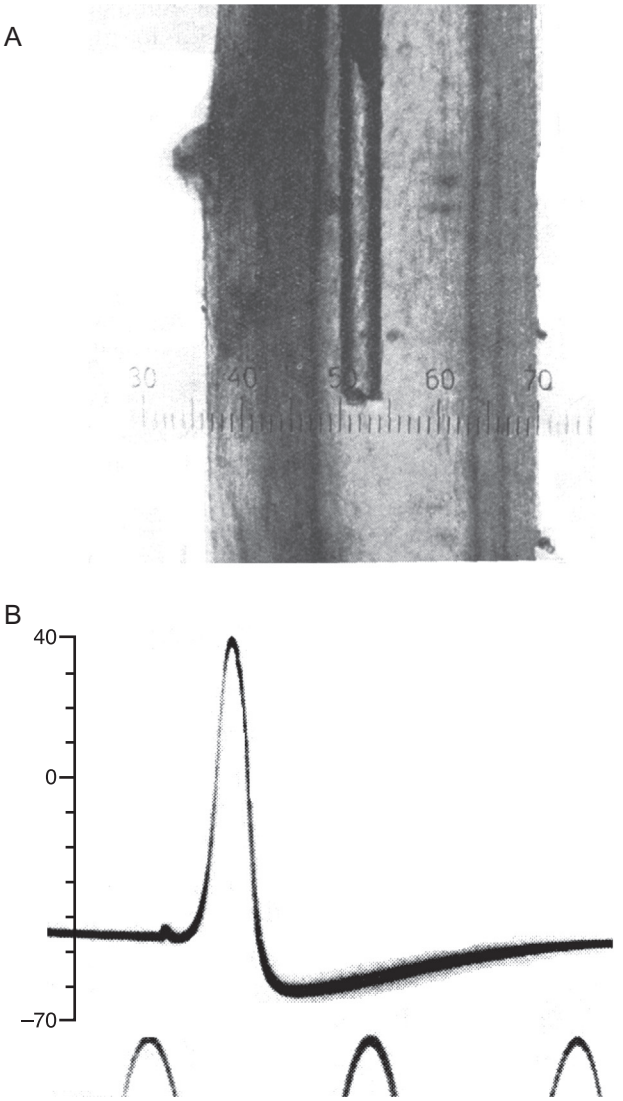

FIGURE 12.1 **Intracellular recording of the membrane potential and action potential generation in the squid giant axon**. (A) A glass micropipette, about 100 μm in diameter, was filled with seawater and lowered into the giant axon of the squid after it had been dissected free. The axon is about 1 mm in diameter and is transilluminated from behind. (B) One action potential recorded between the inside and the outside of the axon. Peaks of a sine wave at the bottom provided a scale for timing, with 2 ms between peaks. *From Hodgkin and Huxley (1939)*.

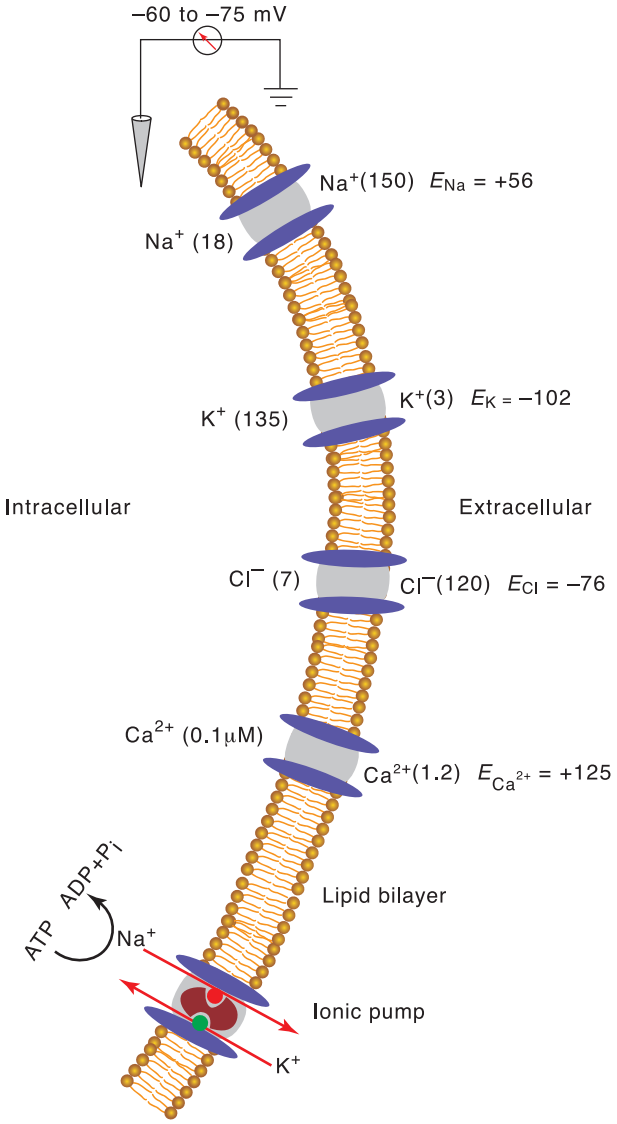

FIGURE 12.2 **Differential distribution of ions inside and outside plasma membrane of neurons and neuronal processes**, showing ionic channels for Na^+, K^+, Cl^-, and Ca^{2+}, as well as an electrogenic Na^+–K^+ ionic pump (also known as Na^+, K^+-ATPase). Concentrations (in millimoles except that for intracellular Ca^{2+}) of the ions are given in parentheses; their equilibrium potentials (*E*) for a typical mammalian neuron are indicated.

the quantitative analysis and dynamic properties of action potentials (Chapter 14).

THE MEMBRANE POTENTIAL

The Membrane Potential is Generated by the Differential Distribution of Ions

Through the operation of ionic pumps and special ionic buffering mechanisms, neurons actively maintain precise internal concentrations of several important ions, including Na^+, K^+, Cl^-, and Ca^{2+}. The mechanisms by which they do so are illustrated in Figs. 12.2 and 12.3. The intracellular and extracellular concentrations of Na^+, K^+, Cl^-, and Ca^{2+} differ markedly (see Fig. 12.2 and Table 12.1); K^+ is actively concentrated inside the cell, and Na^+, Cl^-, and Ca^{2+} are actively extruded to the extracellular space. However, this does not mean that the cell is filled only with positive charge; anions (denoted A^-) to which the plasma membrane is impermeant are also present inside the cell and almost balance the high concentration of K^+. The osmolarity inside the cell is approximately equal to that outside the cell.

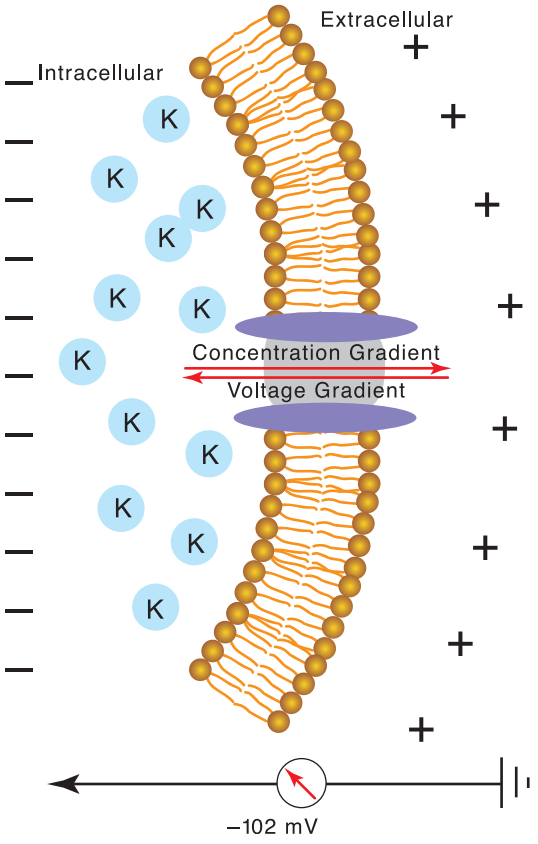

FIGURE 12.3 **The equilibrium potential is influenced by the concentration gradient and the voltage difference across the membrane.** Neurons actively concentrate K^+ inside the cell. These K^+ ions tend to flow down their concentration gradient from inside to outside the cell. However, the negative membrane potential inside the cell provides an attraction for K^+ ions to enter or remain within the cell. These two factors balance one another at the equilibrium potential, which in a typical mammalian neuron is −102 mV for K^+.

TABLE 12.1 Ion Concentrations and Equilibrium Potentials

	Inside (mM)	Outside (mM)	Equilibrium potential (mV)
	Squid giant axon		
Na^+	50	440	+55
K^+	400	20	−76
Cl^-	40	560	−66
Ca^{2+}	0.4	10	+145
	Mammalian neuron		
Na^+	18	145	+56
K^+	140	3	−102
Cl^-	7	120	−76
Ca^{2+}	0.1	1.2	+125

Electrical and Thermodynamic Forces Determine the Passive Distribution of Ions

To understand membrane potential and action potential we must first understand how ions move in solution. The movement of ions in solution is influenced by both local differences in concentration and voltage, since ions are charged molecules. The relationship between ion movement from concentration and voltage differences is given by the Nernst-Planck equation (see Box 12.1), which basically states that the movement of ions in solution is the sum total of movement down the concentration gradient and the movement along electrical gradients. In cells, ions tend to move down their concentration gradients through specialized ionic pores, known as ionic channels, in the plasma membrane. Through simple laws of thermodynamics, the high concentration of K^+ inside glial cells, neurons, and axons results in a tendency for K^+ ions to diffuse down their concentration gradient and leave the cell or cell process (see Fig. 12.3). However, the movement of ions across the membrane also results in a redistribution of electrical charge. As K^+ ions move down their concentration gradient, the intracellular voltage becomes more negative, and this increased negativity results in an electrical attraction between the negative potential inside the cell and the positively charged K^+ ions, thus offsetting the outward flow of these ions. If the membrane were equally permeable to all ions, then these would eventually distribute evenly across the membrane. However, the membrane is selectively permeable; that is, it is impermeable to the large anions inside the cell, which cannot follow the potassium ions across the membrane. At some membrane potential, the "force" of the electrostatic attraction between the negative membrane potential inside the cell and the positively charged K^+ ions exactly balances the thermal "forces" by which K^+ ions tend to flow down their concentration gradient (see Fig. 12.3). In this circumstance, it is equally likely that a K^+ ion exits the cell by movement down the concentration gradient as it is that a K^+ ion enters the cell owing to the attraction between the negative membrane potential and the positive charge of this ion. At this membrane potential, there is no net flow of K^+ (the same number of K^+ ions enter the cell as leave the cell per unit time) and these ions are said to be in equilibrium. The membrane potential at which this occurs is known as the equilibrium potential, and can be calculated by the Nernst equation. (See Box 12.2 for the calculation of the equilibrium potential.)

To illustrate, let us consider the passive distribution of K^+ ions in the squid giant axon as studied by Hodgkin and Huxley. The K^+ concentration inside the squid giant axon $[K^+]_i$ is about 400 mM, whereas that

BOX 12.1

THE NERNST-PLANCK EQUATION

The movement of ions in a solution is influenced by both the concentration gradient and local differences in voltage. Ions tend to flow down their concentration gradient, owing to the higher probability of a random movement being made from a higher to lower region of concentration. At the same time, the electrical charge on ions makes them sensitive to gradients in voltage. The Nernst-Planck equation relates these two processes to reveal how ions diffuse.

$$I = I_{\text{voltage}} + I_{\text{concentration}} = -uz^2F[C]\delta V/\delta x - uzRT\delta[C]/\delta x$$

In other words, total current flow is equal to the partial flow determined by the concentration of the ion [C] times local spatial differences in voltage ($\delta V/\delta x$) (the first term of the equation) and by the partial flow resulting from local differences in concentration ($\delta[C]/\delta x$) (the second term). I is current flow (A/cm^2), u is molar mobility (cm^2/V-sec-mol), z is valence of the ion, F is Faraday's constant (96,485 coulombs per mole), [C] is the concentration of the ion, R is the gas constant (8.315 joules per Kelvin per mole), and T is the temperature in Kelvin. At equilibrium, when there is no net current flow (i.e., $I = 0$) the flow down the concentration gradient is exactly offset by the flow along the voltage gradient and the Nernst-Planck equation reduces to the Nernst equation (see Box 12.2).

