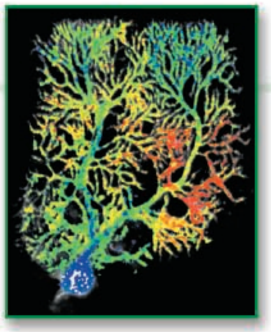

Chapter 3

Voltage-Dependent Membrane Permeability

Overview

The action potential is the primary electrical signal generated by nerve cells and arises from changes in the permeability of the nerve cell's axonal membranes to specific ions. Present understanding of these changes in ionic permeability is based on evidence obtained by the voltage clamp technique, which permits detailed characterization of permeability changes as a function of membrane potential and time. For most types of axons, these changes consist of a rapid and transient rise in sodium ion (Na^+) permeability, followed by a slower but more prolonged rise in permeability to potassium ions (K^+). Both permeabilities are voltage-dependent, increasing as the membrane potential depolarizes. The kinetics and voltage dependence of Na^+ and K^+ permeabilities provide a complete explanation of action potential generation. Depolarizing the membrane potential to the threshold level causes a rapid, self-sustaining increase in Na^+ permeability that produces the rising phase of the action potential; however, the Na^+ permeability increase is short-lived and is followed by a slower increase in K^+ permeability that restores the membrane potential to its usual negative resting level. A mathematical model that describes the behavior of these ionic permeabilities predicts virtually all of the observed properties of action potentials. Importantly, this same ionic mechanism permits action potentials to be propagated along the length of neuronal axons, explaining how electrical signals are conveyed throughout the nervous system.

Ionic Currents Across Nerve Cell Membranes

The previous chapter introduced the idea that nerve cells generate electrical signals by virtue of a membrane that is differentially permeable to various ion species. In particular, a transient increase in the permeability of the neuronal membrane to Na^+ initiates the action potential. This chapter considers exactly how this increase in Na^+ permeability occurs. A key to understanding this phenomenon is the observation that action potentials are initiated *only* when the neuronal membrane potential becomes more positive than a threshold level. This observation suggests that the mechanism responsible for the increase in Na^+ permeability is sensitive to the membrane potential. Therefore, if one could understand how a change in membrane potential activates Na^+ permeability, it should be possible to explain how action potentials are generated.

The fact that the Na^+ permeability that generates the membrane potential change is itself sensitive to the membrane potential presents both conceptual and practical obstacles to studying the mechanism of the action potential. A practical problem is the difficulty of systematically varying the membrane potential to study the permeability change: such changes in membrane potential will produce an action potential, thus causing further uncontrolled changes in the

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membrane potential. Historically, then, it was not possible to understand action potentials until a new technology, the **voltage clamp technique**, was developed that allowed experimenters to control membrane potential *and* simultaneously measure the underlying permeability changes (Box 3A). These features of the voltage clamp technique provide the information needed to define the ionic permeability of the membrane at any level of membrane potential.

In the late 1940s, Alan Hodgkin and Andrew Huxley working at the University of Cambridge used the voltage clamp technique to work out the perme-

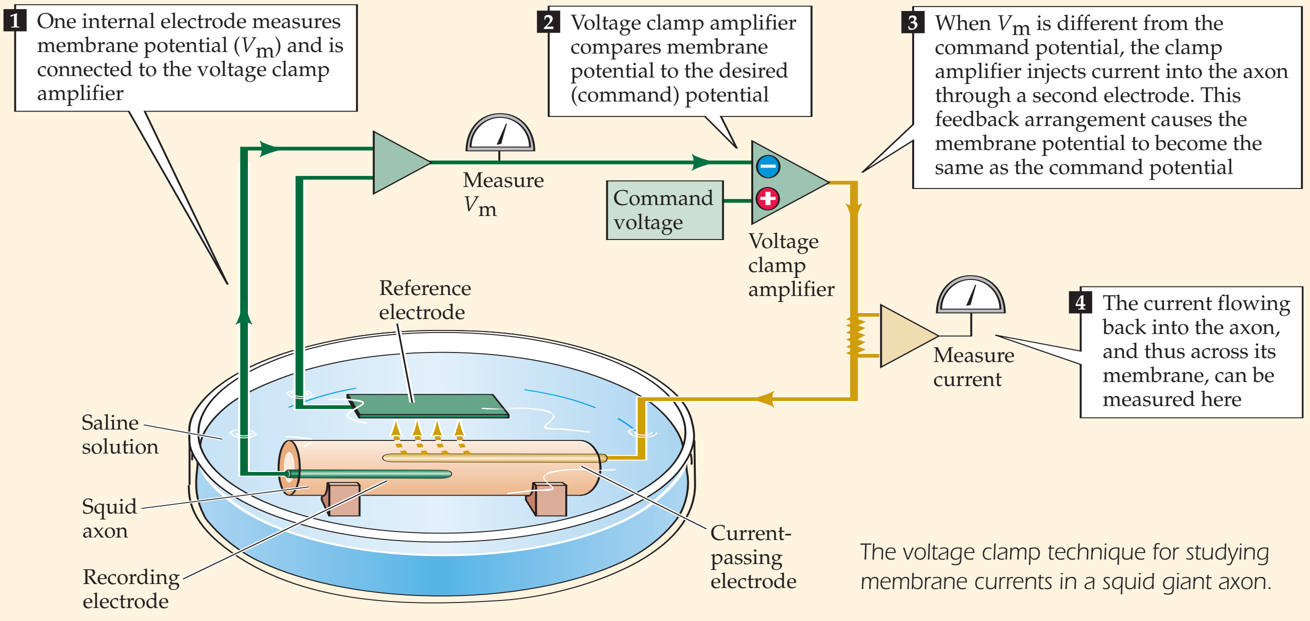
BOX 3A The Voltage Clamp Technique

Breakthroughs in scientific research often rely on the development of new technologies. In the case of the action potential, detailed understanding came only after of the invention of the voltage clamp technique by Kenneth Cole in the 1940s. This device is called a voltage clamp because it controls, or clamps, membrane potential, or voltage, at any level desired by the experimenter. As described in the figure below, voltage clamping measures the membrane potential with a microelectrode (or other type of electrode) placed inside the cell (1) and electronically compares this voltage to the voltage to be maintained, called the *command voltage* (2). The clamp circuitry then passes a current back into the cell though another intracellular electrode (3). This electronic feedback circuit holds the membrane potential at the desired level, even in the face of permeability changes that would normally alter the membrane potential (such as those generated during the action potential). Most importantly, the device permits the simultaneous measurement of the current needed to keep the cell at a given voltage (4). This current is exactly equal to the amount of current flowing across the neuronal membrane, allowing direct measurement of these membrane currents. Therefore, the voltage clamp technique can indicate how membrane potential influences ionic current flow across the membrane. This information gave Hodgkin and Huxley the key insights that led to their model for action potential generation.

Today the voltage clamp method remains widely used to study ionic currents in neurons and other cells. The most popular contemporary version of this approach is the patch clamp technique, a variation of voltage clamping that can be applied to virtually any cell and has a resolution high enough to measure the minute electrical currents flowing through single ion channels (see Box 4A).

Reference

Cole, K. S. (1968) *Membranes, Ions and Impulses: A Chapter of Classical Biophysics*. Berkeley, CA: University of California Press.



1 One internal electrode measures membrane potential (V_m) and is connected to the voltage clamp amplifier

2 Voltage clamp amplifier compares membrane potential to the desired (command) potential

3 When V_m is different from the command potential, the clamp amplifier injects current into the axon through a second electrode. This feedback arrangement causes the membrane potential to become the same as the command potential

4 The current flowing back into the axon, and thus across its membrane, can be measured here

The voltage clamp technique for studying membrane currents in a squid giant axon.

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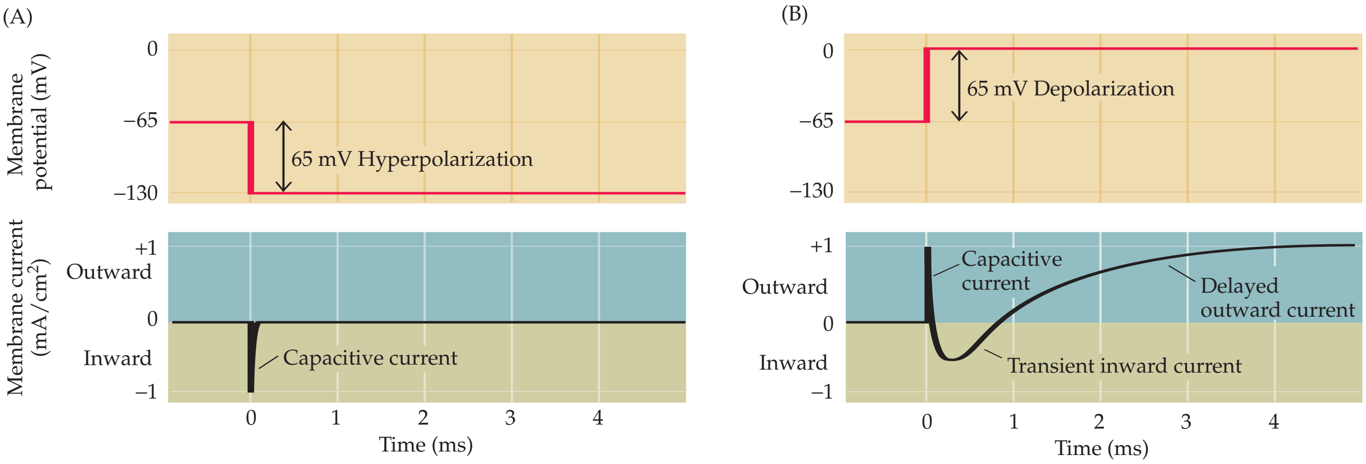

Figure 3.1 Current flow across a squid axon membrane during a voltage clamp experiment. (A) A 65 mV hyperpolarization of the membrane potential produces only a very brief capacitive current. (B) A 65 mV depolarization of the membrane potential also produces a brief capacitive current, which is followed by a longer lasting but transient phase of inward current and a delayed but sustained outward current. (After Hodgkin et al., 1952a.)

ability changes underlying the action potential. They again chose to use the giant neuron of the squid because its large size (up to 1 mm in diameter; see Box 2A) allowed insertion of the electrodes necessary for voltage clamping. They were the first investigators to test the hypothesis that potential-sensitive Na^+ and K^+ permeability changes are both necessary and sufficient to produce action potentials.

Hodgkin and Huxley's first goal was to determine whether neuronal membranes do, in fact, have voltage-dependent permeabilities. To address this issue, they asked whether ionic currents flow across the membrane when its potential is changed. The result of one such experiment is shown in Figure 3.1. Figure 3.1A illustrates the currents produced by a squid axon when its membrane potential, V_m, is hyperpolarized from the resting level of –65 mV to –130 mV. The initial response of the axon results from the redistribution of charge across the axonal membrane. This *capacitive current* is nearly instantaneous, ending within a fraction of a millisecond. Aside from this brief event, however, very little current flows when the membrane is hyperpolarized. But when the membrane potential is depolarized from –65 mV to 0 mV, the response is quite different (Figure 3.1B). Following the capacitive current, the axon produces a rapidly rising inward ionic current (inward refers to a positive charge entering the cell—that is, cations in or anions out), which gives way to a more slowly rising, delayed outward current. The fact that membrane depolarization elicits these ionic currents establishes that the membrane permeability of axons is indeed voltage-dependent.

