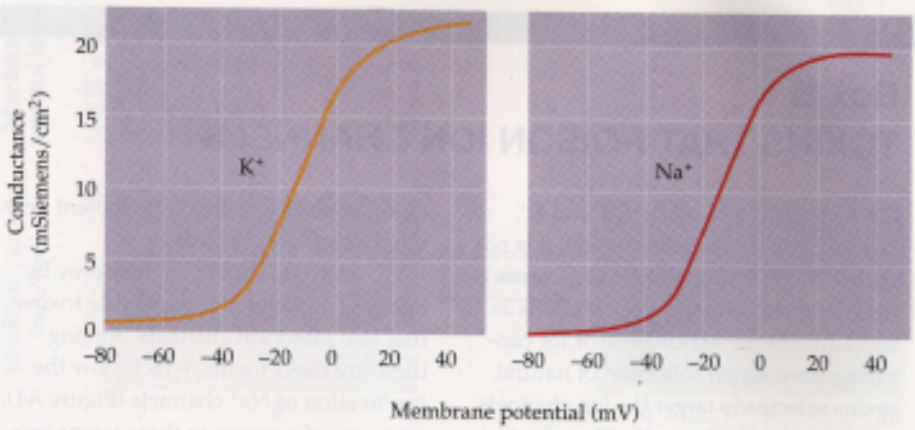


Figure 3.6 Depolarization increases Na^+ and K^+ conductances of the squid giant axon. The peak magnitude of Na^+ conductance and steady-state value of K^+ conductance both increase steeply as the membrane potential is depolarized. (After Hodgkin and Huxley, 1952b.)

both the Na^+ and K^+ conductances increase progressively as the neuron is depolarized. Figure 3.6 illustrates this relationship by plotting each conductance versus the membrane potential. Note the similar voltage dependence for each conductance; both are sigmoidal functions of membrane potential, and are quite small at negative potentials, maximal at very positive potentials, and exquisitely dependent on membrane voltage at intermediate potentials. The observation that these conductances are sensitive to changes in membrane potential shows that the mechanism underlying the conductances somehow "senses" the voltage across the membrane.

The second conclusion derived from the calculations of Hodgkin and Huxley is that the Na^+ and K^+ conductances also change over time. For example, both the Na^+ and K^+ 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; the Na^+ conductance reaches its maximum more rapidly (Figure 3.7). The more rapid activation of the Na^+ conductance allows the resulting inward Na^+ current to precede the delayed outward K^+ current (Figure 3.7A, B). Although the Na^+ conductance rises rapidly, it quickly declines, even though 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 also voltage-dependent, with the speed of both activation and inactivation increasing at more depolarized potentials (see Figure 3.7C, D). This finding accounts for more rapid courses of membrane currents measured at more depolarized potentials.

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.

■ RECONSTRUCTION OF THE ACTION POTENTIAL

From the experimental measurements of these three conductance changes, Hodgkin and Huxley were able to construct a detailed mathematical model of these processes to determine whether conductance changes to Na^+ and K^+