BOX 12.2

THE NERNST EQUATION

The equilibrium potential is determined by (1) the concentration of the ion inside and outside the cell, (2) the temperature of the solution, (3) the valence of the ion, and (4) the amount of work required to separate a given quantity of charge. The equation that describes the equilibrium potential was formulated by a German physical chemist named Walter Nernst, 1888

$$E_{\text{ion}} = RT/zF \ln \{[\text{ion}]_o/[\text{ion}]_i\}$$

Here, E_{ion} is the membrane potential at which the ionic species is at equilibrium, R is the gas constant (8.315 joules per Kelvin per mole), T is the temperature in Kelvin ($T_{\text{Kelvin}} = 273.16 + T_{\text{Celsius}}$), F is Faraday's constant (96,485 coulombs per mole), z is the valence of the ion, and $[\text{ion}]_o$ and $[\text{ion}]_i$ are the concentrations of the ion outside and inside the cell, respectively. For a monovalent, positively charged ion (cation) at room temperature (20 °C), substituting the appropriate numbers and converting natural log (ln) into log base 10 (log) results in the equation

$$E_{\text{ion}} = 58.2 \log \{[\text{ion}]_o/[\text{ion}]_i\};$$

at a body temperature of 37 °C, the Nernst equation is

$$E_{\text{ion}} = 61.5 \log \{[\text{ion}]_o/[\text{ion}]_i\}$$

outside the axon $[K^+]_o$ is about 20 mM (Table 12.1). Because $[K^+]_i$ is greater than $[K^+]_o$ potassium ions tend to flow down their concentration gradient, taking positive charge with them. The equilibrium potential (at which the tendency for K^+ ions to flow down their concentration gradient is exactly offset by the attraction for K^+ ions to enter the cell because of the negative charge inside the cell) at a room temperature of 20 °C can be determined by the Nernst equation as:

$$E_K = 58.2 \log(20/400) = -76 \text{ mV}.$$

Therefore, at a membrane potential of −76 mV, K^+ ions have an equal tendency to flow either into or out of the axon. The concentrations of K^+ in mammalian neurons and glial cells differ considerably from that in the squid giant axon, which is adapted to live in sea water (see Table 12.1). By substituting 3.1 mM for $[K^+]_o$ and 140 mM for $[K^+]_i$ in the Nernst equation, with mammalian body temperature, $T = 37$ °C, we obtain

$$E_K = 61.5 \log (3.1/140) = -102 \text{ mV}.$$

Movements of Ions can Cause Either Hyperpolarization or Depolarization

In mammalian cells, at membrane potentials positive to −102 mV, K^+ ions tend to flow out of the cell. Increasing the ability of K^+ ions to flow across the membrane—that is, increasing the conductance of the membrane to K^+ (g_K)—causes the membrane potential to become more negative, or hyperpolarized, owing to the exit of positively charged ions from inside the cell (Fig. 12.4).

At membrane potentials negative to −102 mV, K^+ ions tend to flow into the cell; increasing the membrane conductance to K^+ causes the membrane potential to become more positive, or depolarized, owing to the flow of positive charge into the cell. The membrane potential at which the net current "flips" direction is referred to as the *reversal potential*. If the channels conduct only one type of ion (e.g., K^+ ions), then the reversal potential and the Nernst equilibrium potential for that ion coincide (see Fig. 12.4A). Increasing the membrane conductance to K^+ ions while the membrane potential is at the equilibrium potential for K^+ (E_K) does not change the membrane potential, because no net driving force causes K^+ ions to either exit or enter the cell. However, this increase in membrane conductance to K^+ decreases the ability of other species of ions to change the membrane potential, because any deviation of the potential from E_K increases the drive for K^+ ions either to exit or to enter the cell, thereby drawing the membrane potential back toward E_K (see Fig. 12.4B). This effect is known as a "shunt" and is important for some effects of inhibitory synaptic transmission.

The exit from and entry into the cell of K^+ ions during generation of the membrane potential give rise to a curious problem. When K^+ ions leave the cell to generate a membrane potential, the concentration of K^+ changes both inside and outside the cell. Why does this change in concentration not alter the equilibrium potential, thus changing the tendency for K^+ ions to flow down their concentration gradient? The reason is that the number of K^+ ions required to leave the cell to achieve the equilibrium potential is quite small. For example, if a cell were at 0 mV and the membrane suddenly became permeable to K^+ ions, only about 10^{-12} mol of K^+ ions per square centimeter of membrane would move from inside to outside the cell in bringing the membrane potential to the equilibrium potential for K^+. In a spherical cell of 25-μm diameter, this would amount to an average decrease in intracellular K^+ of only about 4 μM (e.g., from 140 to 139.996 mM). However, there are instances when significant changes in the concentrations of K^+ may occur, particularly during the generation of pronounced activity, such as an epileptic seizure. During the occurrence of a tonic-clonic generalized (grand mal) seizure, large numbers of neurons discharge throughout the cerebral cortex in a synchronized manner. This synchronous discharge of large numbers of neurons significantly increases the extracellular K^+ concentration, by as much as a couple of millimoles, resulting in a commensurate positive shift in the equilibrium potential for K^+ (Hotson et al., 1973; Prince et al., 1973). This shift in the equilibrium potential can increase the excitability of affected neurons and neuronal processes and thus promote the spread of the seizure activity. Fortunately, the extracellular

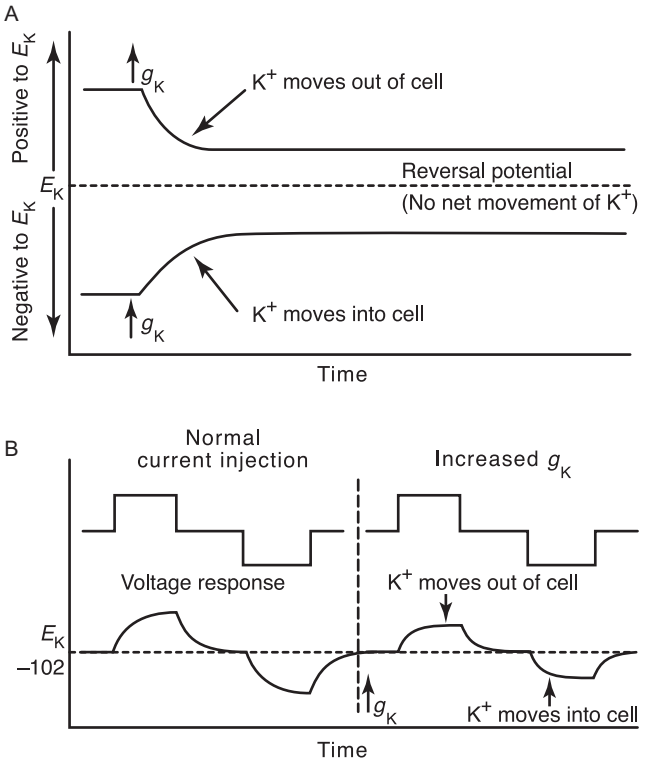

FIGURE 12.4 Increases in K^+ conductance can result in hyperpolarization, depolarization, or no change in membrane potential. (A) Opening K^+ channels increases the conductance of the membrane to K^+, denoted g_K. If the membrane potential is positive to the equilibrium potential (also known as the reversal potential) for K^+, then increasing g_K will cause some K^+ ions to leave the cell, and the cell becomes hyperpolarized. If the membrane potential is negative to E_K when g_K is increased, then K^+ ions enter the cell, making the inside more positive (more depolarized). If the membrane potential is exactly E_K when g_K is increased, then there is no net movement of K^+ ions. (B) Opening K^+ channels when the membrane potential is at E_K does not change the membrane potential; however, it reduces the ability of other ionic currents to move the membrane potential away from E_K. For example, a comparison of the ability of the injection of two pulses of current, one depolarizing and one hyperpolarizing, to change the membrane potential before and after opening K^+ channels reveals that increases in g_K noticeably decrease the responses of the cell.

concentration of K^+ is tightly regulated and kept at normal levels through uptake by glial cells as well as by diffusion through the fluid of the extracellular space (Kuffler and Nicholls, 1966).

As is true for K^+ ions, each of the membrane-permeable species of ions possesses an equilibrium potential that depends on the concentration of the ions inside and outside the cell. Thus, equilibrium potentials may vary between different cell types, such as those found in animals adapted to live in salt water versus mammalian neurons (see Table 12.1). In mammalian neurons, the equilibrium potential is approximately +56 mV for Na^+, approximately −76 mV for Cl^-, and about +125 mV for Ca^{2+} (see Table 12.1 and Fig. 12.2). Thus, increasing the membrane conductance to Na^+ (g_{Na}) through the opening of Na^+ channels depolarizes the membrane potential toward +56 mV; increasing the membrane conductance to Cl^- brings the membrane potential closer to −76 mV; and finally increasing the membrane conductance to Ca^{2+} depolarizes the cell toward +125 mV.

Na^+, K^+, and Cl^- Contribute to the Determination of the Resting Membrane Potential

If a membrane is permeable to only one ion and no electrogenic ionic pumps are operating (see next section), then the membrane potential is necessarily at the equilibrium potential for that ion. If the membrane is permeable to two ions, say K^+ and Cl^-, in the presence of an impermeant ion (neurons contain lots of impermeant anions, for example), then the movement of the two permeant ions influences each other and will come to equilibrium with an unequal distribution across the semipermeable membrane. Donnan equilibrium (Box 12.3) illustrates how the passive distribution of one type of ion may influence that of another. At rest, the plasma membrane of most cell types is not at the equilibrium potential for K^+ ions, indicating that the membrane is also permeable to other types of ions. For example, the resting membrane of the squid giant axon is permeable to Cl^- and Na^+, as well as K^+, owing to the presence of ionic channels that not only allow these ions to pass but also are open at the resting membrane potential. Because the membrane is permeable to K^+, Cl^-, and Na^+, the resting potential of the squid giant axon is not equal to E_K, E_{Na}, or E_{Cl}, but is somewhere within these three. A membrane permeable to more than one ion has a steady-state membrane potential whose value is between those of the equilibrium potentials for each of the permeant ions (Box 12.4) (Goldman, 1943; Hodgkin and Katz, 1949).

Different Types of Neurons have Different Resting Potentials

Intracellular recordings from neurons in the mammalian CNS reveal that different types of neurons exhibit different resting membrane potentials. Indeed, some types of neurons do not even exhibit a true "resting" membrane potential; they spontaneously and continuously generate action potentials even in the total lack of synaptic input. In the visual system, intracellular recordings have shown that the photoreceptor cells of the retina—the rods and cones—have a membrane potential of approximately −40 mV at rest and are hyperpolarized when activated by light (Tomita, 1965). Cells in the dorsal lateral geniculate nucleus, which receive axonal input from the retina and project to the visual cortex, have a resting membrane potential of approximately −70 mV during sleep and −55 mV during waking (Hirsch et al., 1983; Jahnsen and Llinas, 1984a,b), whereas pyramidal neurons of the visual cortex have a resting membrane potential of about −75 mV (McCormick et al., 1985). Presumably, the resting membrane potentials of different cell types in the central and peripheral nervous system are highly regulated and are functionally important. For example, the depolarized membrane potential of photoreceptors presumably allows the membrane potential to move in both negative and positive directions in response to changes in light intensity. The hyperpolarized membrane potential of thalamic neurons during sleep (−70 mV) dramatically decreases the flow of information from the sensory periphery to the cerebral cortex (Livingstone and Hubel, 1981; Steriade and McCarley, 2005), presumably to allow the cortex to be relatively undisturbed during sleep, and the 20 mV membrane potential between the resting potential and the action potential threshold in cortical pyramidal cells may permit the subthreshold computation and integration of multiple neuronal inputs in single neurons (see Chapters 16 and 17).

Ionic Pumps Actively Maintain Ionic Gradients

Because the resting membrane potential of a neuron is not at the equilibrium potential for any particular ion, ions constantly flow down their concentration gradients. This flux becomes considerably larger with the generation of electrical and synaptic potentials, because ionic channels are opened by these events. Although the absolute number of ions traversing the plasma membrane during each action potential or synaptic potential may be small in individual cells, the collective influence of a large neural network of cells, such as in the brain, and the presence of ion fluxes even at rest can substantially change the distribution

BOX 12.3

DONNAN EQUILIBRIUM

If a membrane were equally permeable to all ions and the only source of voltage difference across the membrane were these ions, then they would move down their respective concentration differences until all ions were distributed uniformly and the voltage difference across the membrane would be zero. This is true since each movement of a negatively charged ion could be offset by the comovement of a positively charged ion (or the opposite movement of another negatively charged ion). However, neurons contain within them many impermeant anions (A^-), such as SO_4^{2-} and small charged proteins. How does the presence of these ions affect the passive distribution of other ions? Consider, for example, the passive distribution of K^+ and Cl^- across the membrane in the presence of impermeant anions. The movement of K^+ ions from inside the cell to out will leave behind a negative charge that cannot be offset by the movement of the impermeant anions. This imbalance will create a negative membrane potential, inside relative to outside the cell. This membrane potential will then act as an attractive force for K^+ to stay inside the cell, and as a repellant force for Cl^- to stay outside. The K^+ and Cl^- ions will move according to their concentration and voltage gradients until each comes into equilibrium, as determined by the Nernst equation. Since there can be only one membrane potential, this equilibrium point, which is known as Donnan equilibrium, is given by:

$$-RT/F\ln[K^+]_i/[K^+]_o = RT/F\ln[Cl^-]_i/[Cl^-]_o$$

which simplifies to:

$$[K^+]_o/[K^+]_i = [Cl^-]_i/[Cl^-]_o$$

or

$$[K^+]_o \times [Cl^-]_o = [K^+]_i \times [Cl^-]_i$$

Let's consider an example. Imagine an initial condition in which the inside of the cell contains 150 mM K^+, 50 mM Cl^- and 100 mM A^- (impermeant anions), while the outside of the cell contains 150 mM K^+, 150 mM Cl^- and no impermeant anions. Since the distribution of charge is equal, there is no membrane potential ($V = 0$). If one considered only the K^+ ion distribution, you might expect there to be no movement of K^+ ions, since there is no concentration gradient or voltage gradient for this ion. However, after this initial condition, ions will move. Cl^- is not at equilibrium and will tend to move down its concentration gradient, into the cell, setting up a voltage across the membrane. This voltage difference will then influence the movement of K^+ ions, bringing K^+ also into the cell. Eventually the system will come into equilibrium when $[K^+]_i = 180$ mM, $[Cl^-]_i = 80$ mM, and $[K^+]_o = 120$ mM, $[Cl^-]_o = 120$ mM (e.g., equal amounts, 30 mM, of K^+ and Cl^- have moved into the cell). At this point, the membrane potential across the cell will be -10.2 mV, owing to a very small difference in the balance of charge across the membrane. Note, however, that there is now an osmotic imbalance—the inside of the cell has a much higher total concentration of ions than outside. In a normal neuron, this imbalance would result in the entry of water into the cell, swelling, and eventual bursting of the neuron. However, in real neurons, ions are not distributed to Donnan equilibrium, owing to the permeability of the membrane to multiple types of ions (see Goldman-Hodgkin-Katz equation; Box 12.4) and the active transport of ions across the membrane by ionic pumps and exchangers. Through both passive and active mechanisms the cell maintains the proper distribution of ions while also regulating osmotic balance, keeping the cell healthy and functional.

of ions inside and outside neurons. As described in Chapter 3, cells have solved this problem with the use of active transport of ions against their concentration gradients. The proteins that actively transport ions are referred to as ionic pumps, of which the Na^+–K^+ pump is perhaps the most thoroughly understood (Hodgkin and Keynes, 1955; Skou, 1957, 1988; Thomas, 1972). The Na^+–K^+ pump is stimulated by increases in the intracellular concentration of Na^+ and moves Na^+ out of the cell while moving K^+ into it, achieving this task through the hydrolysis of ATP (see Fig. 12.2). Three Na^+ ions are extruded for every two K^+ ions transported into the cell. Owing to the unequal transport of ions, the operation of this pump generates a hyperpolarizing electrical potential and is said to be electrogenic. The Na^+–K^+ pump typically results in the membrane potential of the cell being a few millivolts more negative than it would be otherwise.