Two Types of Voltage-Dependent Ionic Current

While the results shown in Figure 3.1 demonstrate that the ionic permeability of neuronal membranes is voltage-sensitive, these experiments do not identify how many types of permeability exist, or which ions are involved. As discussed in Chapter 2 (see Figure 2.5), varying the potential across a membrane makes it possible to deduce the equilibrium potential for the fluxes of different ions through the membrane, and thus to identify the ions that are flowing. Because the voltage clamp method allows the membrane potential to be changed while ionic currents are being measured, it was a straightforward matter for Hodgkin and Huxley to determine ionic permeability by examining how the properties of the early inward and late outward currents changed as the membrane potential was varied (Figure 3.2).

As already noted, no appreciable ionic currents flow at membrane potentials more negative than the resting potential. At more positive potentials, however,

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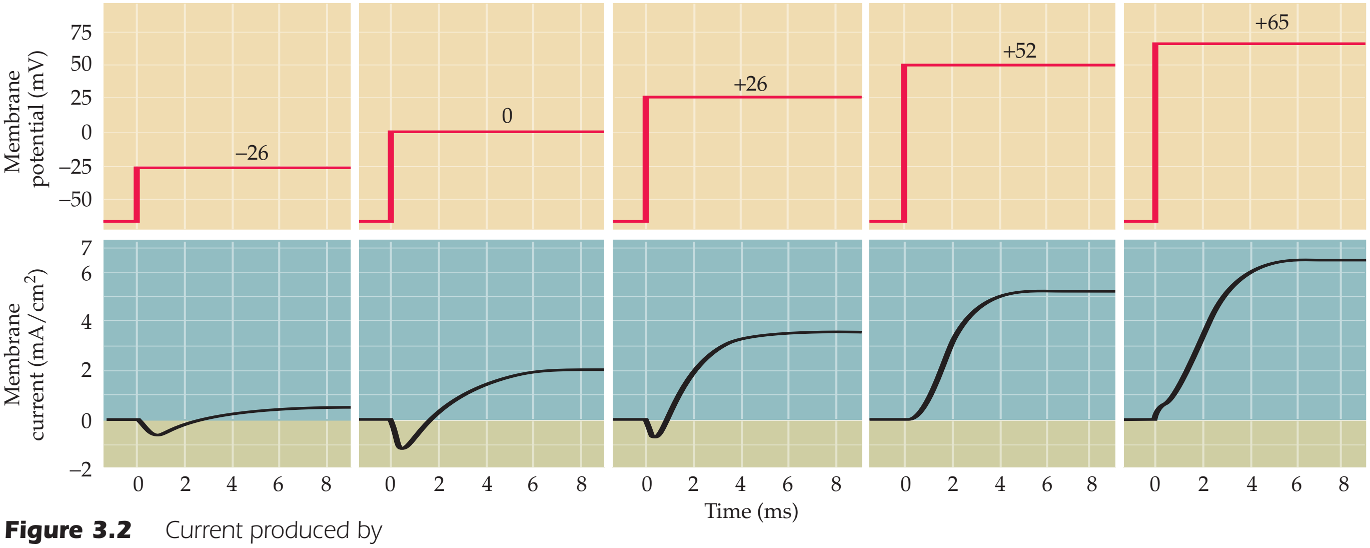

Figure 3.2 Current produced by membrane depolarizations to several different potentials. The early current first increases, then decreases in magnitude as the depolarization increases; note that this current is actually reversed in polarity at potentials more positive than about +55 mV. In contrast, the late current increases monotonically with increasing depolarization. (After Hodgkin et al., 1952a.)

the currents not only flow but they also change in magnitude. The early current has a U-shaped dependence on membrane potential, increasing over a range of depolarizations up to approximately 0 mV but decreasing as the potential is depolarized further. In contrast, the late current increases monotonically with increasingly positive membrane potentials. These different responses to membrane potential can be seen more clearly when the magnitudes of the two current components are plotted as a function of membrane potential, as in Figure 3.3.

The voltage sensitivity of the early inward current gives an important clue about the nature of the ions carrying the current—namely, that no current flows when the membrane potential is clamped at +52 mV. For the squid neurons studied by Hodgkin and Huxley, the external Na^+ concentration is 440 m*M*, and the internal Na^+ concentration is 50 m*M*. For this concentration gradient, the Nernst equation predicts that the equilibrium potential for Na^+ should be +55 mV. Recall further from Chapter 2 that at the equilibrium potential for Na^+ (E_{Na}) there is no net flux of Na^+ across the membrane, even if the membrane is highly permeable to Na^+. Thus, the experimental observation that no current flows at the membrane potential where Na^+ cannot flow is a strong indication that the early inward current is carried by entry of Na^+ into the axon. The fact that the early current reverses its direction at potentials more positive than E_{Na} provides another indication that this current is carried by Na^+: increasing membrane permeability to Na^+ at such potentials should cause current to flow outward as Na^+ leaves the axon, due to the reversed electrochemical gradient.

An even more demanding way to test whether Na^+ carries the early inward current is to examine the behavior of this current after removing external Na^+. Removing the Na^+ outside the axon makes E_{Na} more negative, which should reverse the electrochemical gradient for Na^+ and cause the current to become outward. When Hodgkin and Huxley performed this experiment, they observed just such a reversal of the early current (Figure 3.4). Removing external Na^+

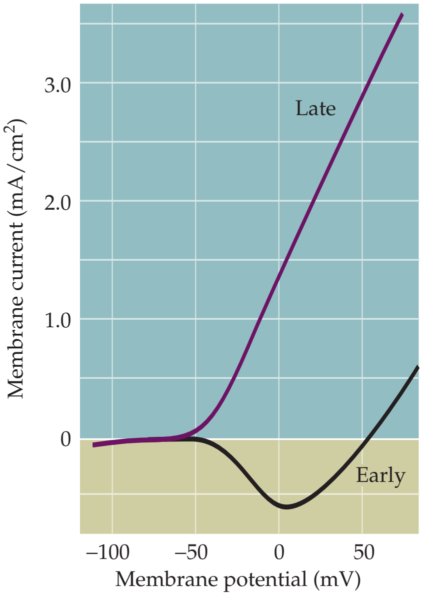

Figure 3.3 Relationship between current amplitude and membrane potential, taken from experiments such as the one shown in Figure 3.2. While the late outward current increases steeply with increasing depolarization, the early inward current first increases in magnitude, but then decreases and reverses to outward current at about +55 mV (the sodium equilibrium potential). (After Hodgkin et al., 1952a.)

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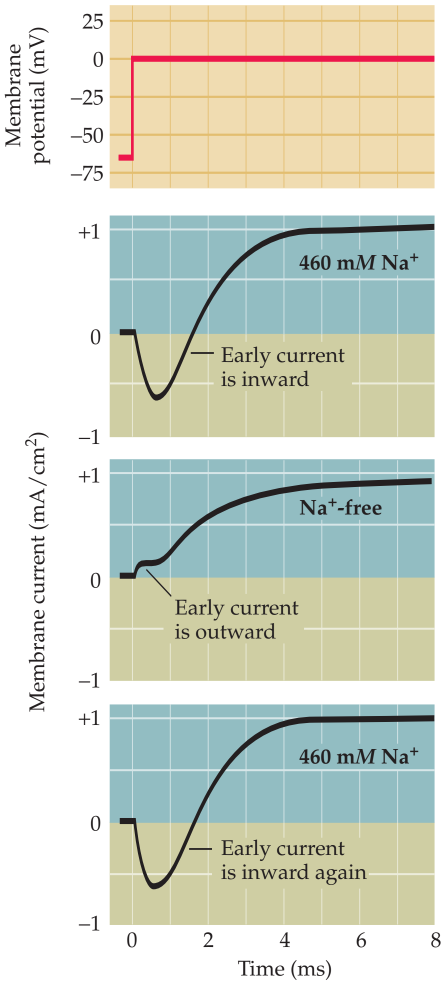

Figure 3.4 Dependence of the early inward current on sodium. In the presence of normal external concentrations of Na^+, depolarization of a squid axon to 0 mV produces an inward initial current. However, removal of external Na^+ causes the initial inward current to become outward, an effect that is reversed by restoration of external Na^+. (After Hodgkin and Huxley, 1952a.)

caused the early inward current to reverse its polarity and become an outward current at the same membrane potential that gave rise to an inward current when external Na^+ was present. This result demonstrates convincingly that the early inward current measured when Na^+ is present in the external medium must be due to Na^+ entering the neuron.

Notice in Figure 3.4 that the experimental removal of external Na^+ has little effect on the outward current that flows after the neuron has been kept at a depolarized membrane voltage for several milliseconds. This further result shows that the late outward current must be due to the flow of an ion other than Na^+. Several lines of evidence presented by Hodgkin, Huxley, and others showed that this late outward current is caused by potassium ions exiting the neuron. Perhaps the most compelling demonstration of K^+ involvement is that the amount of K^+ efflux from the neuron (measured by loading the neuron with radioactive K^+) is closely correlated with the magnitude of the late outward current.

Taken together, these experiments using the voltage clamp show that changing the membrane potential to a level more positive than the resting potential produces two effects: an *early influx of* Na^+ into the neuron, followed by a *delayed efflux of* K^+. The early influx of Na^+ produces a transient inward current, whereas the delayed efflux of K^+ produces a sustained outward current. The differences in the time course and ionic selectivity of the two fluxes suggest that two different

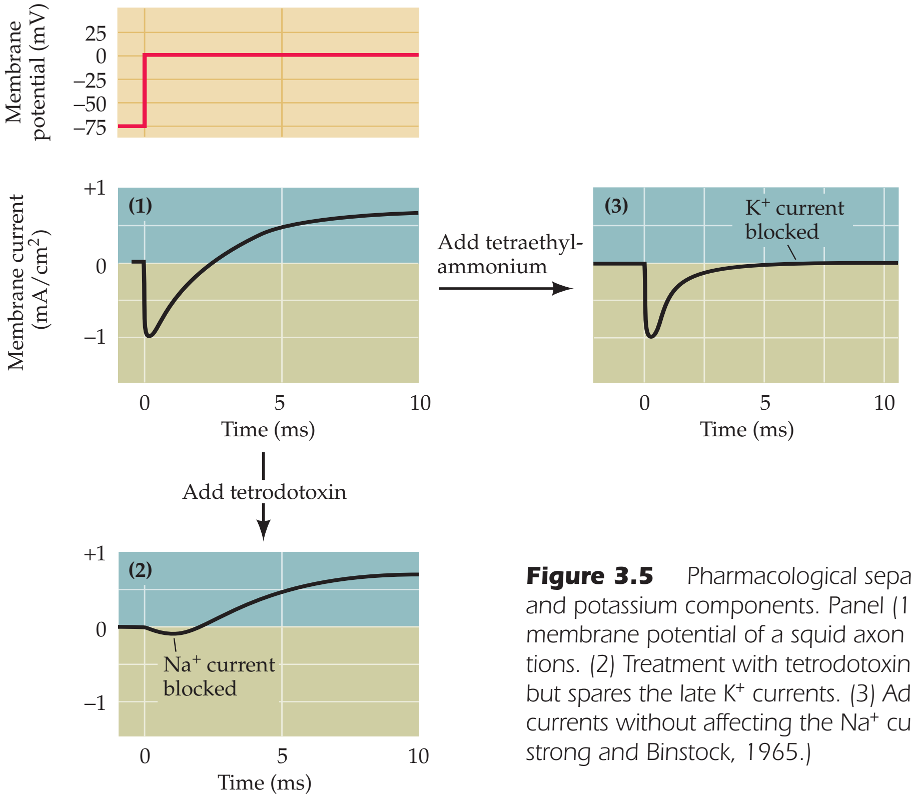

Figure 3.5 Pharmacological separation of Na^+ and K^+ currents into sodium and potassium components. Panel (1) shows the current that flows when the membrane potential of a squid axon is depolarized to 0 mV in control conditions. (2) Treatment with tetrodotoxin causes the early Na^+ currents to disappear but spares the late K^+ currents. (3) Addition of tetraethylammonium blocks the K^+ currents without affecting the Na^+ currents. (After Moore et al., 1967 and Armstrong and Binstock, 1965.)