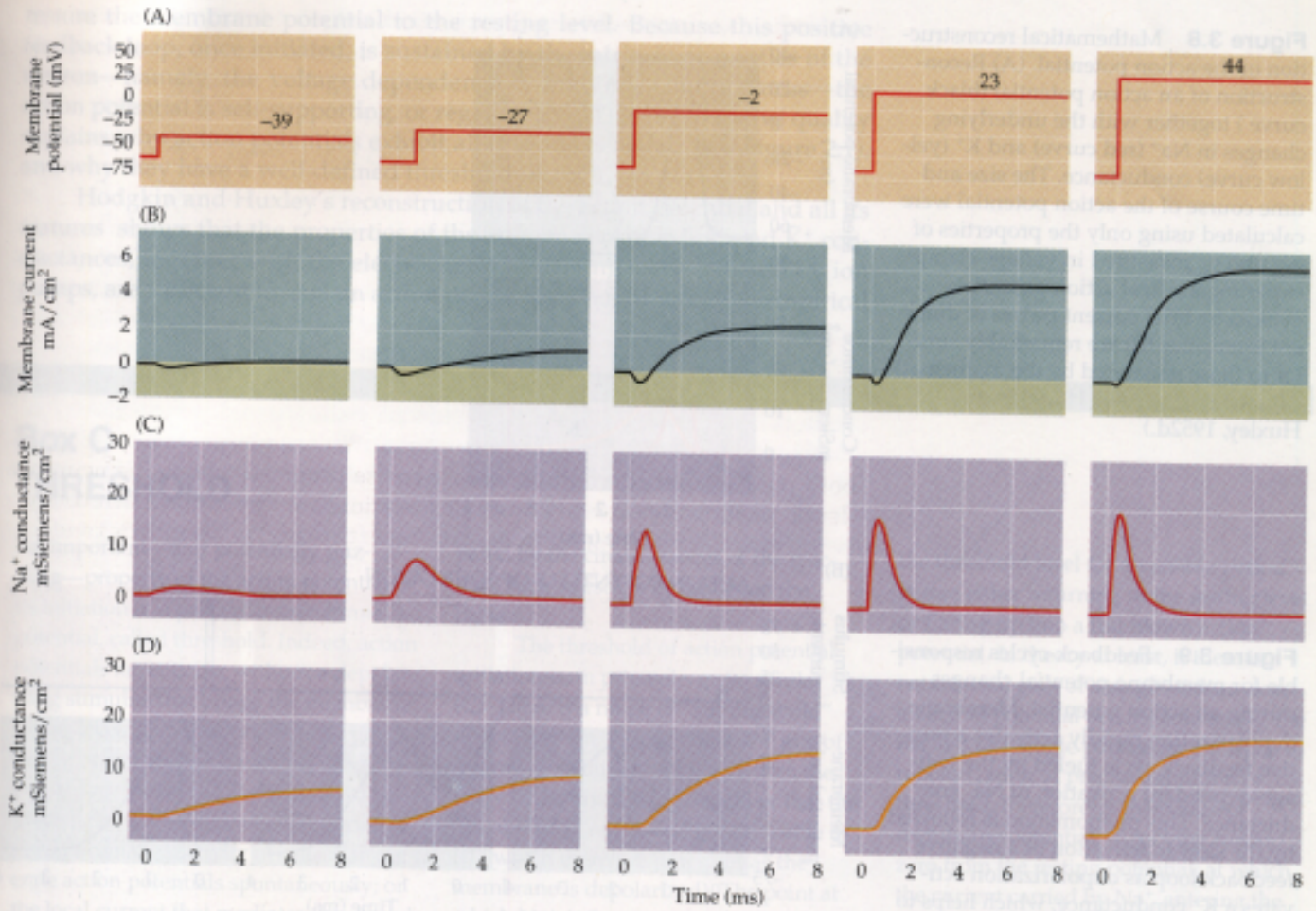


Figure 3.7 Membrane conductance changes underlying the action potential are time- and voltage-dependent. Depolarizations to various membrane potentials (A) elicit different membrane currents (B). Below are shown the Na^+ (C) and K^+ (D) conductances calculated from these currents. Both peak Na^+ conductance 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.)

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). Further, the Hodgkin-Huxley model predicted other features of action potential behavior in the squid axon, such as how the delay before action potential generation changes in response to stimulating currents of different intensities (Figure 3.8B). 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^+ conduc-