The Na^+–K^+ pump consists of two subunits, α and β, arranged in a tetramer $(\alpha\beta)2$. The α subunit has a molecular mass of about 100 kDa and six hydrophobic regions capable of forming transmembrane helices (Horisberger et al., 1991; Mercer, 1993). The β subunit is smaller (about 38 kDa) and has only one hydrophobic

BOX 12.4

THE GOLDMAN–HODGKIN–KATZ EQUATION

An equation developed by Goldman (1943) and later used by Hodgkin and Katz (1949) describes the steady-state membrane potential for a given set of ionic concentrations inside and outside the cell and the relative permeabilities of the membrane to each of those ions:

$$V_m = RT/F \ln \{(p_K[K^+]_o + p_{Na}[Na^+]_o + p_{Cl}[Cl^-]_i)/(p_K[K^+]_i + p_{Na}[Na^+]_i + p_{Cl}[Cl^-]_o)\}$$

The relative contribution of each ion is determined by its concentration differences across the membrane and the relative permeability (p_K, p_{Na}, p_{Cl}) of the membrane to each type of ion. If a membrane is permeable to only one ion, then the Goldman–Hodgkin–Katz equation reduces to the Nernst equation. In the squid giant axon, at resting membrane potential, the permeability ratios are:

$$p_K : p_{Na} : p_{Cl} = 1.00:0.04:0.45.$$

The membrane of the squid giant axon, at rest, is most permeable to K^+ ions, less so to Cl^-, and least permeable to Na^+. (Chloride appears to contribute considerably less to the determination of the resting potential of mammalian neurons.) These results indicate that the resting membrane potential is determined by the resting permeability of the membrane to K^+, Na^+, and Cl^-. In theory, this resting membrane potential may be anywhere between E_K (e.g., −76 mV) and E_{Na} (+55 mV). For the three ions at 20 °C, the equation is

$$V_m = 58.2 \log\{(1 \cdot 20 + 0.04 \cdot 440 + 0.45 \cdot 40)/(1 \cdot 400 + 0.04 \cdot 50 + 0.45 \cdot 560)\} = -62 \text{ mV}.$$

This suggests that the squid giant axon should have a resting membrane potential of −62 mV. In fact, the resting membrane potential may be a few millivolts hyperpolarized to this value through the operation of the electrogenic Na^+–K^+ pump.

membrane-spanning region. The Na^+–K^+ pump is believed to operate through conformational changes that alternatively expose a Na^+ binding site to the interior of the cell (followed by the release of Na^+) and a K^+ binding site to the extracellular fluid (see Fig. 12.2). Such a conformation change may be due to the phosphorylation and dephosphorylation of the protein.

The membranes of neurons and glia contain multiple types of ionic pumps, used to maintain the proper distribution of each ionic species important for cellular signaling (Pedersen and Carafoli, 1987; Läuger, 1991). Many of these pumps are operated by the Na^+ gradient across the cell, whereas others operate through a mechanism similar to that of the Na^+–K^+ pump (i.e., the hydrolysis of ATP). For example, the calcium concentration inside neurons is kept at very low levels (typically 50–100 nM) through the operation of both types of ionic pumps as well as special intracellular Ca^{2+} buffering mechanisms. Ca^{2+} is extruded from neurons through both a Ca^{2+}, Mg^{2+}-ATPase and a Na^+–Ca^{2+} exchanger. The Na^+–Ca^{2+} exchanger is driven by the Na^+ gradient across the membrane and extrudes one Ca^{2+} ion for each Na^+ ion allowed to enter the cell.

The Cl^- concentration in neurons is actively maintained at a low level through the operation of a chloride–bicarbonate exchanger, which brings in one ion of Na^+ and one ion of $HCO3^-$ for each ion of Cl^- extruded (Thompson et al., 1988; Reithmeier, 1994). Intracellular pH also can markedly affect neuronal excitability and is therefore tightly regulated, in part by a Na^+–H^+ exchanger that extrudes one proton for each Na^+ allowed to enter the cell.

Summary

The membrane potential is generated by the unequal distribution of ions, particularly K^+, Na^+, and Cl^-, across the plasma membrane. This unequal distribution of ions is maintained by ionic pumps and exchangers. K^+ ions are concentrated inside the neuron and tend to flow down their concentration gradient, leading to a hyperpolarization of the cell. At the equilibrium potential, the tendency of K^+ ions to flow out of the cell is exactly offset by the tendency of K^+ ions to enter the cell owing to the attraction of the negative potential inside the cell. The resting membrane is also permeable to Na^+ and Cl^- and therefore the resting membrane potential is approximately −75 to −40 mV, in other words, substantially positive to E_K.

THE ACTION POTENTIAL

An Increase in Na^+ and K^+ Conductance Generates Action Potentials

Hodgkin and Huxley not only recorded the action potential with an intracellular microelectrode (see

BOX 12.5

THE VOLTAGE-CLAMP TECHNIQUE

In the voltage-clamp technique, two independent electrodes are inserted into the squid giant axon: one for recording the voltage difference across the membrane and the other for intracellularly injecting current (Fig. 12.5). These electrodes are then connected to a feedback circuit that compares the measured voltage across the membrane with the voltage desired by the experimenter. If these two values differ, then current is injected into the axon to compensate for this difference. This continuous feedback cycle, in which the voltage is measured and current is injected, effectively "clamps" the membrane at a particular voltage. If ionic channels were to open, then the resultant flow of ions into or out of the axon would be compensated for by the injection of positive or negative current into the axon through the current-injection electrode. The current injected through this electrode is necessarily equal to the current flowing through the ionic channels. It is this injected current that is measured by the experimenter. The benefits of the voltage-clamp technique are twofold. First, the current injected into the axon to keep the membrane potential "clamped" is necessarily equal to the current flowing through the ionic channels in the membrane, thereby giving a direct measurement of this current. Second, ionic currents are both voltage and time dependent; they become active at certain membrane potentials and do so at a particular rate. Keeping the voltage constant in the voltage clamp allows these two variables to be separated; the voltage dependence and the kinetics of the ionic currents flowing through the plasma membrane can be directly measured.

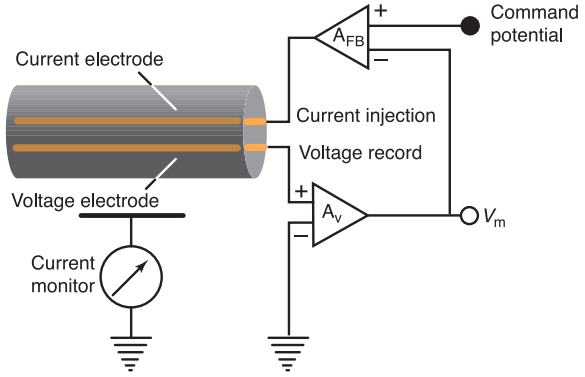

FIGURE 12.5 **Voltage-clamp technique.** The voltage-clamp technique keeps the voltage across the membrane constant so that the amplitude and time course of ionic currents can be measured. In the two-electrode voltage-clamp technique, one electrode measures the voltage across the membrane while the other injects current into the cell to keep the voltage constant. The experimenter sets a voltage to which the axon or neuron is to be stepped (the command potential). Current is then injected into the cell in proportion to the difference between the present membrane potential and the command potential. This feedback cycle occurs continuously, thereby clamping the membrane potential to the command potential. By measuring the amount of current injected, the experimenter can determine the amplitude and time course of the ionic currents flowing across the membrane.

Fig. 12.1), but also went on to perform a remarkable series of experiments that qualitatively and quantitatively explained the ionic mechanisms by which the action potential is generated (Hodgkin and Huxley, 1952a,b,c,d; Hodgkin et al., 1952). As mentioned earlier, these investigators found that during the action potential, the membrane potential of the cell rapidly overshoots 0 mV and approaches the equilibrium potential for Na^+. After generation of the action potential, the membrane potential repolarizes and becomes more negative than before, generating an afterhyperpolarization. Cole and Curtis (1939) had previously shown that these changes in membrane potential during the generation of the action potential are associated with a large increase in conductance of the plasma membrane. But to what does the membrane become conductive to generate the action potential? The prevailing hypothesis was that there was a nonselective increase in conductance causing the negative resting potential to increase toward 0 mV. Since publication of the experiments of Overton, 1902, the action potential had been known to depend on the presence of extracellular Na^+. Reducing the concentration of Na^+ in the artificial seawater bathing the axon resulted in a marked reduction in the amplitude of the action potential. On the basis of these and other data, Hodgkin and Katz proposed that the action potential is generated through a rapid increase in the conductance of the membrane to Na^+ ions. Quantitative proof of this theory was lacking, however, because ionic currents could not be observed directly. The development of the voltage-clamp technique by Cole (1949) at the Marine Biological Laboratory in Massachusetts resolved this problem and allowed quantitative measurement of the Na^+ and K^+ currents underlying the action potential (Box 12.5).

Hodgkin and Huxley used the voltage-clamp technique to investigate the mechanisms of generation of the action potential in the squid giant axon. Neurons have a threshold for the initialization of an action potential of about −45 to −55 mV. Increasing the voltage from −60 to 0 mV produces a large, but transient, flow of positive charge into the cell (known as inward current). This transient inward current is followed by a sustained flow of positive charge out of the cell (the outward current). By voltage-clamping the cell and substituting different ions inside or outside the axon or both, Hodgkin, Huxley, and colleagues demonstrated that the transient inward current is carried by Na^+ ions flowing into the cell and the sustained outward current is mediated by a sustained flux of K^+ ions moving out of the cell (Fig. 12.6) (Hodgkin and Huxley, 1952a,b,c,d; Hodgkin et al., 1952; Hille, 1977).

The Na^+ and K^+ currents (I_{Na} and I_K, respectively) can be blocked, allowing each current to be examined in isolation (see Fig. 12.6B) (see also Chapter 14). Tetrodotoxin (TTX), a powerful poison found in the puffer fish *Spheroides rubripes* (Kao, 1966), selectively blocks voltage-dependent Na^+ currents (the puffer fish remains a delicacy in Japan and must be prepared with the utmost care by the chef). Using TTX, one can selectively isolate I_K and examine its voltage dependence and time course (see Fig. 12.6B).

Armstrong and Hille (1972) and others demonstrated that tetraethylammonium (TEA) is a useful pharmacological tool for selectively blocking I_K (see Fig. 12.6B). The use of TEA to examine the voltage dependence and time course of the Na^+ current underlying action-potential generation (see Fig. 12.6B) reveals some fundamental differences between Na^+ and the K^+ currents. First, the inward Na^+ current activates, or "turns on," much more rapidly than does the K^+ current (giving rise to the name "delayed rectifier" for this K^+ current). Second, the Na^+ current is transient; it inactivates, even if the membrane potential is maintained at 0 mV (see Fig. 12.6A). In contrast, the outward K^+ current, once activated, remains "on" as long as the membrane potential is clamped to positive levels; that is, the K^+ current does not inactivate; it is sustained. Remarkably, from one experiment, we see that the Na^+ current both rapidly activates and inactivates, whereas the K^+ current only slowly activates. These fundamental properties of the underlying Na^+ and K^+ channels allow the generation of action potentials.