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ionic permeability mechanisms are activated by changes in membrane potential. Confirmation that there are indeed two distinct mechanisms has come from pharmacological studies of drugs that specifically affect these two currents (Figure 3.5). **Tetrodotoxin** (**TTX**), an alkaloid neurotoxin found in certain puffer fish, tropical frogs, and salamanders, blocks the Na^+ current without affecting the K^+ current. Conversely, **tetraethylammonium ions** block K^+ currents without affecting Na^+ currents. The differential sensitivity of Na^+ and K^+ currents to these drugs provides strong additional evidence that Na^+ and K^+ flow through independent permeability pathways. It is now known that these pathways are membrane proteins known as ion channels that are selectively permeable to either Na^+ or K^+. In fact, tetrodotoxin, tetraethylammonium, and other drugs that interact with specific types of ion channels have been extraordinarily useful tools in characterizing these channel molecules, as discussed in Chapter 4.

Two Voltage-Dependent Membrane Conductances

The next goal Hodgkin and Huxley set for themselves was to describe Na^+ and K^+ permeability changes mathematically. To do this, they assumed that the ionic currents are due to a change in **membrane conductance**, defined as the reciprocal of the membrane resistance. Membrane conductance is thus closely related, although not identical, to membrane permeability. When evaluating ionic movements from an electrical standpoint, it is convenient to describe them in terms of ionic conductances rather than ionic permeabilities. For present pur-

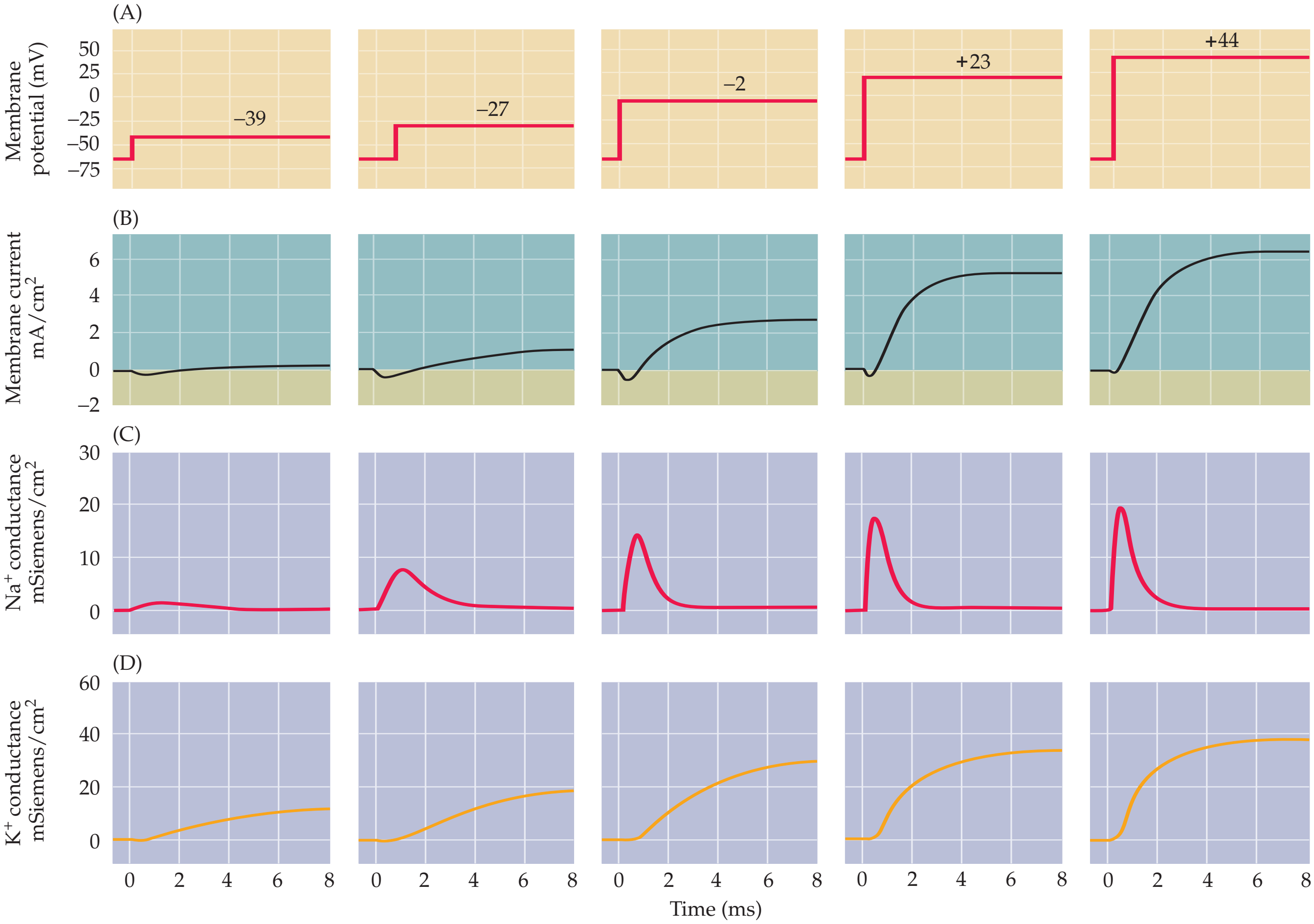

Figure 3.6 Membrane conductance changes underlying the action potential are time- and voltage-dependent. Depolarizations to various membrane potentials (A) elicit different membrane currents (B). Below are shown the Na^+ (C) and K^+ (D) conductances calculated from these currents. Both peak Na^+ conductance and steady-state K^+ conductance increase as the membrane potential becomes more positive. In addition, the activation of both conductances, as well as the rate of inactivation of the Na^+ conductance, occur more rapidly with larger depolarizations. (After Hodgkin and Huxley, 1952b.)

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poses, permeability and conductance can be considered synonymous. If membrane conductance (g) obeys Ohm's Law (which states that voltage is equal to the product of current and resistance), then the ionic current that flows during an increase in membrane conductance is given by

$$I_{\text{ion}} = g_{\text{ion}}\,(V_{\text{m}} - E_{\text{ion}})$$

where I_{ion} is the ionic current, V_{m} is the membrane potential, and E_{ion} is the equilibrium potential for the ion flowing through the conductance, g_{ion}. The difference between V_{m} and E_{ion} is the electrochemical driving force acting on the ion.

Hodgkin and Huxley used this simple relationship to calculate the dependence of Na^+ and K^+ conductances on time and membrane potential. They knew V_{m}, which was set by their voltage clamp device (Figure 3.6A). They could determine E_{Na} and E_{K} from the ionic concentrations on the two sides of the axonal membrane (see Table 2.1). The currents carried by Na^+ and K^+—I_{Na} and I_{K}—could be determined separately from recordings of the membrane currents resulting from depolarization (Figure 3.6B) by measuring the difference between currents recorded in the presence and absence of external Na^+ (as shown in Figure 3.4). From these measurements, Hodgkin and Huxley were able to calculate g_{Na} and g_{K} (Figure 3.6C,D), from which they drew two fundamental conclusions. Their first conclusion was that the Na^+ and K^+ conductances change over time. For example, both conductances require some time to **activate**, or turn on. In particular, the K^+ conductance has a pronounced delay, requiring several milliseconds to reach its maximum (Figure 3.6D), while the Na^+ conductance reaches its maximum more rapidly (Figure 3.6C). The more rapid activation of the Na^+ conductance allows the resulting inward Na^+ current to precede the delayed outward K^+ current (see Figure 3.6B).

Although the Na^+ conductance rises rapidly, it quickly declines, even when the membrane potential is kept at a depolarized level. This fact shows that depolarization not only causes the Na^+ conductance to activate, but also causes it to decrease over time, or **inactivate**. The K^+ conductance of the squid axon does not inactivate in this way; thus, while the Na^+ and K^+ conductances share the property of time-dependent activation, only the Na^+ conductance inactivates. (Inactivating K^+ conductances have since been discovered in other types of nerve cells; see Chapter 4.) The time courses of the Na^+ and K^+ conductances are voltage-dependent, with the speed of both activation and inactivation increasing at more depolarized potentials. This finding accounts for more rapid time courses of membrane currents measured at more depolarized potentials.

The second conclusion derived from Hodgkin and Huxley's calculations is that both the Na^+ and K^+ conductances are voltage-dependent—that is, both increase progressively as the neuron is depolarized. Figure 3.7 illustrates this by plotting

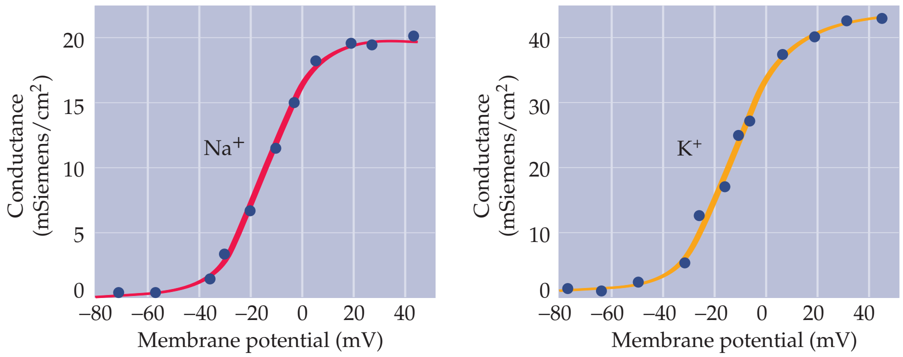

Figure 3.7 Depolarization increases Na^+ and K^+ conductances of the squid giant axon. The peak magnitude of Na^+ conductance and steady-state value of K^+ conductance both increase steeply as the membrane potential is depolarized. (After Hodgkin and Huxley, 1952b.)