Hodgkin and co-workers (Hodgkin and Huxley, 1952a,b,c,d; Hodgkin et al., 1952) proposed that the K^+ channels possess a voltage-sensitive "gate" that opens by the depolarization and closes by the subsequent repolarization of the membrane potential. This process of "turning on" and "turning off" the K^+ current came to be known as *activation* and *deactivation*. The Na^+ current also exhibits voltage-dependent activation and

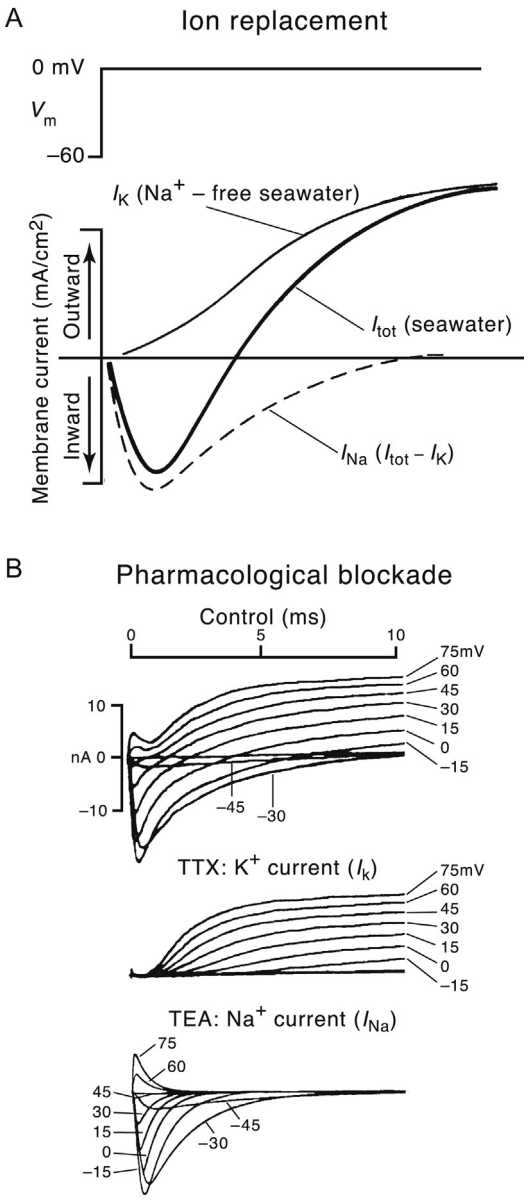

FIGURE 12.6 Voltage-clamp analysis reveals the ionic currents underlying action potential generation. (A) Increasing the potential from −60 to 0 mV across the membrane of the squid giant axon activates an inward current followed by an outward current. If the Na^+ in seawater is replaced by choline (which does not pass through Na^+ channels), then increasing the membrane potential from −60 to 0 mV results in only the outward current, which corresponds to I_K. Subtracting I_K from the recording in normal seawater illustrates the amplitude–time course of the inward Na^+ current, I_{Na}. Note that I_K activates more slowly than I_{Na} and that I_{Na} inactivates with time. (B) These two ionic currents can also be isolated from one another through the use of pharmacological blockers. (1) Increasing the membrane potential from −45 to +75 mV in 15-mV steps reveals the amplitude–time course of the inward Na^+ and outward K^+ currents. (2) After the block of I_{Na} with the poison tetrodotoxin (TTX), increasing the membrane potential to positive levels activates I_K only. (3) After the block of I_K with tetraethylammonium (TEA), increasing the membrane potential to positive levels activates I_{Na} only. *(A) from Hodgkin and Huxley (1952); part (B) from Hille (1977).*

deactivation (see Fig. 12.6), but the Na^+ channels also become inactive despite maintained depolarization. Thus, the Na^+ current not only activates and deactivates, but also exhibits a separate process known as *inactivation*, whereby the channels become blocked even though they are activated. The removal of this inactivation is achieved by relief of the depolarization and is a process known as *deinactivation*. Thus, the Na^+ channels possess two voltage-sensitive processes: activation–deactivation and inactivation–deinactivation. The kinetics of these two properties of Na^+ channels are different: inactivation takes place at a slower rate than activation.

The functional consequence of the two mechanisms is that Na^+ ions are allowed to flow across the membrane only when the channel is activated but not inactivated. Accordingly, Na^+ ions do not flow at resting membrane potentials, because the activation gate is closed (even though the inactivation gate is not). On depolarization, the activation gate opens, allowing Na^+ ions to flow into the cell. However, this depolarization also results in closure (at a slower rate) of the inactivation gate, which then blocks the flow of Na^+ ions. On repolarization of the membrane potential, the activation gate once again closes and the inactivation gate once again opens, preparing the axon for generation of the next action potential (Fig. 12.7) (see also Chapter 14, Fig. 14.6). Depolarization allows ionic current to flow by virtue of activation of the channel. The rush of Na^+ ions into the cell further depolarizes the membrane potential and more Na^+ channels become activated, forming a positive feedback loop that rapidly (within 100 μs or so) brings the membrane potential toward E_{Na}. However, the depolarization associated with the generation of the action potential also inactivates Na^+ channels, and, as a larger and larger percentage of Na^+ channels become inactivated, the rush of Na^+ into the cell diminishes. This inactivation of the Na^+ channels and activation of K^+ channels result in repolarization of the action potential. This repolarization deactivates the Na^+ channels. Then, the inactivation of the channel is slowly removed, and the channels are ready, once again, for generation of another action potential (see Fig. 12.7).

By measuring the voltage sensitivity and kinetics of these two processes, activation–deactivation and inactivation–deinactivation of the Na^+ current, as well as activation–deactivation of the delayed rectifier K^+ current, Hodgkin and Huxley generated a series of mathematical equations (see Chapter 14 for details) that quantitatively describe the generation of the action potential (calculation of the propagation of a single action potential required an entire week of cranking a mechanical calculator). According to these early experimental and computational neuroscientists, the action

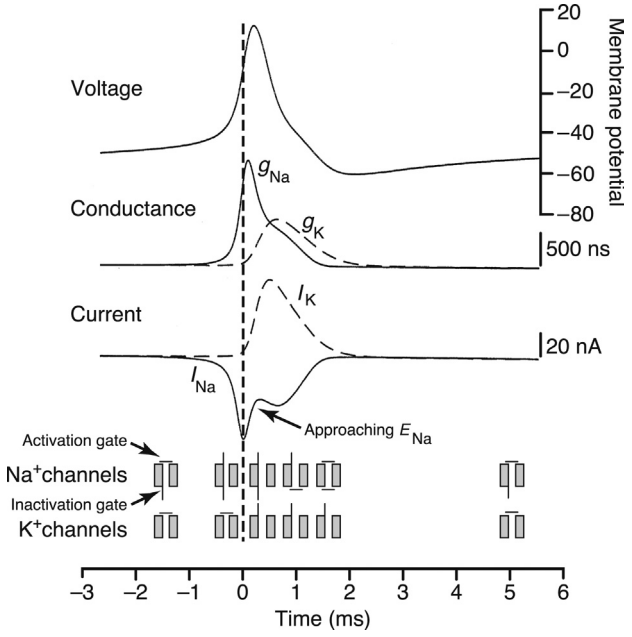

FIGURE 12.7 Generation of the action potential is associated with an increase in membrane Na^+ conductance and Na^+ current followed by an increase in K^+ conductance and K^+ current. Before action potential generation, Na^+ channels are neither activated nor inactivated (illustrated at the bottom of the figure). Activation of the Na^+ channels allows Na^+ ions to enter the cell, depolarizing the membrane potential. This depolarization also activates K^+ channels. After activation and depolarization, the inactivation particle on the Na^+ channels closes and the membrane potential repolarizes. The persistence of the activation of K^+ channels (and other membrane properties) generates an afterhyperpolarization. During this period, the inactivation particle of the Na^+ channel is removed and the K^+ channels close. *From Huguenard and McCormick (1994).*

potential is generated as follows. Depolarization of the membrane potential increases the probability of Na^+ channels being in the activated, but not yet inactivated, state. At a particular membrane potential, the resulting inflow of Na^+ ions tips the balance of the net ionic current from outward to inward (remember that depolarization also increases K^+ and Cl^- currents by moving the membrane potential away from E_K and E_{Cl}). At this membrane potential, known as the action potential threshold (typically about −55 mV), the movement of Na^+ ions into the cell depolarizes the axon and opens more Na^+ channels, causing yet more depolarization of the membrane; repetition of this process yields a rapid, positive feedback loop that brings the axon close to E_{Na}. However, even as more and more Na^+ channels are becoming activated, some of these channels are also inactivating and, therefore, no longer conducting Na^+ ions. In addition, the delayed rectifier K^+ channels also are opening, owing to the depolarization of the membrane potential, and allowing positive charge to exit the cell. At some point, close to the peak of the action potential, the inward movement of Na^+

ions into the cell is exactly offset by the outward movement of K^+ ions out of the cell. After this point, the outward movement of K^+ ions dominates, and the membrane potential is repolarized, corresponding to the fall of the action potential. The persistence of the K^+ current for a few milliseconds following generation of the action potential generates the afterhyperpolarization. During this afterhyperpolarization, which is lengthened by the membrane time constant, the inactivation of the Na^+ channels is removed, preparing the axon for generation of the next action potential (see Fig. 12.7).

The occurrence of an action potential is *not* associated with substantial changes in the intracellular or extracellular concentrations of Na^+ or K^+, as we saw earlier for the generation of the resting membrane potential. For example, the generation of a single action potential in a 25-μm-diameter hypothetical spherical cell should increase the intracellular concentration of Na^+ by only approximately 6 μM (from about 18 to 18.006 mM). Thus, the action potential is an electrical event generated by a change in the distribution of charge across the membrane and not by a marked change in the intracellular or extracellular concentration of Na^+ or K^+.

Action Potentials Typically Initiate in the Axon Initial Segment and Propagate Down the Axon and Backwards through the Dendrites

Neurons have complex morphologies including dendritic arbors, a cell body, and typically one axonal output that branches extensively. In many cells, all of these parts of the neuron are capable of independently generating action potentials. The activity of most neurons is dictated by barrages of synaptic potentials generated at each moment by a variable subset of the thousands of synapses impinging upon the cell's dendrites and soma. Where then is the action potential initiated? In most cells, each action potential is initiated in the initial portion of the axon, known as the axon initial segment (Coombs et al., 1957; Stuart et al., 1997; Shu et al., 2007). The initial segment of the axon has the lowest threshold for action potential generation because it typically contains a moderately high density of Na^+ channels and it is a small compartment which is easily depolarized by the in-rush of Na^+ ions. Once a spike is initiated (e.g., about 30–50 microns down the axon from the cell body in cortical pyramidal cells), this action potential then propagates orthodromically down the axon to the synaptic terminals, where it causes release of transmitter, as well as antidromically back through the cell body and into the cell dendrites, where it can modulate intracellular processes.

Refractory Periods Prevent "Reverberation"

The ability of depolarization to activate an action potential varies as a function of the time since the last generation of an action potential, owing to the inactivation of Na^+ channels and the activation of K^+ channels. Immediately after generation of an action potential, another action potential usually cannot be generated regardless of the amount of current injected into the axon. This period corresponds to the absolute refractory period and is mediated largely by the inactivation of Na^+ channels. The relative refractory period occurs during the action potential afterhyperpolarization and follows the absolute refractory period. The relative refractory period is characterized by a requirement for the increased injection of ionic current into the cell to generate another action potential and results from the persistence of the outward K^+ current. The practical implication of refractory periods is that action potentials are not allowed to "reverberate" between the soma and the axon terminals.

The Speed of Action Potential Propagation is Affected by Myelination

Axons may be either myelinated or unmyelinated. Invertebrate axons or small vertebrate axons are typically unmyelinated, whereas larger vertebrate axons are often myelinated. As described in Chapter 1, sensory and motor axons of the peripheral nervous system are myelinated by specialized cells (Schwann cells) that form a spiral wrapping of multiple layers of myelin around the axon (Fig. 12.8). Several Schwann cells wrap around an axon along its length; between the ends of successive Schwann cells are small gaps (nodes of Ranvier). In the central nervous system, a single oligodendrocyte, a special type of glial cell, typically ensheathes several axonal processes (Bunge, 1968).

In unmyelinated axons, the Na^+ and K^+ channels taking part in action potential generation are distributed along the axon, and the action potential propagates along the length of the axon through local depolarization of each neighboring patch of membrane (see Chapter 17), causing that patch of membrane also to generate an action potential (Fig. 12.8). In myelinated axons, on the other hand, the Na^+ channels are concentrated at the nodes of Ranvier (Ritchie and Rogart, 1977). The generation of an action potential at each node results in the depolarization of the next node and subsequently the generation of an action potential with an internode delay of only about 20 μs, referred to as *saltatory conduction* (from the Latin *saltare*, "to dance"). Growing evidence indicates that, near the nodes of Ranvier and underneath the myelin

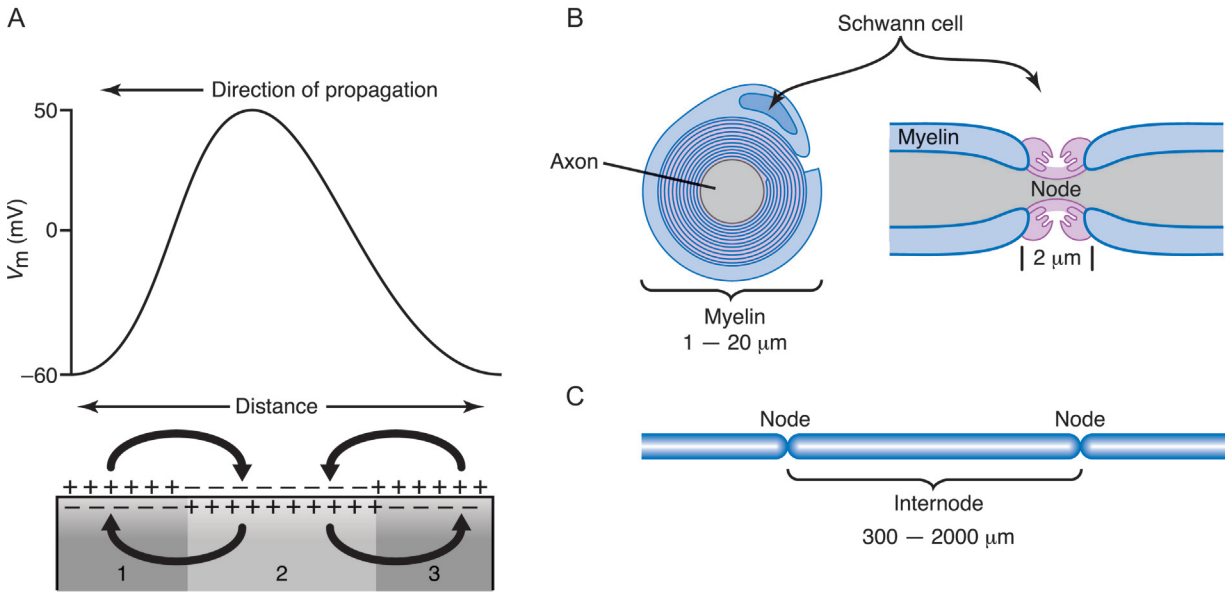

FIGURE 12.8 **Propagation of the action potential in unmyelinated and myelinated axons.** (A) Action potentials propagate in unmyelinated axons through the depolarization of adjacent regions of membrane. In the illustrated axon, region 2 is undergoing depolarization during the generation of the action potential, while region 3 has already generated the action potential and is now hyperpolarized. The action potential will propagate further by depolarizing region 1. (B) Vertebrate myelinated axons have a specialized Schwann cell that wraps around them in many spiral turns. The axon is exposed to the external medium at the nodes of Ranvier (Node). (C) Action potentials in myelinated fibers are regenerated at the nodes of Ranvier, where there is a high density of Na^+ channels. Action potentials are induced at each node through the depolarizing influence of the generation of an action potential at adjacent nodes, thereby increasing the conduction velocity.

covering, K^+ channels may play a role in determining the resting membrane potential and the repolarization of the action potential. A cause of some neurological disorders, such as multiple sclerosis and Guillain–Barré syndrome, is the demyelination of axons, resulting in a block of conduction of action potentials (see Chapter 1).

Ion Channels are Membrane-Spanning Proteins with Water-Filled Pores

The generation of ionic currents useful for the propagation of action potentials requires the movement of significant numbers of ions across the membrane in a relatively short time. The rate of ionic flow during the generation of an action potential is far too high to be achieved by an active transport mechanism and results instead from the opening of ion channels. Although the existence of ionic channels in the membrane has been postulated for decades, their properties and structure have only recently become known in detail. The powerful combination of electrophysiological and molecular techniques, and, most recently, X-ray crystallography, has greatly enhanced the knowledge of the structure–function relations of ionic channels (Catterall, 1995, 2000a,b; Jiang et al., 2002a,b; Yellen, 2002; Gouaux and MacKinnon, 2005) (Box 12.6).

Various neural toxins were particularly useful in the initial isolation of ionic channels. For example, three subunits (α, β1, β2) of the voltage-dependent Na^+ channel were isolated with the use of a derivative of a scorpion toxin (Beneski and Catterall, 1980; Catterall, 2000b). The α-subunit of the Na^+ channel is a large glycoprotein with a molecular mass of 270 kDa, whereas the β1 and β2 subunits are smaller polypeptides of molecular masses 39 and 37 kDa, respectively (Fig. 12.9). The α subunit, of which there are at least nine different isoforms, is the building block of the water-filled pore of the ionic channel, whereas the β subunits have some other role, such as in the regulation or structure of the native channel.

The α subunit of the Na^+ channel contains four internal repetitions (see Fig. 12.9B). Hydrophobicity analysis of these four components reveals that each contains six hydrophobic domains that may span the membrane as an α-helix. Of these six membrane-spanning components, the fourth (S4) has been proposed to be critical to the voltage sensitivity of the Na^+ channels. Voltage-sensitive gating of Na^+ channels is accomplished by the redistribution of ionic charge ("gating charge") in the Na^+ channel (Armstrong, 1992). Positive charges in the S4 region may act as voltage sensors such that an increase in the positivity of the inside of the cell results in a conformational change of the ionic channel. In support of this

BOX 12.6

ION CHANNELS AND DISEASE

Frances M. Ashcroft

Cells cannot survive without functional ion channels. It is therefore not surprising that an ever-increasing number of diseases have been found to be associated with defective ion channel function. There are a number of different mechanisms by which this may occur.

1. Mutations in the coding region of ion channel genes may lead to gain or loss of channel function, either of which may have deleterious consequences. For example, mutations producing enhanced activity of the epithelial Na^+ channel are responsible for Liddle syndrome, an inherited form of hypertension, whereas other mutations in the same protein that cause reduced channel activity give rise to hypotension. The most common inherited disease in Caucasians is also an ion channel mutation. This disease is cystic fibrosis (CF), which results from mutations in the epithelial chloride channel, known as CFTR. The most common mutation, the deletion of a phenylalanine at position 508, results in defective processing of the protein and prevents it from reaching the surface membrane. CFTR regulates chloride fluxes across epithelial cell membranes, and this loss of CFTR activity leads to reduced fluid secretion in the lung, resulting in potentially fatal lung infections.
2. Mutations in the promoter region of the gene may cause under- or overexpression of a given ion channel.
3. Other diseases result from defective regulation of channel activity by cellular constituents or extracellular ligands. This defective regulation may be caused by mutations in the genes encoding the regulatory molecules themselves or defects in the pathways leading to their production. Some forms of maturity-onset diabetes of the young (MODY) may be attributed to such a mechanism. ATP-sensitive potassium (K-ATP) channels play a key role in the glucose-induced insulin secretion from pancreatic β cells, and their defective regulation is responsible for two forms of MODY.
4. Autoantibodies to channel proteins may cause disease by downregulating channel function—often by causing internalization of the channel protein itself. Well-known examples are myasthenia gravis, which results from antibodies to skeletal muscle acetylcholine channels, and Eaton–Lambert myasthenic syndrome, in which patients produce antibodies against presynaptic Ca channels.
5. Finally, a number of ion channels are secreted by cells as toxic agents. They insert into the membrane of the target cell and form large nonselective pores, leading to cell lysis and death. The hemolytic toxin produced by the bacterium *Staphylococcus aureus* and the toxin secreted by the protozoan *Entamoeba histolytica*, which causes amebic dysentery, are examples.

Natural mutations in ion channels have been invaluable in studying the relationship between channel structure and function. In many cases, genetic analysis of a disease has led to cloning of the relevant ion channel. The first K channel to be identified (Shaker), for example, came from the cloning of the gene that caused *Drosophila* to shake when exposed to ether. Likewise, the gene encoding the primary subunit of a cardiac potassium channel (KVLQT1) was identified by positional cloning in families carrying mutations that caused a cardiac disorder known as long QT syndrome (see below). Conversely, the large number of studies on the relationship between Na channel structure and function has greatly assisted our understanding of how mutations in Na channels produce their clinical phenotypes.

Many diseases are genetically heterogeneous, and the same clinical phenotype may be caused by mutations in different genes. Long QT syndrome is a relatively rare inherited cardiac disorder that causes abrupt loss of consciousness, seizures, and sudden death from ventricular arrhythmia in young people. Mutations in three different genes, two types of cardiac muscle K channels (HERG and KVLQT1) and the cardiac muscle sodium channel (SCN1A), give rise to long QT syndrome. The disorder is characterized by a long QT interval in the electrocardiogram, which reflects the delayed repolarization of the cardiac action potential. As might therefore be expected, the mutations in the cardiac Na channel gene that cause long QT syndrome enhance the Na current (by reducing Na channel inactivation), while those in the potassium channel genes cause loss of function and reduce the K current.

Mutations in many different types of ion channels have been shown to cause human diseases. In addition to the examples listed above, mutations in water channels cause nephrogenic diabetes insipidus; mutations in gap junction channels cause Charcot–Marie–Tooth disease (a form of peripheral neuropathy) and hereditary deafness; mutations in the skeletal muscle Na channel cause a range of disorders known as the periodic

BOX 12.6 *(continued)*

paralyses; mutations in intracellular Ca-release channels cause malignant hyperthermia (a disease in which inhalation anesthetics trigger a potentially fatal rise in body temperature); and mutations in neuronal voltage-gated Ca channels cause migraine and episodic ataxia. The list increases daily. As is the case with all single-gene disorders, the frequency of these diseases in the general population is very low. However, the insight they have provided into the relationship between ion channel structure and function, and into the physiological role of the different ion channels, has been invaluable. As William Harvey said in 1657, "nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual form of nature, by careful investigation of the rarer forms of disease."

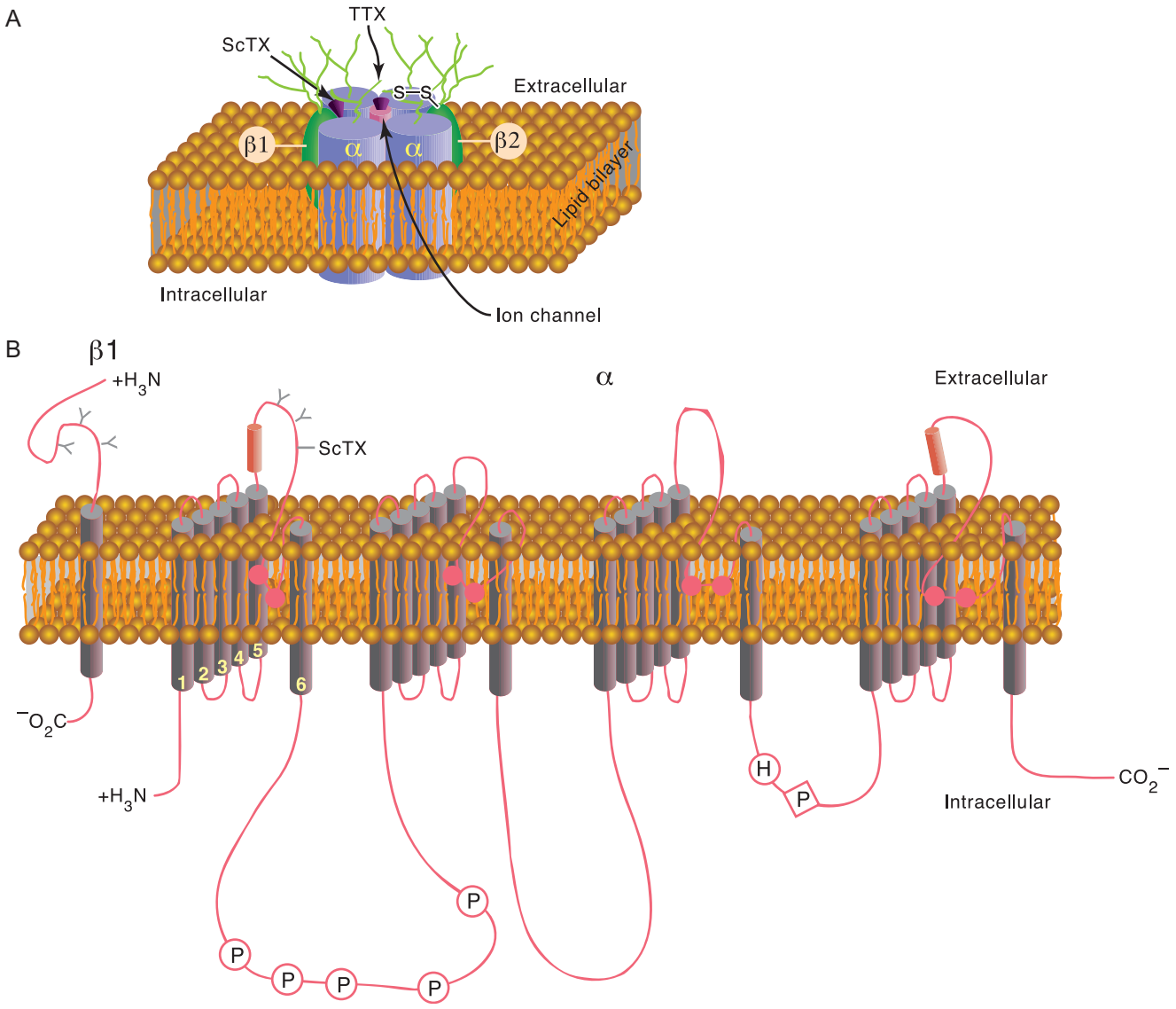

FIGURE 12.9 **Structure of the sodium channel.** (A) Cross section of a hypothetical sodium channel consisting of a single transmembrane α subunit in association with a β1 subunit and a β2 subunit. The α subunit has receptor sites for α-scorpion toxin (ScTX) and tetrodotoxin (TTX). (B) Primary structures of α and β1 subunits of sodium channel illustrated as transmembrane folding diagrams. Cylinders represent probable transmembrane α-helices.

hypothesis, site-directed mutagenesis of the S4 region of the Na^+ channel to reduce the positive charge of this portion of the pore also reduces the voltage sensitivity of activation of the ionic channel (Catterall, 2000b).