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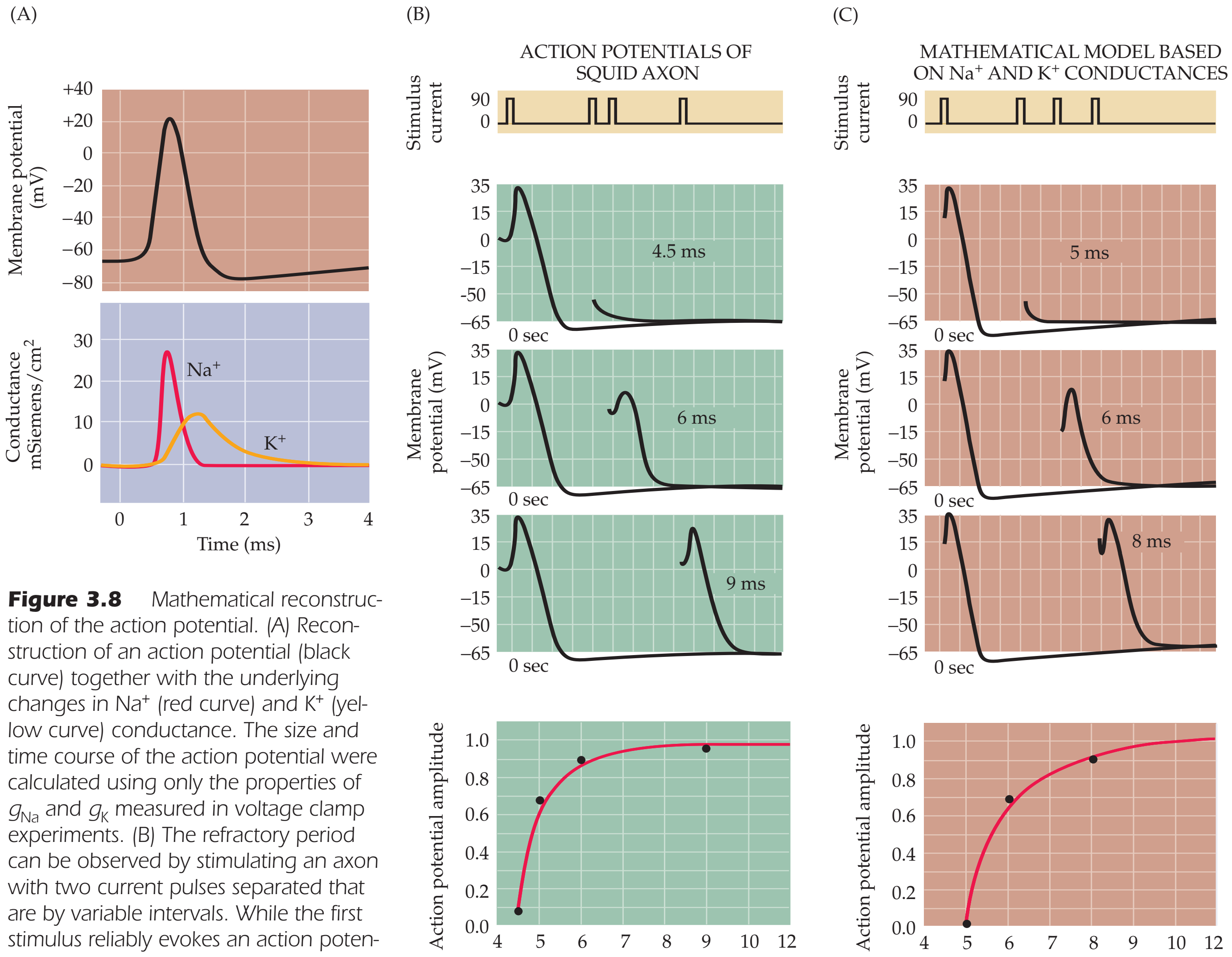

Figure 3.8 Mathematical reconstruction of the action potential. (A) Reconstruction of an action potential (black curve) together with the underlying changes in Na$^+$ (red curve) and K$^+$ (yellow curve) conductance. The size and time course of the action potential were calculated using only the properties of g_{Na} and g_K measured in voltage clamp experiments. (B) The refractory period can be observed by stimulating an axon with two current pulses separated that are by variable intervals. While the first stimulus reliably evokes an action potential, during the refractory period the second stimulus generates only a small action potential (or no response at all). (C) The mathematical model accurately simulates responses of the axon during the refractory period. (After Hodgkin and Huxley, 1952d.)

the relationship between peak value of the conductances (from Figure 3.6C and D) against the membrane potential. Note the similar voltage dependence for each conductance; both conductances are quite small at negative potentials, maximal at very positive potentials, and exquisitely dependent on membrane voltage at intermediate potentials. The observation that Na$^+$ and K$^+$ conductances are sensitive to changes in membrane potential shows that the mechanism underlying these conductances somehow "senses" the voltage across the membrane.

All told, the voltage clamp experiments carried out by Hodgkin and Huxley showed that the ionic currents that flow when the neuronal membrane is depolarized are due to three different voltage-sensitive processes: (1) activation of Na$^+$ conductance; (2) activation of K$^+$ conductance; and (3) inactivation of Na$^+$ conductance.

Reconstructing the Action Potential

From their experimental measurements, Hodgkin and Huxley were able to construct a detailed mathematical model of the Na$^+$ and K$^+$ conductance changes. The goal of these modeling efforts was to determine whether the Na$^+$ and K$^+$

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conductances alone are sufficient to produce an action potential. Using this information, they could in fact generate the form and time course of the action potential with remarkable accuracy (Figure 3.8A). The Hodgkin-Huxley model could simulate many other features of action potential behavior in the squid axon. For example, it was well known that, following an action potential, the axon experiences a brief **refractory period** during which it is resistant to further excitation (Figure 3.8B). The model was capable of closely mimicking such behavior (Figure 3.8C).

The Hodgkin-Huxley model also provided many insights into how action potentials are generated. Figure 3.8A shows a reconstructed action potential, together with the time courses of the underlying Na^+ and K^+ conductances. The coincidence of the initial increase in Na^+ conductance with the rapid rising phase of the action potential demonstrates that a selective increase in Na^+ conductance is responsible for action potential initiation. The increase in Na^+ conductance causes Na^+ to enter the neuron, thus depolarizing the membrane potential, which approaches E_{Na}. The rate of depolarization subsequently falls, both because the electrochemical driving force on Na^+ decreases and because the Na^+ conductance inactivates. At the same time, depolarization slowly activates the voltage-dependent K^+ conductance, causing K^+ to leave the cell and repolarizing the membrane potential toward E_K. Because the K^+ conductance becomes temporarily higher than it is in the resting condition, the membrane potential actually becomes briefly more negative than the normal resting potential (the undershoot). The hyperpolarization of the membrane potential causes the voltage-dependent K^+ conductance (and any Na^+ conductance not inactivated) to turn off, allowing the membrane potential to return to its resting level. The relatively slow time course of turning off the K^+ conductance, as well as the persistence of Na^+ conductance inactivation, is responsible for the refractory period (see also Figure 3.12 below).

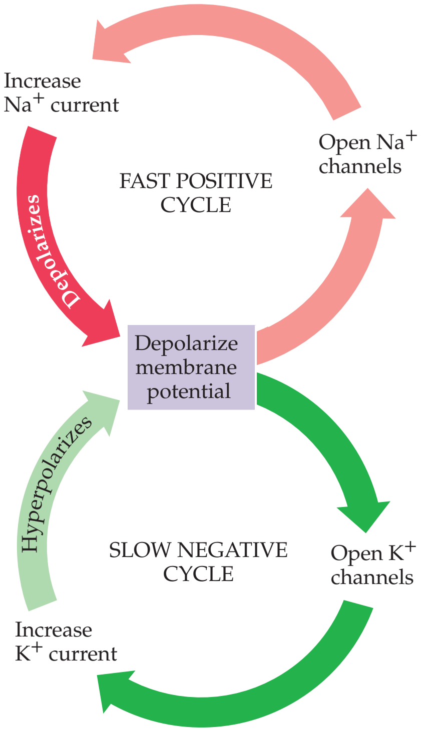
This mechanism of action potential generation represents a positive feedback loop: Activating the voltage-dependent Na^+ conductance increases Na^+ entry into the neuron, which makes the membrane potential depolarize, which leads to the activation of still more Na^+ conductance, more Na^+ entry, and still further depolarization (Figure 3.9). Positive feedback continues unabated until Na^+ conductance inactivation and K^+ conductance activation restore the membrane potential to the resting level. Because this positive feedback loop, once initiated, is sustained by the intrinsic properties of the neuron—namely, the voltage dependence of the ionic conductances—the action potential is self-supporting, or **regenerative**. This regenerative quality explains why action potentials exhibit all-or-none behavior (see Figure 2.2), and why they have a threshold (see Box 3B). The delayed activation of the K^+ conductance represents a negative feedback loop that eventually restores the membrane to its resting state.

Hodgkin and Huxley's reconstruction of the action potential and all its features shows that the properties of the voltage-sensitive Na^+ and K^+ conductances, together with the electrochemical driving forces created by ion transporters, are sufficient to explain action potentials. Their use of both empirical and theoretical methods brought an unprecedented level of rigor to a long-standing problem, setting a standard of proof that is achieved only rarely in biological research.

Long-Distance Signaling by Means of Action Potentials

The voltage-dependent mechanisms of action potential generation also explain the long-distance transmission of these electrical signals. Recall from Chapter 2 that neurons are relatively poor conductors of electricity, at least compared to a

Figure 3.9 Feedback cycles responsible for membrane potential changes during an action potential. Membrane depolarization rapidly activates a positive feedback cycle fueled by the voltage-dependent activation of Na^+ conductance. This phenomenon is followed by the slower activation of a negative feedback loop as depolarization activates a K^+ conductance, which helps to repolarize the membrane potential and terminate the action potential.



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BOX 3B Threshold

An important—and potentially puzzling—property of the action potential is its initiation at a particular membrane potential, called **threshold**. Indeed, action potentials never occur without a depolarizing stimulus that brings the membrane to this level. The depolarizing "trigger" can be one of several events: a synaptic input, a receptor potential generated by specialized receptor organs, the endogenous pacemaker activity of cells that generate action potentials spontaneously, or the local current that mediates the spread of the action potential down the axon.

Why the action potential "takes off" at a particular level of depolarization can be understood by comparing the underlying events to a chemical explosion (Figure A). Exogenous heat (analogous to the initial depolarization of the membrane potential) stimulates an exothermic chemical reaction, which produces more heat, which further enhances the reaction (Figure B). As a result of this positive feedback loop, the rate of the reaction builds up exponentially—the definition of an explosion.