The mechanisms of inactivation of ionic channels have been analyzed with a combination of molecular and electrophysiological techniques. The most convincing hypothesis is that inactivation is achieved by a block of the inner mouth of the aqueous pore. Ionic channels are inactivated without detectable movement of ionic current through the membrane; thus inactivation is probably not directly gated by changes in the membrane potential alone. Rather, inactivation may be triggered or facilitated as a secondary consequence of activation. Site-directed mutagenesis or the use of antibodies has shown that the part of the molecule between regions III and IV may be allowed to move to block the cytoplasmic side of the ionic pore after the conformational change associated with activation (Vassilev et al., 1988, 1989; Stuhmer et al., 1989). Additional information on the molecular properties of voltage-gated ion channels is provided in Chapter 13.

Neurons of the Central Nervous System Exhibit a Wide Variety of Electrophysiological Properties

The first intracellular recordings of action potentials in mammalian neurons by Sir John Eccles and colleagues revealed a remarkable similarity to those of the squid giant axon and gave rise to the assumption that the electrophysiology of neurons in the CNS was really rather simple: when synaptic potentials brought the membrane potential positive to action potential threshold, action potentials were produced through an increase in Na^+ conductance followed by an increase in K^+ conductance, as in the squid giant axon. The assumption, therefore, was that the complicated patterns of activity generated by the brain during the resting, sleeping, or active states were brought about as an interaction of the very large numbers of neurons present in the mammalian CNS (Brock et al., 1952; Eccles, 1957). However, intracellular recordings of invertebrate neurons revealed that different cell types exhibit a wide variety of different electrophysiological behaviors, indicating that neurons may be significantly more complicated than the squid giant axon (Arvanitaki and Chalazonitis, 1961; Alving, 1968; Jackelet, 1989). Elucidation of the basic electrophysiology and synaptic physiology of different types of neurons and neuronal pathways within the mammalian CNS was facilitated by the *in vitro* slice technique, in which thin ($\sim$0.5 mm) slices of brain can be maintained for several hours. Intracellular recordings from identified cells revealed that neurons of the mammalian nervous system, similar to those of invertebrate networks, can generate complex patterns of action potentials entirely through intrinsic ionic mechanisms and without synaptic interaction with other cell types. For example, Rodolfo Llinás and colleagues discovered that Purkinje cells of the cerebellum can generate high-frequency ($>$200 Hz) trains of Na^+- and K^+-mediated action potentials interrupted by Ca^{2+} spikes in the dendrites (Llinás and Sugimori, 1980a,b), whereas a major afferent to these neurons, the inferior olivary cell, can generate rhythmic sequences of broad action potentials only at low frequencies ($<$15 Hz) through an interaction between various Ca^{2+}, Na^+, and K^+ conductances (Llinás and Yarom, 1981a,b) (Fig. 12.10). These *in vitro* recordings confirmed a major finding obtained with earlier intracellular recordings *in vivo*: each morphologically distinct class of neuron in the brain exhibits distinct electrophysiological features (Llinás, 1988). Just as cortical pyramidal cells are morphologically distinct from cerebellar Purkinje cells, which are distinct from thalamic relay cells, the electrophysiological properties of each of these different cell types also are markedly distinct.

Although no uniform classification scheme has been formulated in which all the different types of neurons of the brain can be classified, a few characteristic patterns of activity seem to recur. The first general class of action potential generation is characterized by those cells that generate trains of action potentials one spike at a time. The more prolonged the depolarization of these cells, the more prolonged their discharge. The more intensely these cells are depolarized, the higher the frequency of action potential generation. This type of relatively linear behavior is typical of brainstem and spinal cord motor neuron functioning in muscle contraction. A modification of this basic pattern of "regular firing" is characterized by the generation of trains of action potentials that exhibit a marked tendency to slow down in frequency with time, a process known as spike frequency adaptation. Examples of cells that discharge in this manner are cortical and hippocampal pyramidal cells (Madison and Nicoll, 1984; McCormick et al., 1985; Pennefather et al., 1985).

In addition to these regular firing cells, many neurons in the central nervous system exhibit the intrinsic propensity to generate rhythmic bursts of action potentials (Fig. 12.10) (see also Chapter 14). Examples of such neurons are thalamic relay neurons, inferior olivary neurons, and some types of cortical and hippocampal pyramidal cells (Llinás and Yarom, 1981a,b; Jahnsen and Llinás, 1984a,b; Wang and McCormick, 1993). In these cells, clusters of action potentials can occur together when the membrane is brought above firing threshold. These clusters of action potentials are

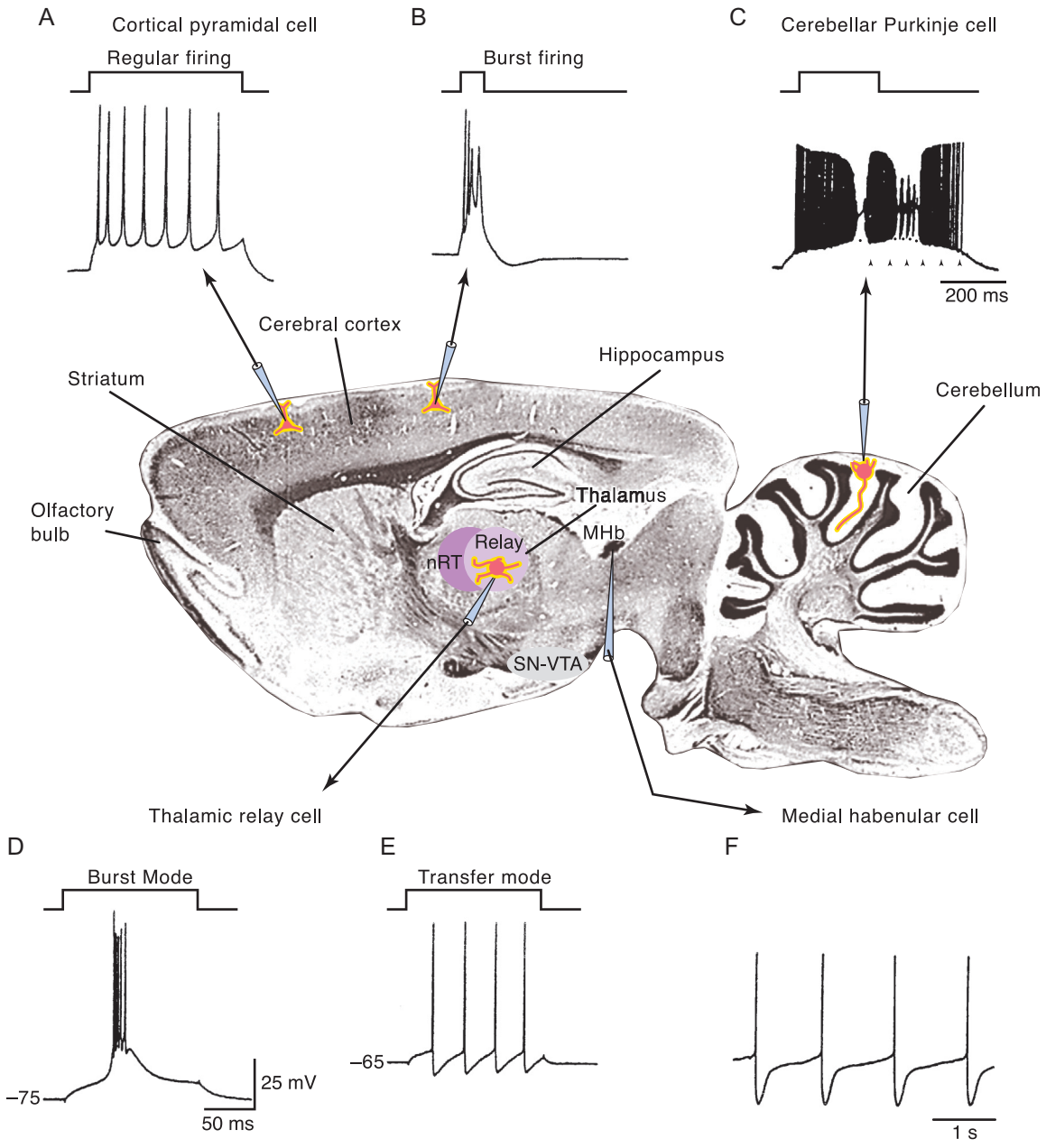

FIGURE 12.10 **Neurons in the mammalian brain exhibit widely varying electrophysiological properties.** (A) Intracellular injection of a depolarizing current pulse in a cortical pyramidal cell results in a train of action potentials that slow down in frequency. This pattern of activity is known as "regular firing." (B) Some cortical cells generate bursts of three or more action potentials, even when depolarized only for a short period. (C) Cerebellar Purkinje cells generate high-frequency trains of action potentials in their cell bodies that are disrupted by the generation of Ca^{2+} spikes in their dendrites. These cells can also generate "plateau potentials" from the persistent activation of Na^{+} conductances (arrowheads). (D) Thalamic relay cells may generate action potentials either as bursts (D) or as tonic trains (E) of action potentials owing to the presence of a large low-threshold Ca^{2+} current. (F) Medial habenular cells generate action potentials at a steady and slow rate, in a "pacemaker" fashion.

typically generated through the activation of specialized Ca^{2+} currents that, through their slower kinetics, allow the membrane potential to be depolarized for a sufficient period to result in the generation of a burst of regular, Na^{+}- and K^{+}-dependent action potentials (discussed in the next section).

Yet another general category of neurons in the brain comprises cells that generate relatively short duration (<1 ms) action potentials and can discharge at relatively high frequencies (>300 Hz). Such electrophysiological properties are often found in neurons that release the inhibitory amino acid γ-aminobutyric acid

TABLE 12.2 Neuronal Ionic Currents

Current	Description	Function
Na^+ currents		
*I*Na,t	Transient; rapidly activating and inactivating	Action potentials
*I*Na,p	Persistent; noninactivating	Enhances depolarization; contributes to steady-state firing
Ca^{2+} currents		
*I*T, low threshold	Transient; rapidly inactivating; threshold negative to −65 mV	Underlies rhythmic burst firing
*I*L, high threshold	Long-lasting; slowly inactivating; threshold around −20 mV	Underlies Ca^{2+} spikes that are prominent in dendrites; involved in synaptic transmission
*I*N	Neither; rapidly inactivating; threshold around −20 mV	Underlies Ca^{2+} spikes that are prominent in dendrites; involved in synaptic transmission
*I*P	Purkinje; threshold around −50 mV	
K^+ currents		
*I*K	Activated by strong depolarization	Repolarization of action potential
*I*C	Activated by increases in $[Ca^{2+}]i$	Action potential repolarization and interspike interval
*I*AHP	Slow afterhyperpolarization; sensitive to increases in $[Ca^{2+}]i$	Slow adaptation of action potential discharge; the block of this current by neuromodulators enhances neuronal excitability
*I*A	Transient; inactivating	Delayed onset of firing; lengthens interspike interval; action potential repolarization
*I*M	Muscarine sensitive; activated by depolarization; noninactivating	Contributes to spike frequency adaptation; the block of this current by neuromodulators enhances neuronal excitability
*I*h	Depolarizing (mixed cation) current that is activated by hyperpolarization	Contributes to rhythmic burst firing and other rhythmic activities
*I*K,leak	Contributes to neuronal resting membrane potential	Block of this current by neuromodulators can result in a sustained change in membrane potential

(Llinás and Sugimori, 1980a,b) (see Fig. 12.10) including some types of interneurons in the cerebral cortex, thalamus, and hippocampus (Schwartzkroin and Mathers, 1978; McCormick et al., 1985; Pape and McCormick, 1995). Finally, the last general category of neurons consists of those that spontaneously generate action potentials at relatively low frequencies (e.g., 1−10 Hz). This type of electrophysiological behavior is often associated with neurons that release neuromodulatory transmitters, such as acetylcholine, norepinephrine, serotonin, and histamine (Vandermaelen and Aghajanian, 1983; Williams et al., 1984; Reiner and McGeer, 1987). Neurons that release these neuromodulatory substances often innervate wide regions of the brain and appear to set the "state" of the different neural networks of the CNS, in a manner similar to the modulation of the different organs of the body by the sympathetic and parasympathetic nervous systems (McCormick, 1992; Steriade and McCarley, 2005).

Each of these unique intrinsic patterns of activity in the nervous system is due to the presence of a distinct mixture and distribution of different ionic currents in the cells. As in the classic studies of the squid giant axon, these different ionic currents have been characterized, at least in part, with voltage-clamp and pharmacological techniques, and the basic electrophysiological properties have been replicated with computational simulations (Belluzzi and Sacchi, 1991; McCormick and Huguenard, 1992; Huguenard and McCormick, 1994) (see Figs. 12.7 and 12.12).

Neurons have Multiple Active Conductances

The search for the electrophysiological basis of the varying intrinsic properties of different types of neurons of vertebrates and invertebrates revealed a wide variety of ionic currents. Each type of ionic current is characterized by several features: (1) the type of ions conducted by the underlying ionic channels (e.g., Na^+, K^+, Ca^{2+}, Cl^-, or mixed cations), (2) their voltage and time dependence, and (3) their sensitivity to second messengers. In vertebrate neurons, two distinct Na^+ currents have been identified and six distinct Ca^{2+} currents and more than seven distinct K^+ currents are known (Table 12.2 and

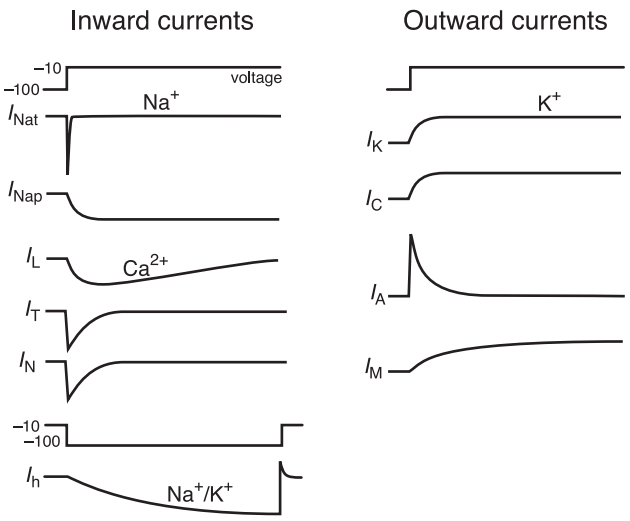

FIGURE 12.11 **Voltage dependence and kinetics of different ionic currents in the mammalian brain.** Depolarization of the membrane potential from −100 to −10 mV results in the activation of currents entering or leaving neurons.