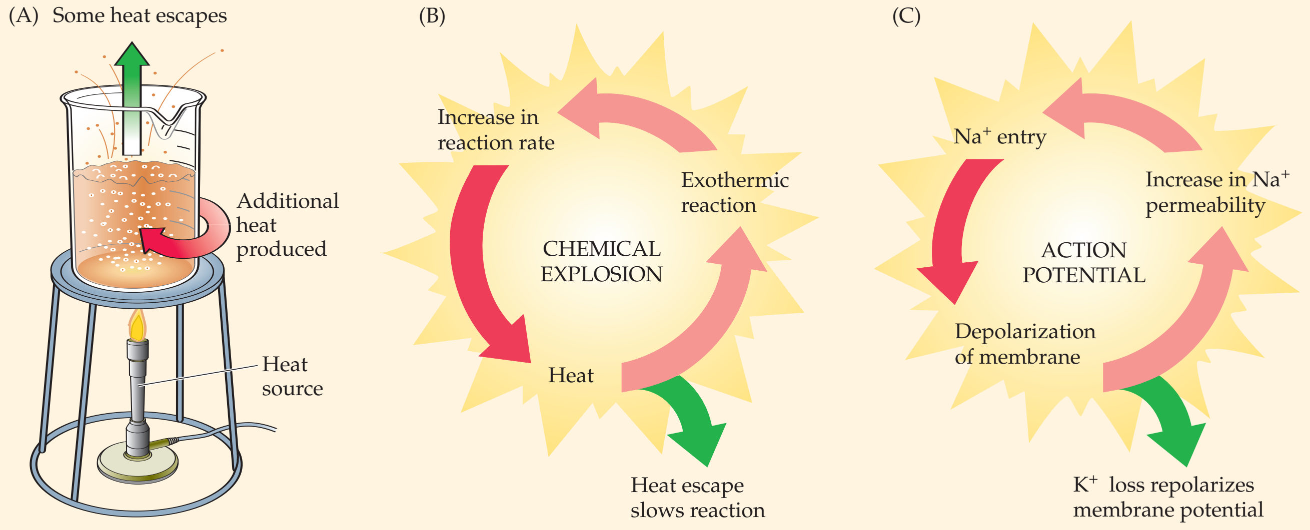
In any such process, however, there is a threshold, that is, a point up to which heat can be supplied without resulting in an explosion. The threshold for the chemical explosion diagrammed here is the point at which the amount of heat supplied exogenously is just equal to the amount of heat that can be dissipated by the circumstances of the reaction (such as escape of heat from the beaker).

The threshold of action potential initiation is, in principle, similar (Figure C). There is a range of "subthreshold" depolarization, within which the rate of increased sodium entry is less than the rate of potassium exit (remember that the membrane at rest is highly permeable to K$^+$, which therefore flows out as the membrane is depolarized). The point at which Na$^+$ inflow just equals K$^+$ outflow represents an unstable equilibrium analogous to the ignition point of an explosive mixture.

The behavior of the membrane at threshold reflects this instability: the membrane potential may linger at the threshold level for a variable period before either returning to the resting level or flaring up into a full-blown action potential. In theory at least, if there is a net internal gain of a single Na$^+$ ion, an action potential occurs; conversely, the net loss of a single K$^+$ ion leads to repolarization. A more precise definition of threshold, therefore, is that value of membrane potential, in depolarizing from the resting potential, at which the current carried by Na$^+$ entering the neuron is exactly equal to the K$^+$ current that is flowing out. Once the triggering event depolarizes the membrane beyond this point, the positive feedback loop of Na$^+$ entry on membrane potential closes and the action potential "fires."

Because the Na$^+$ and K$^+$ conductances change dynamically over time, the threshold potential for producing an action potential also varies as a consequence of the previous activity of the neuron. For example, following an action potential, the membrane becomes temporarily refractory to further excitation because the threshold for firing an action potential transiently rises. There is, therefore, no specific value of membrane potential that defines the threshold for a given nerve cell in all circumstances.

A positive feedback loop underlying the action potential explains the phenomenon of threshold.



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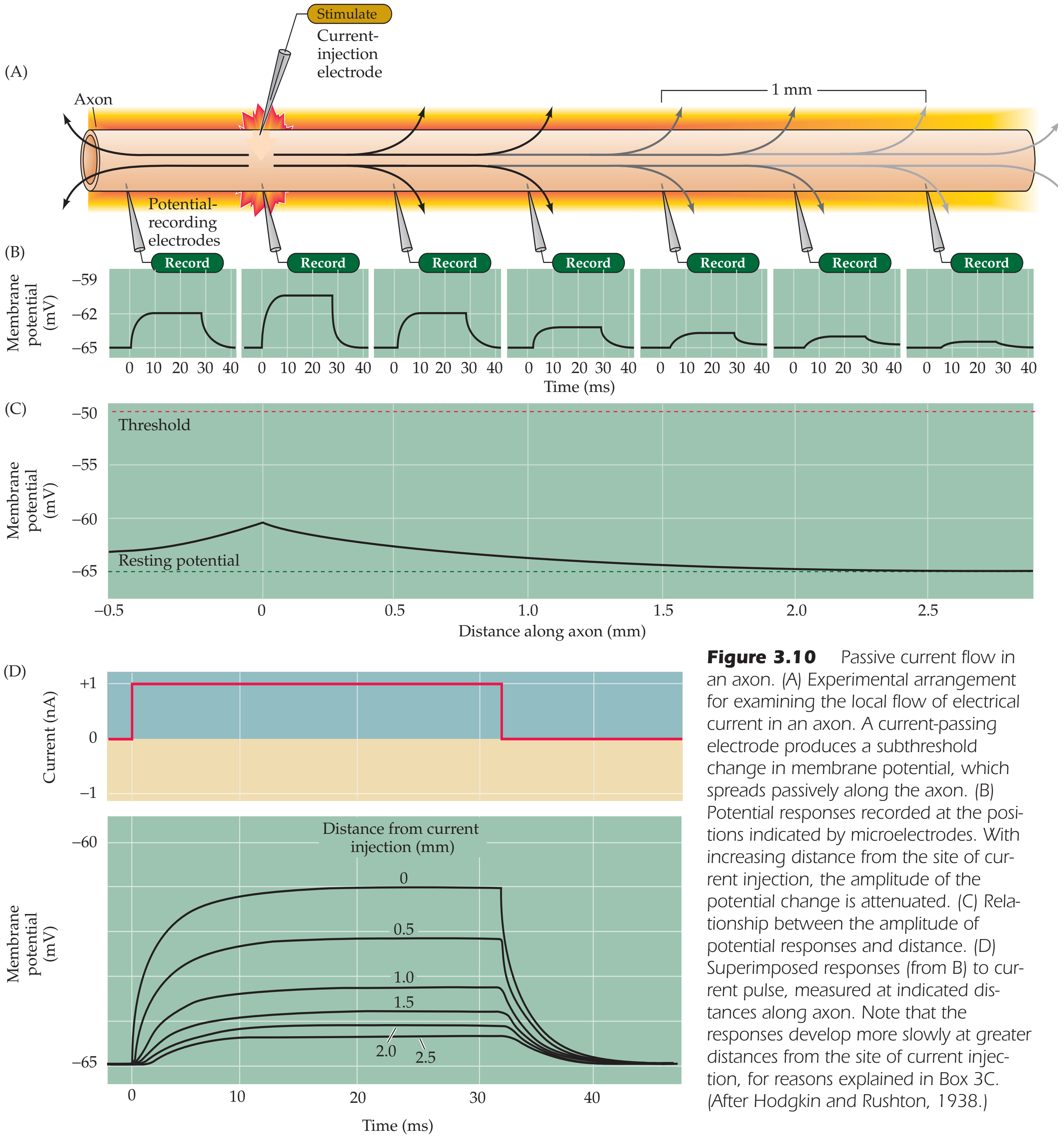

Figure 3.10 Passive current flow in an axon. (A) Experimental arrangement for examining the local flow of electrical current in an axon. A current-passing electrode produces a subthreshold change in membrane potential, which spreads passively along the axon. (B) Potential responses recorded at the positions indicated by microelectrodes. With increasing distance from the site of current injection, the amplitude of the potential change is attenuated. (C) Relationship between the amplitude of potential responses and distance. (D) Superimposed responses (from B) to current pulse, measured at indicated distances along axon. Note that the responses develop more slowly at greater distances from the site of current injection, for reasons explained in Box 3C. (After Hodgkin and Rushton, 1938.)

wire. Current conduction by wires, and by neurons in the absence of action potentials, is called **passive current flow** (Figure 3.10). The passive electrical properties of a nerve cell axon can be determined by measuring the voltage change resulting from a current pulse passed across the axonal membrane (Figure 3.10A). If this current pulse is not large enough to generate action potentials, the magnitude of the resulting potential change decays exponentially with increasing distance from the site of current injection (Figure 3.10B). Typically, the

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BOX 3C Passive Membrane Properties

The passive flow of electrical current plays a central role in action potential propagation, synaptic transmission, and all other forms of electrical signaling in nerve cells. Therefore, it is worthwhile understanding in quantitative terms how passive current flow varies with distance along a neuron. For the case of a cylindrical axon, such as the one depicted in Figure 3.10, subthreshold current injected into one part of the axon spreads passively along the axon until the current is dissipated by leakage out across the axon membrane. The decrement in the current flow with distance (Figure A) is described by a simple exponential function:

$$V_x = V_0 \, e^{-x/\lambda}$$

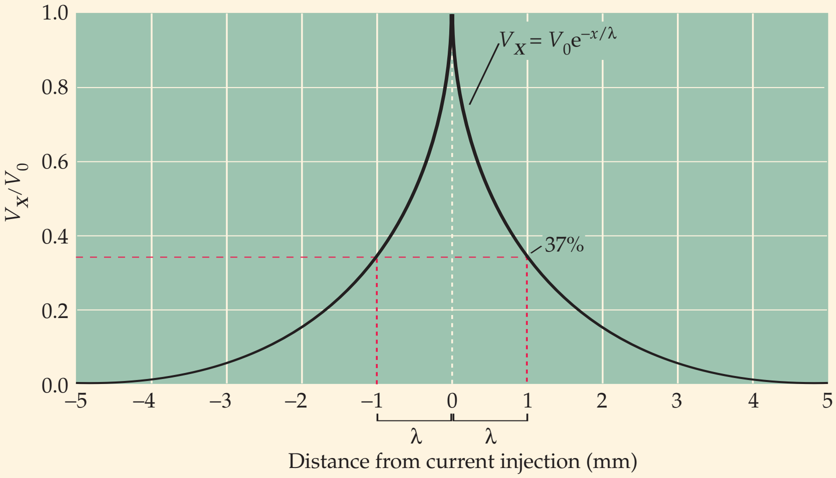
where V_x is the voltage response at any distance x along the axon, V_0 is the voltage change at the point where current is injected into the axon, e is the base of natural logarithms (approximately 2.7), and λ is the length constant of the axon. As evident in this relationship, the length constant is the distance where the initial voltage response (V_0) decays to 1/e (or 37%) of its value. The length constant is thus a way to characterize how far passive current flow spreads before it leaks out of the axon, with leakier axons having shorter length constants.

The length constant depends upon the physical properties of the axon, in particular the relative resistances of the plasma membrane (r_m), the intracellular axoplasm (r_i), and the extracellular medium (r_0). The relationship between these parameters is

$$\lambda = \sqrt{\frac{r_m}{r_o + r_i}}$$

Hence, to improve the passive flow of current along an axon, the resistance of the plasma membrane should be as high as possible and the resistances of the axoplasm and extracellular medium should be low.

Another important consequence of the passive properties of neurons is that currents flowing across a membrane do not immediately change the membrane potential. For example, when a rectangular current pulse is injected into the axon shown in the experiment illustrated in Figure 3.10A, the membrane potential depolarizes slowly over a few milliseconds and then repolarizes over a similar time course when the current pulse ends (see Figure 3.10D). These delays in changing the membrane potential are due to the fact that the plasma membrane behaves as a capacitor, storing the initial charge that flows at the beginning and end of the current pulse. For the



(A) Spatial decay of membrane potential along a cylindrical axon. A current pulse injected at one point in the axon (0 mm) produces voltage responses (V_x) that decay exponentially with distance. The distance where the voltage response is 1/e of its initial value (V_0) is the length constant, λ.

potential falls to a small fraction of its initial value at a distance of no more than a couple of millimeters away from the site of injection (Figure 3.10C). The progressive decrease in the amplitude of the induced potential change occurs because the injected current leaks out across the axonal membrane; accordingly, less current is available to change the membrane potential farther along the axon (Box 3C). Thus, the leakiness of the axonal membrane prevents effective passive transmission of electrical signals in all but the shortest axons (those 1 mm or less in length). Likewise, the leakiness of the membrane slows the time course of the responses measured at increasing distances from the site where current was injected (Figure 3.10D).

If the experiment shown in Figure 3.10 is repeated with a depolarizing current pulse large enough to produce an action potential, the result is dramatically different (Figure 3.11A). In this case, an action potential occurs without decre-

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case of a cell whose membrane potential is spatially uniform, the change in the membrane potential at any time, V_t after beginning the current pulse (Figure B) can also be described by an exponential relationship:

$$V_t = V_\infty(1 - e^{-t/\tau})$$

where V_∞ is the steady-state value of the membrane potential change, t is the time after the current pulse begins, and τ is the membrane time constant. The time constant is thus defined as the time when the voltage response (V_t) rises to 1 – (1/e) (or 63%) of V_∞. After the current pulse ends, the membrane potential change also declines exponentially according to the relationship

$$V_t = V_\infty e^{-t/\tau}$$

During this decay, the membrane potential returns to 1/e of V_∞ at a time equal to t. For cells with more complex geometries than the axon in Figure 3.10, the time courses of the changes in membrane potential are not simple exponentials, but nonetheless depend on the membrane time constant. Thus, the time constant characterizes how rapidly current flow changes the membrane potential. The membrane time constant also depends on the physical properties of the nerve cell, specifically on the resistance (r_m) and capacitance (c_m) of the plasma membrane such that:

$$\tau = r_m c_m$$

The values of r_m and c_m depend, in part, on the size of the neuron, with larger cells having lower resistances and larger capacitances. In general, small nerve cells tend to have long time constants and large cells brief time constants.

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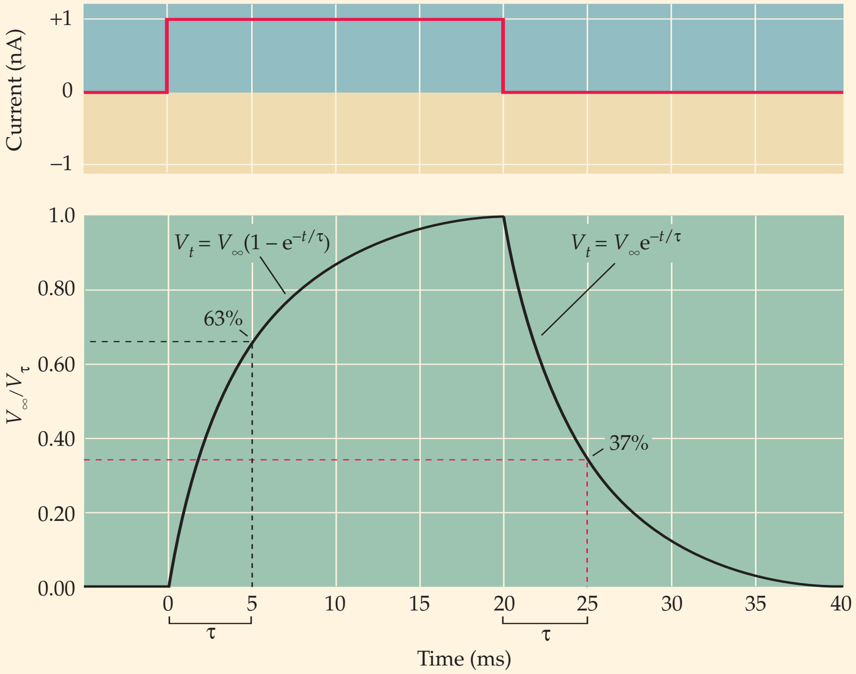

(B) Time course of potential changes produced in a spatially uniform cell by a current pulse. The rise and fall of the membrane potential (V_τ) can be described as exponential functions, with the time constant τ defining the time required for the response to rise to 1 – (1/e) of the steady-state value (V_∞), or to decline to 1/e of V_∞.

ment along the entire length of the axon, which in humans may be a distance of a meter or more (Figure 3.11B). Thus, action potentials somehow circumvent the inherent leakiness of neurons.

How do action potentials traverse great distances along such a poor passive conductor? A partial answer is provided by the observation that the amplitude of the action potentials recorded at different distances is constant. This all-or-none behavior indicates that more than simple passive flow of current must be involved in action potential propagation. A second clue comes from examination of the time of occurrence of the action potentials recorded at different distances from the site of stimulation: action potentials occur later and later at greater distances along the axon (see Figure 3.11B). Thus, the action potential has a measurable rate of transmission, called the **conduction velocity**. The delay in the arrival of the action potential at successively more distant points

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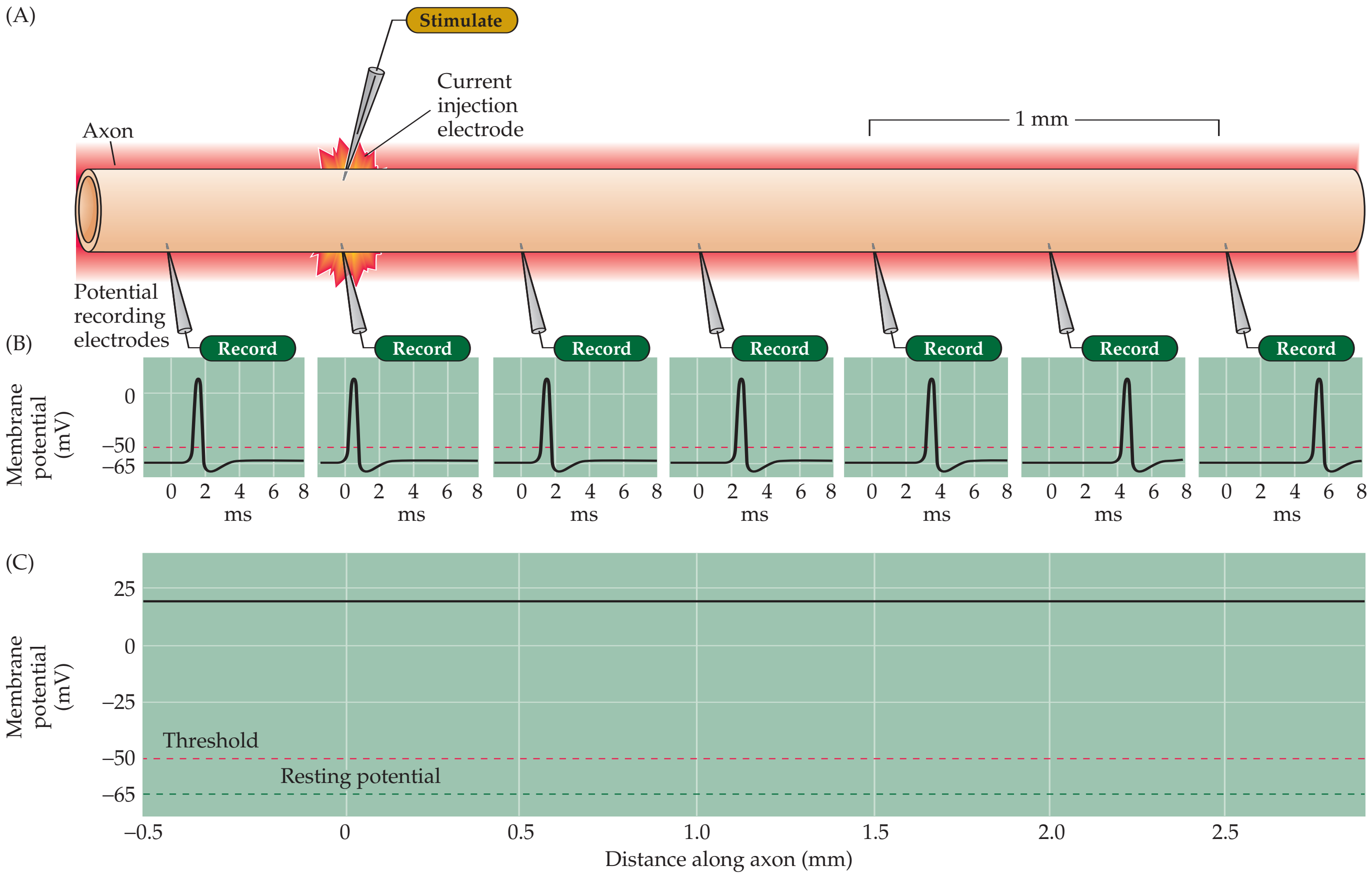

Figure 3.11 Propagation of an action potential. (A) In this experimental arrangement, an electrode evokes an action potential by injecting a suprathreshold current. (B) Potential responses recorded at the positions indicated by microelectrodes. The amplitude of the action potential is constant along the length of the axon, although the time of appearance of the action potential is delayed with increasing distance. (C) The constant amplitude of an action potential (solid black line) measured at different distances.

along the axon differs from the case shown in Figure 3.10, in which the electrical changes produced by passive current flow occur at more or less the same time at successive points.

The mechanism of action potential propagation is easy to grasp once one understands how action potentials are generated and how current passively flows along an axon (Figure 3.12). A depolarizing stimulus—a synaptic potential or a receptor potential in an intact neuron, or an injected current pulse in an experiment—locally depolarizes the axon, opening the voltage-sensitive Na^+ channels in that region. The opening of Na^+ channels causes inward movement of Na^+, and the resultant depolarization of the membrane potential generates an action potential at that site. Some of the local current generated by the action potential then flows passively down the axon, in the same way that subthreshold currents spread along the axon (see Figure 3.10). Note that this passive current flow does not require the movement of Na^+ along the axon, but instead occurs by a shuttling of charge, somewhat similar to what happens when wires passively conduct electricity by transmission of electron charge. This passive current flow depolarizes the membrane potential in the adjacent region of the axon, thus opening the Na^+ channels in the neighboring membrane. The local depolarization triggers an action potential in this region, which then spreads again in a continuing cycle until the end of the axon is reached. Thus, action potential propagation requires the coordinated action of two forms of current flow—the passive flow of current as well as active currents flowing through voltage-dependent ion channels. The regenerative properties of Na^+ channel opening allow action potentials to propagate in an all-or-none fashion by acting