Fig. 12.11). This is a minimal number, as these currents are formed from a much greater pool of channel subunits. The following sections briefly review these classes of ionic currents and their ionic channels, relating them to the different patterns of behavior mentioned earlier for neurons in the mammalian CNS.

Na^+ Currents are Both Transient and Persistent

Depolarization of many different types of vertebrate neurons results not only in the activation of the rapidly activating and inactivating Na^+ current (I_{Nat}) underlying action potential generation but also in the rapid activation of a Na^+ current that does not inactivate and is therefore known as the "persistent" Na^+ current (I_{Nap}) (Hotson et al., 1979; Stafstrom et al., 1982; Llinás, 1988; Alzheimer et al., 1993). The threshold for activation of the persistent Na^+ current is typically about −65 mV, that is, below the threshold for the generation of action potentials. This property gives this current the interesting ability to enhance or facilitate the response of the neuron to depolarizing, yet subthreshold, inputs. For example, synaptic events that depolarize the cell activate I_{Nap}, resulting in an extra influx of positive charge and therefore a larger depolarization than otherwise would occur. Likewise, hyperpolarizations may result in deactivation of I_{Nap}, again resulting in larger hyperpolarizations than would otherwise occur. In this manner, the persistent Na^+ current may play an important regulatory role in the control of the functional responsiveness of the neuron to synaptic inputs and may contribute to the dynamic coupling of the dendrites to the soma.

Persistent activation of I_{Nap} may also contribute to another electrophysiological feature of neurons, namely, the generation of plateau potentials (Llinás, 1988). A plateau potential refers to the ability of many different types of neurons to generate, through intrinsic ionic mechanisms, a prolonged (from tens of milliseconds to seconds) depolarization and action potential discharge in response to a short-lasting depolarization (see Fig. 12.10C). One can wonder whether such plateau potentials contribute to persistent firing in neurons during the performance of visual memory tasks, as has been found in some types of neurons in the prefrontal neocortex and superior colliculus of behaving primates (Goldman-Rakic, 1995).

K^+ Currents Vary in Their Voltage Sensitivity and Kinetics

Potassium currents that contribute to the electrophysiological properties of neurons are numerous and exhibit a wide range of voltage-dependent and kinetic properties (Jan and Jan, 1990; Storm, 1990; Johnston and Wu, 1995; Coetzee et al., 1999; Yellen, 2002) (see also Chapter 13 for additional details). Perhaps the simplest K^+ current is that characterized by Hodgkin and Huxley: this K^+ current, I_K, rapidly activates on depolarization and does not inactivate (see Fig. 12.11). Other K^+ currents activate with depolarization but also inactivate with time. For example, the rapid activation and inactivation of I_A give this current a transient appearance (see Fig. 12.11), and I_A is believed to be important in controlling the rate of action potential generation, particularly at low frequencies (Connor and Stevens, 1971a,b) (Fig. 12.12). Like the Na^+ channel, I_A channels are inactivated by the plugging of the inner mouth of the pore through the movement of an inactivation particle (Hoshi et al., 1990; Zagotta et al., 1990; Yellen, 2002).

Another broad class of K^+ channel consists of those that are sensitive to changes in the intracellular concentration of Ca^{2+} (Blatz and Magleby, 1987; Latorre et al., 1989). These K^+ currents are collectively referred to as I_{KCa}, with two examples being the K^+ currents that underlie slow afterhyperpolarizations following repetitive action potential discharge, I_{AHP} and a fast K^+ current that helps repolarize action potentials, I_C (see Fig. 12.11). Still other K^+ channels are not only activated by voltage but also modulated by activation of various modulatory neurotransmitter receptors, such as the M current (see Fig. 12.11). By investigating the ionic mechanisms by which the release of acetylcholine from preganglionic neurons in the brain results in prolonged changes in the excitability of neurons of the sympathetic ganglia, Brown and Adams (1980)

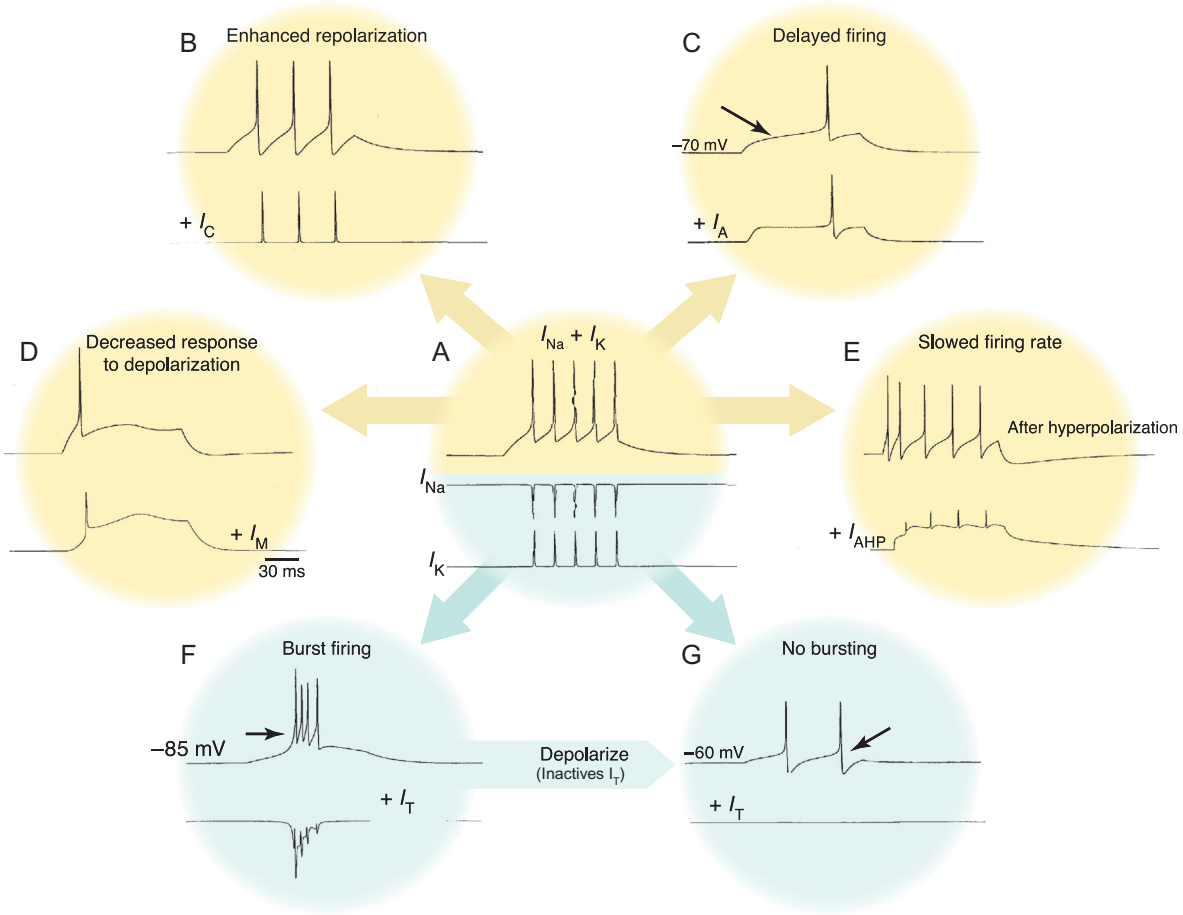

FIGURE 12.12 **Simulation of the effects of the addition of various ionic currents to the pattern of activity generated by neurons in the mammalian CNS.** (A) The repetitive impulse response of the classic Hodgkin–Huxley model (voltage recordings above, current traces below). With only *I*Na and *I*K, the neuron generates a train of five action potentials in response to depolarization. Addition of *I*C (B) enhances action potential repolarization. Addition of *I*A (C) delays the onset of action potential generation. Addition of *I*M (D) decreases the ability of the cell to generate a train of action potentials. Addition of *I*AHP (E) slows the firing rate and generates a slow afterhyperpolarization. Finally, addition of the transient Ca^{2+} current *I*T results in two states of action potential firing: (F) burst firing at −85 mV and (G) tonic firing at −60 mV. *From Huguenard and McCormick (1994).*

discovered a unique K^+ current (I_M) that slowly (over tens of milliseconds) turns on with depolarization of the neuron (see Fig. 12.12D). The slow activation of this K^+ current results in a decrease in the responsiveness of the cell to depolarization and, therefore, regulates how the cell responds to excitation. This K^+ current, like I_{AHP}, is reduced by the activation of a wide variety of receptors, including muscarinic receptors, for which it is named. Reduction of I_M results in a marked increase in responsiveness of the affected cell to depolarizing inputs and again may contribute to the mechanisms by which neuromodulatory systems control the state of activity in cortical and hippocampal networks (Nicoll, 1988; Nicoll et al., 1990; McCormick, 1992).

Between these classic examples of K^+ currents are a variety of other types that have not been fully characterized, including K^+ currents that vary from one another in their voltage sensitivity, kinetics, and response to various second messengers. Molecular biological studies of voltage-sensitive K^+ channels, first done in *Drosophila* and later in mammals, have revealed a large number of genes that generate K^+ channels. The voltage-gated K^+ channel subunits are contained within nine distinct subfamilies: Kv1–9 (reviewed in Coetzee et al., 1999). These genes generate a wide variety of different K^+ channels due not only to the large number of genes involved, but also to alternative RNA splicing, gene duplication, and other post-translational mechanisms. Functional expression of different K^+ channels reveals remarkable variation in the rate of inactivation, such that some are rapidly inactivating (A-current-like), whereas others inactivate more slowly. Finally, some K^+ channels do not inactivate, such as I_K. One of the largest subfamilies of K^+ channels comprises those that give rise to the resting membrane potential, so-called "leak channels."

Interestingly, these channels appear to be opened by gaseous anesthetics, indicating that the hyperpolarization of central neurons is a major component of general anesthesia. MacKinnon and colleagues have succeeded in crystallizing different types of K^+ channels, leading to a great leap in our knowledge of their structure and how they function, including the mechanisms by which channels are opened by voltage and ligands (Jiang et al., 2002a,b; Gouaux and MacKinnon, 2005). It is now clear that each type of neuron in the nervous system is likely to contain a unique set of functional voltage-sensitive K^+ channels, perhaps selected, modified, and placed in particular spatial locations in the cell in a manner to facilitate the unique role of that cell type in neuronal processing.

Ca^{2+} Currents Control Electrophysiological Properties and Ca^{2+}-Dependent Second-Messenger Systems

Ionic channels that conduct Ca^{2+} are present in all neurons. These channels are special in that they serve two important functions. First, Ca^{2+} channels are present throughout the different parts of the neuron (dendrites, soma, synaptic terminals) and contribute greatly to the electrophysiological properties of these processes (Llinás, 1988; Regehr and Tank, 1994; Johnston et al., 1996). Second, Ca^{2+} channels are unique in that Ca^{2+} is an important second messenger in neurons, and entry of Ca^{2+} into the cell can affect numerous physiological functions, including neurotransmitter release, synaptic plasticity, neurite outgrowth during development, and even gene expression.

On the basis of their voltage sensitivity, their kinetics of activation and inactivation, and their ability to be blocked by various pharmacological agents, Ca^{2+} currents can be separated into at least four distinct categories, three of which are I_T ("transient"), I_L ("long lasting"), and I_N ("neither") (Carbonne and Lux, 1984; Nowycky et al., 1985), illustrated in Fig. 12.11A. A fourth, I_P, is found in the Purkinje cells of the cerebellum, as well as in many different cell types of the CNS (Llinás et al., 1992) Calcium channels are formed from at least 10 different α subunits as well as a variety of β and γ subunits, indicating that an even greater number of Ca^{2+} currents are yet to be characterized (Tsien et al., 1991; Ertel et al., 2000).

Neurons Possess Multiple Subtypes of High-Threshold Ca^{2+} Currents

High-voltage-activated Ca^{2+} channels are activated at membrane potentials more positive than approximately -40 mV and include the currents I_L, I_N, and I_P. The L-type calcium currents exhibit a high threshold for activation (about -10 mV) and give rise to rather persistent, or long-lasting, ionic currents (see Fig. 12.11A). Dihydropyridines, Ca^{2+} channel antagonists, are clinically useful for their effects on the heart and vascular smooth muscle (e.g., for the treatment of arrhythmias, angina, and migraine headaches) and selectively block L-type Ca^{2+} channels (Stea et al., 1985; Bean, 1989). In contrast with I_L, I_N is not blocked by dihydropyridines; rather it is selectively blocked by a toxin found in Pacific cone shells (ω-conotoxin-GVIA). The N-type Ca^{2+} channels have a threshold for activation of about -20 mV, inactivate with maintained depolarization, and are modulated by a variety of neurotransmitters. In some cell types, I_N has a role in the Ca^{2+}-dependent release of neurotransmitters at presynaptic terminals (Wheeler et al., 1994). The P-type calcium channel is distinct from N and L types in that it is not blocked by either dihydropyridines or ω-conotoxin-GVIA but is blocked by a toxin (ϖ-agatoxin-IVA) present in the venom of the funnel web spider (Llinás et al., 1992; Stea et al., 1995). This type of calcium channel activates at relatively high thresholds and does not inactivate. Prevalent in Purkinje cells as well as other cell types, as mentioned earlier, the P-type Ca^{2+} channel participates in the generation of dendritic Ca^{2+} spikes, which can strongly modulate the firing pattern of the neuron in which it resides (see Fig. 12.10C).