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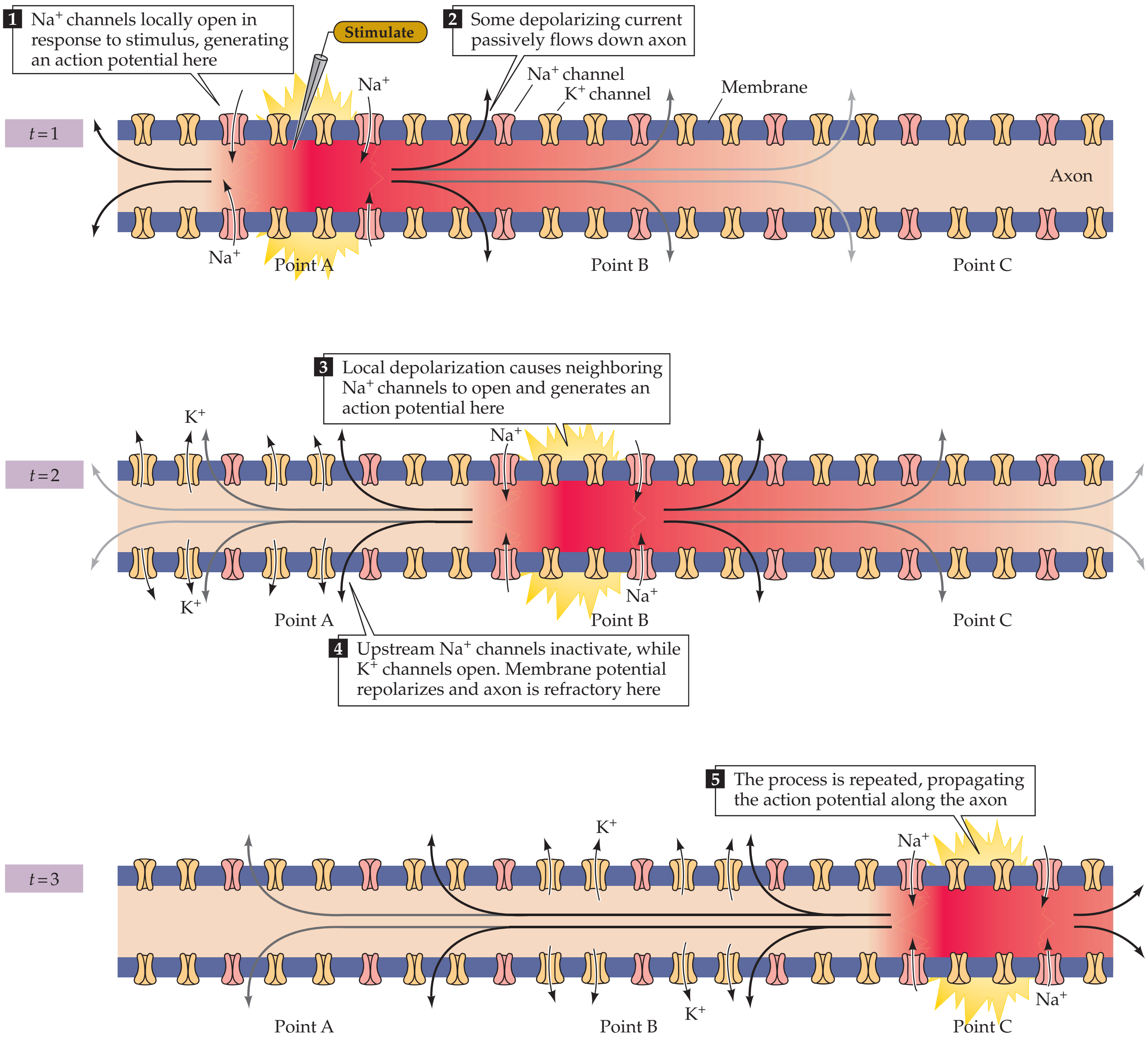

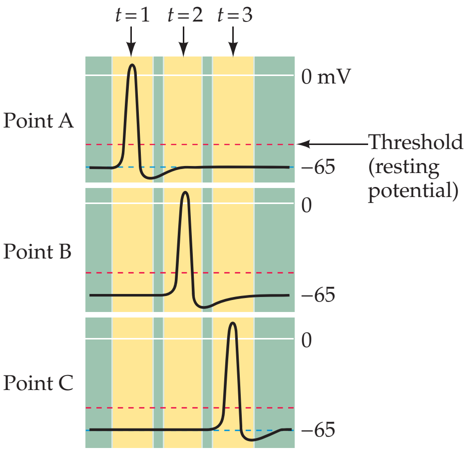

Figure 3.12 Action potential conduction requires both active and passive current flow. Depolarization opens Na⁺ channels locally and produces an action potential at point A of the axon (time $t = 1$). The resulting inward current flows passively along the axon, depolarizing the adjacent region (point B) of the axon. At a later time ($t = 2$), the depolarization of the adjacent membrane has opened Na⁺ channels at point B, resulting in the initiation of the action potential at this site and additional inward current that again spreads passively to an adjacent point (point C) farther along the axon. At a still later time ($t = 3$), the action potential has propagated even farther. This cycle continues along the full length of the axon. Note that as the action potential spreads, the membrane potential repolarizes due to K⁺ channel opening and Na⁺ channel inactivation, leaving a "wake" of refractoriness behind the action potential that prevents its backward propagation (panel 4). The panel to the left of this figure legend shows the time course of membrane potential changes at the points indicated.

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as a booster at each point along the axon, thus ensuring the long-distance transmission of electrical signals.

The Refractory Period Ensures Polarized Propagation of Action Potentials

Recall that axons become refractory following an action potential: that is, the generation of an action potential briefly makes it harder for the axon to produce subsequent action potentials (see Figure 3.8B). Refractoriness limits the number of action potentials that a neuron can produce per unit time, with different types of neurons having different maximum rates of action potential firing (a result of their different types and densities of ion channels). As described in a previous section, the refractory period arises because the depolarization that produces Na^+ channel opening also results in the delayed activation of K^+ channels and Na^+ channel inactivation, which temporarily makes it more difficult for the axon to produce another action potential. The action potential sweeps along the length of an axon, and in its wake Na^+ channels are inactivated and K^+ channels activated for a brief time. Refractoriness of the membrane region where an action potential has been generated prevents subsequent re-excitation of this membrane (see Figure 3.12)—an important feature that prevents action potentials from propagating backward (i.e., back toward their point of initiation). Thus, refractory behavior ensures polarized propagation of action potentials from their usual point of initiation near the neuronal cell body, toward the synaptic terminals at the distal end of the axon.

Myelination Results in Increased Conduction Velocity

The rate of action potential conduction limits the flow of information within the nervous system. It is not surprising, then, that various mechanisms have evolved that optimize the propagation of action potentials. Because action potential conduction requires passive and active flow of current (see Figure 3.12), the rate of action potential propagation is determined by both of these phenomena. One way of improving passive current flow is to increase the diameter of an axon, which effectively decreases the internal resistance to passive current flow (see Box 3C). The consequent increase in action potential conduction velocity presumably explains why giant axons evolved in invertebrates such as squid, and why rapidly conducting axons in all animals tend to be larger than slowly conducting ones.

Another strategy to improve the passive flow of electrical current is to insulate the axonal membrane, reducing the ability of current to leak out of the axon and thus increasing the distance along the axon that a given local current can flow passively. Among vertebrates, this strategy is evident in the **myelination** of axons, a process by which oligodendrocytes in the central nervous system (and Schwann cells in the peripheral nervous system) wrap the axon in **myelin**, which consists of multiple layers of closely opposed glial membranes (Figure 3.13A). By acting as an electrical insulator, myelin greatly speeds up action potential conduction (Figure 3.14). For example, whereas unmyelinated axon conduction velocities range from about 0.5 to 10 m/s, myelinated axons can conduct at velocities of up to 150 m/s. The major reason underlying this marked increase in speed is that the time-consuming process of action potential generation occurs only at specific points along the axon, called **nodes of Ranvier**, where there is a gap in the myelin wrapping. If the entire surface of an axon were insulated, there would be no place for current to flow out of the axon and action potentials could not be generated. Thus, the voltage-gated Na^+ channels required for action

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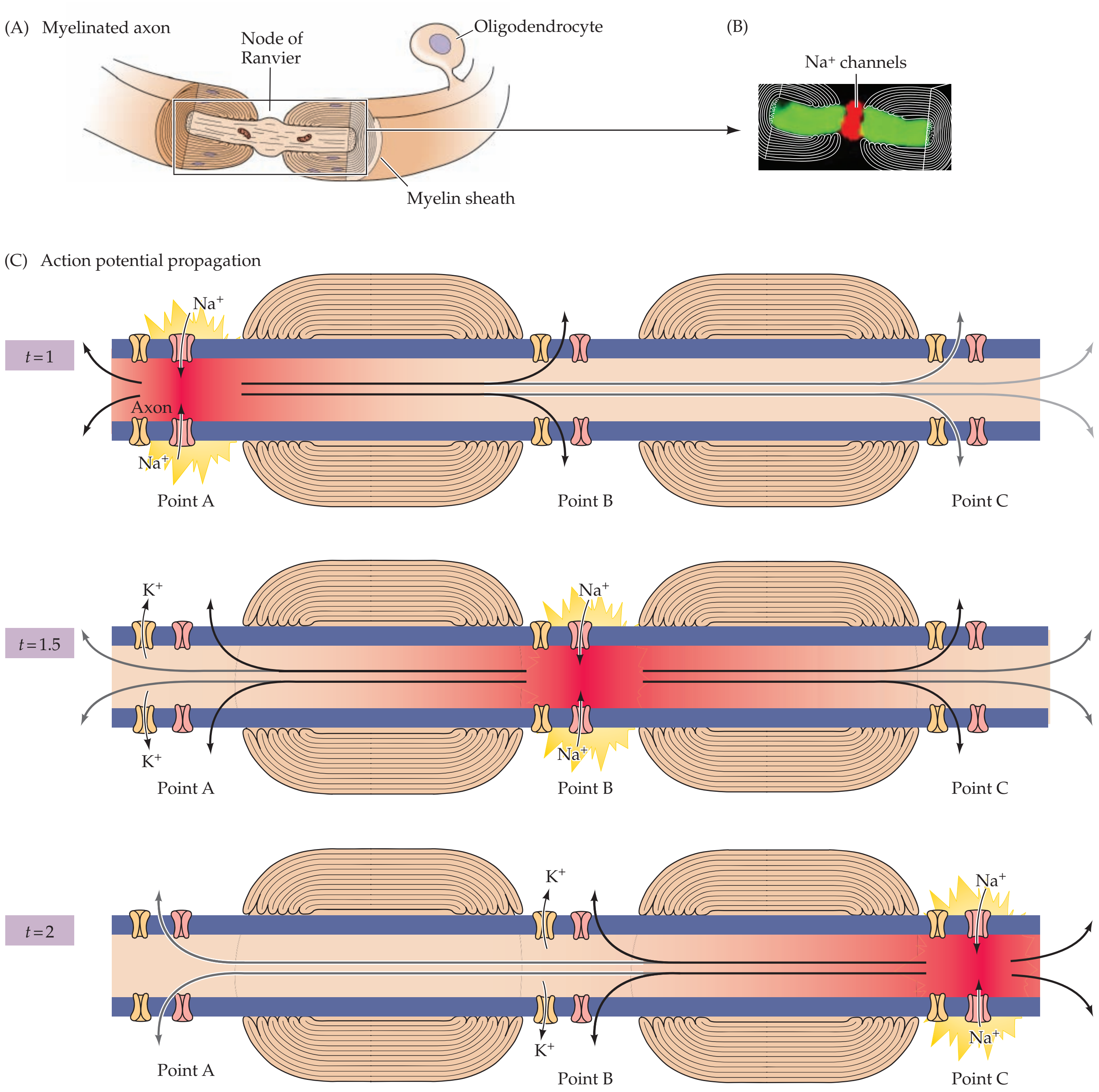

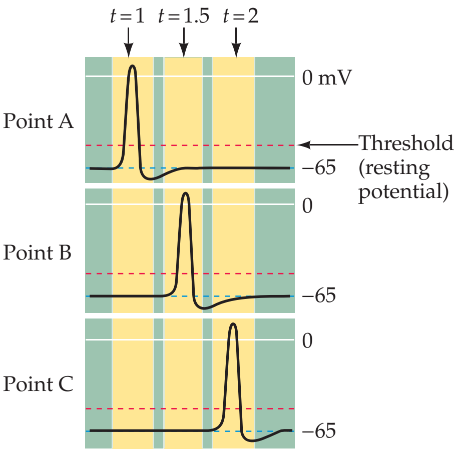

Figure 3.13 Saltatory action potential conduction along a myelinated axon. (A) Diagram of a myelinated axon. (B) Localization of voltage-gated Na channels (red) at a node of Ranvier in a myelinated axon of the optic nerve. Green color indicates a protein called Caspr, which is located adjacent to the node of Ranvier. (C) Local current in response to action potential initiation at a particular site flows locally, as described in Figure 3.12. However, the presence of myelin prevents the local current from leaking across the internodal membrane; it therefore flows farther along the axon than it would in the absence of myelin. Moreover, voltage-gated Na^+ channels are present only at the nodes of Ranvier (K^+ channels are present at the nodes of some neurons but not others). This arrangement means that the generation of active, voltage-gated Na^+ currents need only occur at these unmyelinated regions. The result is a greatly enhanced velocity of action potential conduction. The panel to the left of this figure legend shows the time course of membrane potential changes at the points indicated. (B from Chen et al., 2004.)

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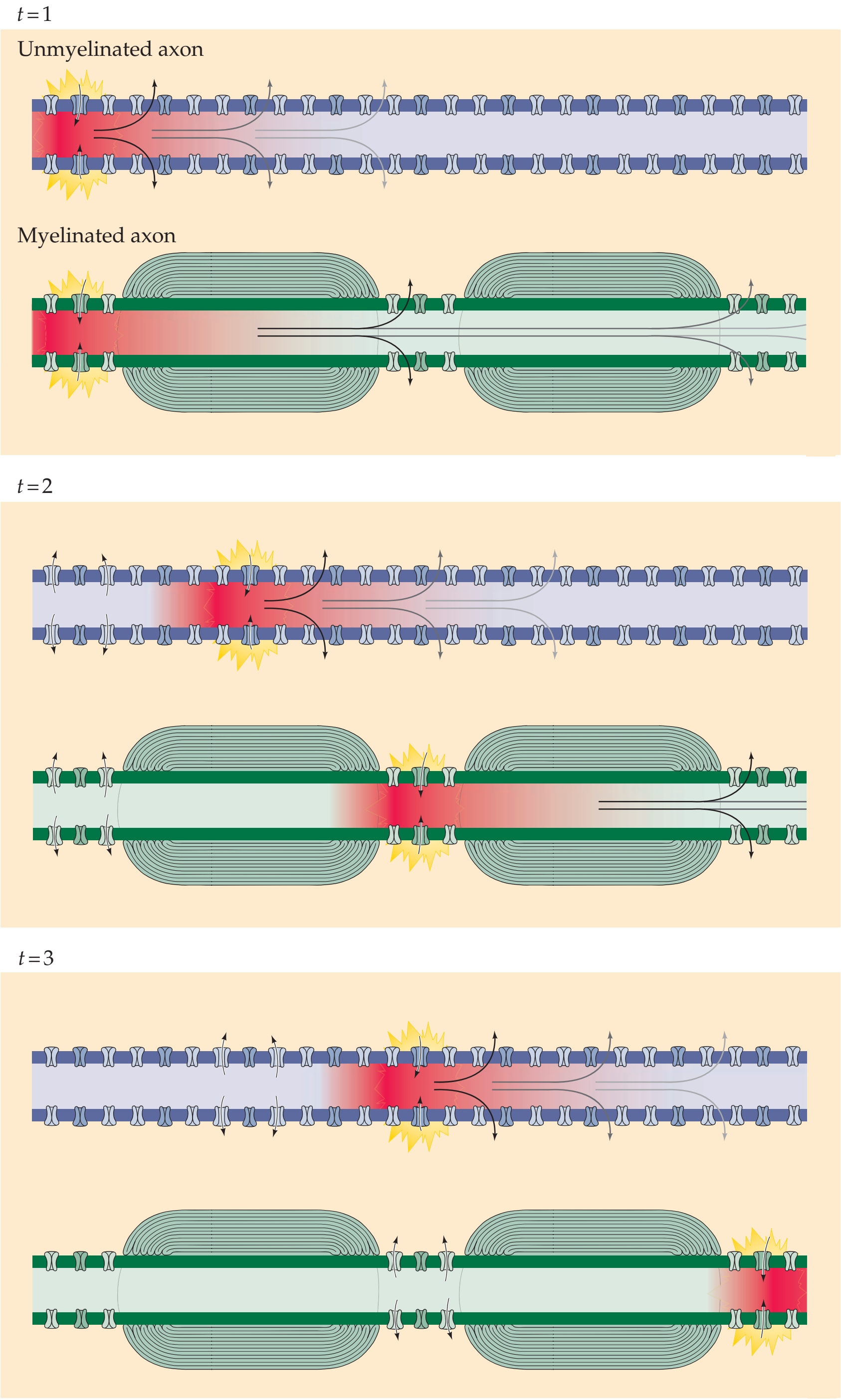

Figure 3.14 Comparison of speed of action potential conduction in unmyelinated (upper) and myelinated (lower) axons. For clarity, passive current flow is shown only for the direction of action potential propagation.

potentials are found only at these nodes of Ranvier (Figure 3.13B). An action potential generated at one node of Ranvier elicits current that flows passively within the myelinated segment until the next node is reached. This local current flow then generates an action potential in the neighboring segment, and the cycle is repeated along the length of the axon. Because current flows across the neuronal membrane only at the nodes (Figure 3.13C), this type of propagation is called **saltatory**, meaning that the action potential jumps from node to node. Not surprisingly, loss of myelin, as occurs in diseases such as multiple sclerosis, causes a variety of serious neurological problems (Box 3D).

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BOX 3D Multiple Sclerosis

Multiple sclerosis (MS) is a disease of the central nervous system characterized by a variety of clinical problems arising from multiple regions of demyelination and inflammation along axonal pathways. The disorder commonly begins between ages 20 and 40 and is characterized by the abrupt onset of neurological deficits that typically persist for days or weeks and then subside. The clinical course ranges from patients with no persistent neurological loss, some of whom experience only occasional later recurrences, to others who progressively deteriorate as a result of extensive and relentless central nervous system involvement.

The signs and symptoms of MS are determined by the location of the affected regions. Particularly common are monocular blindness (due to lesions of the optic nerve), motor weakness or paralysis (due to lesions of the corticospinal tracts), abnormal somatic sensations (due to lesions of somatic sensory pathways, often in the posterior columns), double vision (due to lesions of medial longitudinal fasciculus), and dizziness (due to lesions of vestibular pathways). Abnormalities are often apparent in the cerebrospinal fluid, which usually contains an abnormal number of cells associated with inflammation and an increased content of antibodies (a sign of an altered immune response).

The diagnosis of MS generally relies on the presence of a neurological problem that remits and then returns at an unrelated site. Confirmation can sometimes be obtained from magnetic resonance imaging (MRI) or from functional evidence of lesions in a particular pathway by abnormal evoked potentials. The histological hallmark of MS (seen at postmortem exam) is multiple lesions at different sites showing loss of myelin associated with infiltration of inflammatory cells and, in some instances, loss of axons themselves.

The concept of MS as a demyelinating disease is deeply embedded in the clinical literature, although precisely how the demyelination translates into functional deficits is poorly understood. The loss of myelin sheath surrounding many axons compromises action potential conduction, and the abnormal patterns of nerve conduction that result presumably produce most of the clinical deficits in the disease. However, MS may have effects that extend beyond loss of the myelin sheath. It is clear that some axons are actually destroyed, probably as a result of the inflammation targeting the overlying myelin and/or loss of trophic support of the axon by oligodendrocytes. Thus, axon loss also contributes to the functional deficits in MS, especially in the chronic, progressive forms of the disease.

The ultimate cause of MS remains unclear. The immune system undoubtedly contributes to the damage, and immunomodulatory therapies provide substantial benefits to some patients. Precisely how the immune system is activated to cause the injury is not known. The most popular hypothesis is that MS is an autoimmune disease (i.e., a disease in which the immune system attacks the body's proper constituents). The fact that immunization of experimental animals with any one of several molecular constituents of the myelin sheath can induce a demyelinating disease (called experimental allergic encephalomyelitis) shows that an autoimmune attack on the myelin membrane is sufficient to produce a clinical picture similar to that of MS. A possible explanation of the human disease is that a genetically susceptible individual becomes transiently infected (by a minor viral illness, for example) with a microorganism that expresses a molecule structurally similar to a component of myelin. An immune response to this antigen is mounted to attack the invader, but the failure of the immune system to discriminate between the foreign protein and self results in destruction of otherwise normal myelin; this exact scenario occurs in mice infected with Theiler's virus.

An alternative hypothesis is that MS is caused by a persistent infection by a virus or other microorganism. In this scenario, the immune system's ongoing efforts to get rid of the pathogen cause the damage to myelin. Tropical spastic paraparesis (TSP) provides a precedent for this idea. TSP is a disease characterized by the gradual progression of weakness of the legs and impaired control of bladder function associated with increased deep tendon reflexes and a positive Babinski sign (see Chapter 17). This clinical picture is similar to that of rapidly advancing MS, and TSP is known to be caused by persistent infection with a retrovirus (human T lymphotropic virus-1). This precedent notwithstanding, proving the persistent viral infection hypothesis for MS requires unambiguous demonstration of the presence of a virus. Despite periodic reports of a virus associated with MS, convincing evidence has not been forthcoming, and multiple sclerosis remains a daunting clinical challenge.

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Summary

The action potential and all its complex properties can be explained by time- and voltage-dependent changes in the Na^+ and K^+ permeabilities of neuronal membranes. This conclusion derives primarily from evidence obtained by a device called the voltage clamp. The voltage clamp technique is an electronic feedback method that allows control of neuronal membrane potential and, simultaneously, direct measurement of the voltage-dependent fluxes of Na^+ and K^+ that produce the action potential. Voltage clamp experiments show that a transient rise in Na^+ conductance activates rapidly and then inactivates during a sustained depolarization of the membrane potential. Such experiments also demonstrate a rise in K^+ conductance that activates in a delayed fashion and, in contrast to the Na^+ conductance, does not inactivate. Mathematical modeling of the properties of these conductances indicates that they, and they alone, are responsible for the production of all-or-none action potentials in the squid axon. Action potentials propagate along the nerve cell axons initiated by the voltage gradient between the active and inactive regions of the axon by virtue of the local current flow. In this way, action potentials compensate for the relatively poor passive electrical properties of nerve cells and enable neural signaling over long distances. These classic electrophysiological findings provide a solid basis for considering the functional and ultimately molecular variations on neural signaling described in the next chapter.

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