Collectively, the high-threshold-activated Ca^{2+} channels contribute to the generation of action potentials in mammalian neurons. The activation of Ca^{2+} currents adds somewhat to the depolarizing part of the action potential, but, more importantly, these channels allow Ca^{2+} to enter the cell and this has the secondary consequence of activation of various Ca^{2+}-activated K^+ currents (Latorre et al., 1989) and protein kinases (see Chapters 4 and 18). As mentioned earlier, the activation of these K^+ currents modifies the pattern of action potentials generated in the cell (see Figs. 12.10 and 12.12). High-threshold Ca^{2+} channels are similar to the Na^+ channel in that they are composed of a central $\alpha 1$ subunit that forms the aqueous pore and several regulatory or auxiliary subunits. As in the Na^+ channel, the primary structure of the $\alpha 1$ subunit of the Ca^{2+} channel consists of four homologous domains (I–IV), each containing six regions (S1–S6) that may generate transmembrane α-helices. Genes for at least 10 different Ca^{2+} channel α subunits have been cloned and are separated into three families (CaV1, CaV2, CaV3). The properties of the products of these genes indicate that I_L is likely to correspond to the CaV1 subfamily, whereas I_N corresponds to CaV2.2 and I_T is formed by the CaV3 subfamily (Bean, 1989; Catterall, 2000a).

BOX 12.7

JELLYFISH—WHAT A NERVE!

Robert W. Meech

Research on jellyfish provides intriguing insight into how the properties and distribution of ion channels within a nerve membrane can affect the behavior of the whole animal. *Aglantha digitale* can swim slowly when feeding or quickly if escaping predators just through the "behavior" of a single muscle sheet coupled to a simply organized nervous system.

The jellyfish does this through an unusual form of signaling. Each "giant" motor nerve axon not only has voltage-dependent sodium channels and three types of potassium channels, but also crucial T-type calcium channels. *Aglantha* motor axons are unusual because they develop two entirely different propagating action potentials (Mackie and Meech, 1985). The T-type calcium channels contribute to a low-amplitude calcium-dependent spike that propagates along the motor axon without gaining amplitude or decrementing in the way that electrotonic potentials do (Meech and Mackie, 1995). The motor axon makes direct synaptic contact with the muscle epithelium that makes up the bell of the jellyfish and so the propagating calcium spike induces the weak contractions responsible for propulsion during the regular slow swimming the animal performs when feeding.

Aglantha lives in the colder waters of the world at a depth of about 100 m. Studied in their natural habitat, they are seen to avoid predators by generating an altogether stronger form of swimming. In the laboratory this "escape" swimming can be reproduced by stimulating vibration-sensitive receptors at the base of the bell of the animal (Arkett et al., 1988). The strong synaptic depolarization that this stimulus induces in each of the eight giant motor axons drives its membrane potential beyond the peak of the calcium spike and induces a full-sized sodium action potential. As the sodium spike propagates more rapidly than the slow swim calcium spike, there is a coordinated contraction of the body wall that drives the animal forward.

Sodium and calcium spikes like those seen in *Aglantha* have been recorded from a variety of sites in the mammalian CNS (Llinás and Yarom, 1981b; Llinás and Jahnsen, 1982). However, unlike in *Aglantha*, the peak of the calcium spike always exceeds the threshold of the sodium spike and the two impulses form a single complex signal. Patch-clamp analysis of *Aglantha* axons has revealed a family of potassium channels that are responsible for setting thresholds and repolarizing each of the two different impulses. Each potassium channel class has an identical unitary conductance and appears to be organized in a mosaic fashion over the surface of the axon (Meech and Mackie, 1993). Sodium and T-type calcium channels are clustered together into well-defined "hot spots." George Mackie and I have suggested that the mosaic organization facilitates the turnover of ion channels; channels inserted into the membrane in clusters age together and are eliminated together.

Low-Threshold Ca^{2+} Currents Generate Bursts of Action Potentials

Low-threshold Ca^{2+} currents (see Fig. 12.11A) often take part in the generation of rhythmic bursts of action potentials (see Figs. 12.10 and 12.12). The low-threshold Ca^{2+} current is characterized by a threshold for activation of about -65 mV, which is below the threshold for generation of typical Na^+–K^+-dependent action potentials (-55 mV). This current inactivates with maintained depolarization. Owing to these properties, the role of low-threshold Ca^{2+} currents differs markedly from that of the high-threshold Ca^{2+} currents. Through activation and inactivation of the low-threshold Ca^{2+} current, neurons can generate slow (about 100 ms) Ca^{2+} spikes, which can result, owing to their prolonged duration, in the generation of a high-frequency "burst" of short-duration Na^+–K^+ action potentials (see Fig. 12.10 and Box 12.7) (Llinás and Jahnsen, 1982).

In the mammalian brain, this pattern is especially well exemplified by the activity of thalamic relay neurons; in the visual system, these neurons receive direct input from the retina and transmit this information to the visual cortex. During periods of slow wave sleep, the membrane potential of these relay neurons is relatively hyperpolarized, resulting in the removal of inactivation (deinactivation) of the low-threshold Ca^{2+} current. This deinactivation allows these cells to spontaneously generate low-threshold Ca^{2+} spikes and bursts of from two to five action potentials (Fig. 12.13) (McCormick and Pape, 1990). The large number of thalamic relay cells bursting during sleep in part gives rise to the spontaneous synchronized activity that early investigators were so surprised to find during recordings from the brains of sleeping animals (Steriade

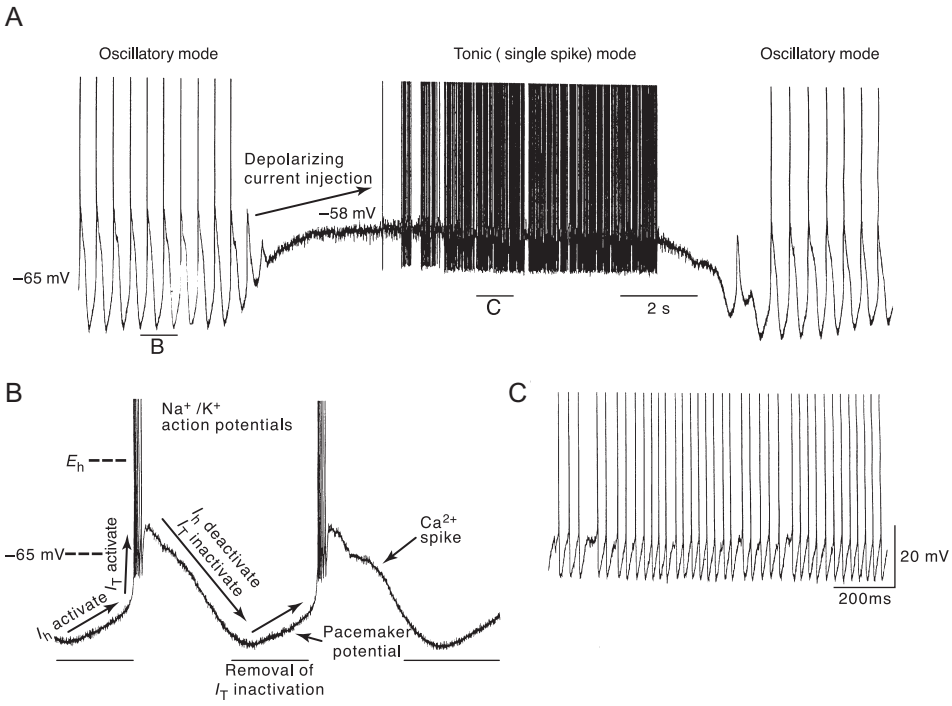

FIGURE 12.13 Two different patterns of activity generated in the same neuron, depending on membrane potential. (A) The thalamic neuron spontaneously generates rhythmic bursts of action potentials owing to the interaction of the Ca^{2+} current I_T and the inward "pacemaker" current I_h. Depolarization of the neuron changes the firing mode from rhythmic burst firing to tonic action potential generation in which spikes are generated one at a time. Removal of this depolarization reinstates the rhythmic burst firing. This transition from rhythmic burst firing to tonic activity is similar to that which occurs in the transition from sleep to waking. (B) Expansion of detail of rhythmic burst firing. (C) Expansion of detail of tonic firing. *From McCormick and Pape (1990).*

et al., 1993). It has even proved possible to maintain one of the sleep-related brain rhythms (spindle waves) intact in slices of thalamic tissue maintained *in vitro*, owing to the generation of this rhythm by the interaction of a local network of thalamic cells and their electrophysiological properties (von Krosigk et al., 1993).

The transition to waking or the period of sleep when vivid dreams are prevalent (rapid eye movement sleep) is associated with a maintained depolarization of thalamic relay cells to membrane potentials ranging from about −60 to −55 mV. The low-threshold Ca^{2+} current is inactivated and therefore the burst discharges are abolished. In this way, the properties of a single ionic current (I_T) help to explain in part the remarkable changes in brain activity taking place in the transition from sleep to waking (Fig. 12.13).

Low-threshold Ca^{2+} channels were recently cloned and shown to have some similarities to other Ca^{2+} channels (Perez-Reyes et al., 1998). Evidence suggests that some antiepileptic drugs may exert their therapeutic actions through a reduction in I_T. This is especially true of the drugs useful in the treatment of generalized absence (petit mal) seizures, which are known to rely on the thalamus for their generation (Coulter et al., 1990).

Hyperpolarization-Activated Ionic Currents are Involved in Rhythmic Activity

In most types of neurons, hyperpolarization negative to approximately −50 mV activates an ionic current, known as I_h, that conducts both Na^+ and K^+ ions (see Fig. 12.11A). This current typically has very slow kinetics, turning on with a time constant on the order of tens of milliseconds to seconds. Because the channels underlying this current allow the passage of both Na^+ and K^+ ions, the reversal potential of I_h is typically about −35 mV—between E_{Na} and E_K. Because this current is activated by hyperpolarization below approximately −60 mV, it is typically dominated by the inward movement of Na^+ ions and is therefore depolarizing. For what purpose could neurons use a depolarizing current that activates when the cell is hyperpolarized? A clue comes from cardiac cells in which this current, known as I_f for "funny," is important for determining heart rate (DiFrancesco, 2005). Activation of I_f results in a slow depolarization of the membrane potential between adjacent cardiac action potentials. The more that I_f is activated, the faster the membrane depolarizes between beats and, therefore, the sooner the threshold for the next action potential is reached and the next beat is generated. In this manner, the amplitude, or sensitivity to voltage, of I_f can modify heart rate. Interestingly, the sensitivity of I_f to voltage is adjusted by the release of noradrenaline and acetylcholine; the activation of β-adrenoceptors by noradrenaline increases I_f and therefore increases the heart rate, whereas the activation of muscarinic receptors decreases I_f, thereby decreasing the heart rate (DiFrancesco et al., 1989; DiFrancesco, 2005). This continual adjustment of I_f results from a "push–pull" arrangement between adrenergic and muscarinic cholinergic receptors and is

mediated by the adjustment of intracellular levels of cyclic AMP. Indeed, the cloning of H channels revealed that their structure is similar to that of cyclic nucleotide-gated channels (Ludwig et al., 1998).

Could I_h play a role in neurons similar to that of I_f in the heart? Possibly. Synchronized rhythmic oscillations in the membrane potential of large numbers of neurons, in some respects similar to those of the heart, are characteristic of the mammalian brain. Oscillations of this type are particularly prevalent in thalamic relay neurons during some periods of sleep, as mentioned earlier. Intracellular recordings from these thalamic neurons reveal that they often generate rhythmic "bursts" of action potentials mediated by the activation of a slow spike that is generated through the activation of the low-threshold, or transient, Ca^{2+} current, I_T (McCormick and Pape, 1990; McCormick and Huguenard, 1992) (see Fig. 12.13). Between the low-threshold Ca^{2+} spikes is a slowly depolarizing membrane potential generated by activation of the mixed Na^+–K^+ current I_h, as with I_f in the heart. The amplitude, or voltage sensitivity, of I_h controls the rate at which the thalamic cells oscillate, and, as with the heart, this sensitivity is adjusted by the release of modulatory neurotransmitters (see Fig. 12.13). In a sense, the thalamic neurons are "beating" in a manner similar to that of the heart.

Summary

An action potential is generated by the rapid influx of Na^+ ions followed by a slightly slower efflux of K^+ ions. Although the generation of an action potential does not disrupt the concentration gradients of these ions across the membrane, the movement of charge is sufficient to generate a large and brief deviation in the membrane potential. Action potentials are typically initiated in the axon initial segment and the propagation of the action potential along the axon allows communication of the output of the cell to its distal synapses. Neurons possess many different types of ionic channels in their membranes, allowing complex patterns of action potentials to be generated and complex computations to occur within single neurons.

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