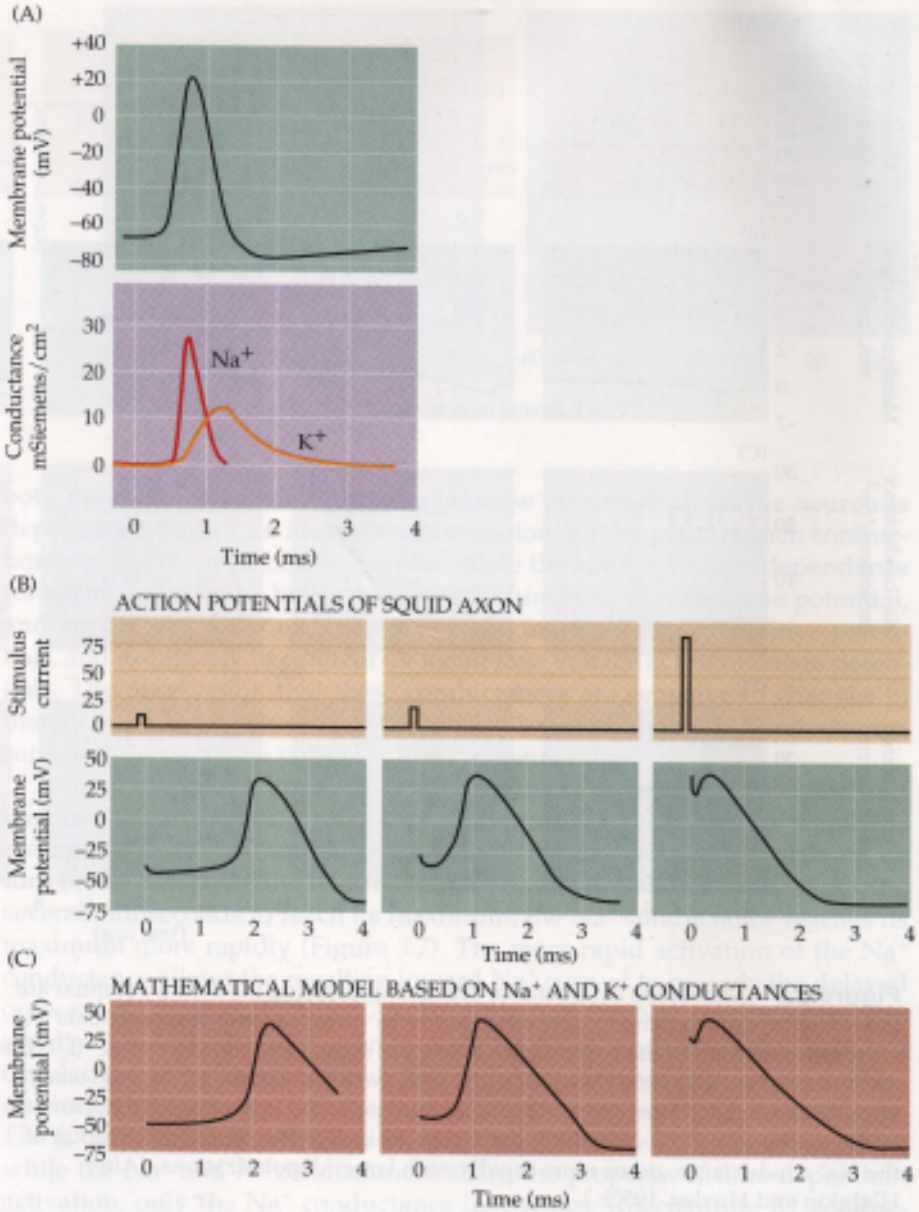


Figure 3.8 Mathematical reconstruction of the action potential. (A) Reconstruction of an action potential (black curve) together with the underlying changes in Na^+ (red curve) and K^+ (yellow curve) conductance. The size and time course of the action potential were calculated using only the properties of g_{Na} and g_K measured in voltage clamp experiments. Real action potentials evoked by brief current pulses of different intensities (B) are remarkably similar to those generated by the mathematical model (C). (After Hodgkin and Huxley, 1952d.)

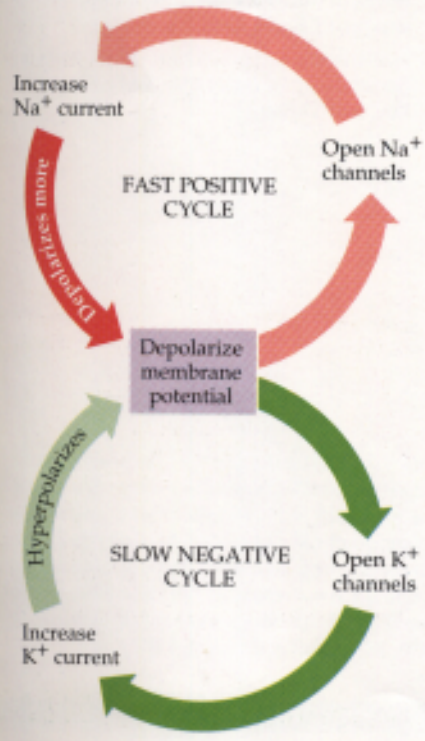


Figure 3.9 Feedback cycles responsible for membrane potential changes during an action potential. Membrane depolarization rapidly activates a positive feedback cycle fueled by the voltage-dependent activation of Na^+ conductance. This phenomenon is followed by the slower activation of a negative feedback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

tance 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.

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 2.1), and why they have a well-defined threshold (Box C).

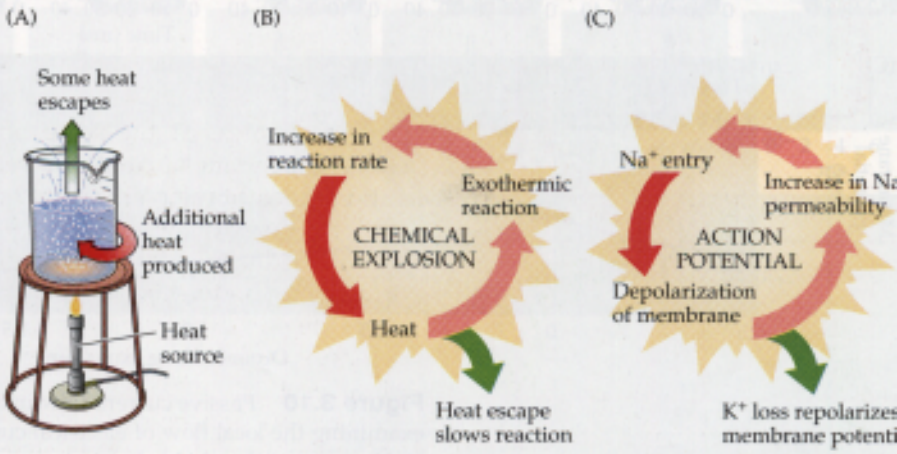
Hodgkin and Huxley's reconstruction of the action potential and all its features shows that the properties of the voltage-sensitive Na^+ and K^+ conductances, together with the electrochemical driving forces created by ion pumps, are sufficient to explain action potentials. Their use of both empirical

Box C
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 (see next section).

Why the action potential "takes off" at a particular level of depolarization can be understood by comparing the underlying events to a chemical explosion (A). As shown in the figure, exogenous heat (analogous to the depolarizing trigger event) stimulates an exothermic chemical reaction, which produces more heat, which further enhances the reaction (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, quite similar (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 sodium ion, an action potential occurs; conversely, the net loss of a single potassium ion leads to repolarization. A precise definition of threshold, therefore, is that value of membrane potential, in moving toward zero 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."



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

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 wire. Current conduction by wires, and by neurons in the absence of action potentials, is called **passive current flow**. 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 potential change that results decays exponentially with increasing distance from the site of current injection (Figure 3.10B). Typically, the potential falls to a small fraction of its ini-

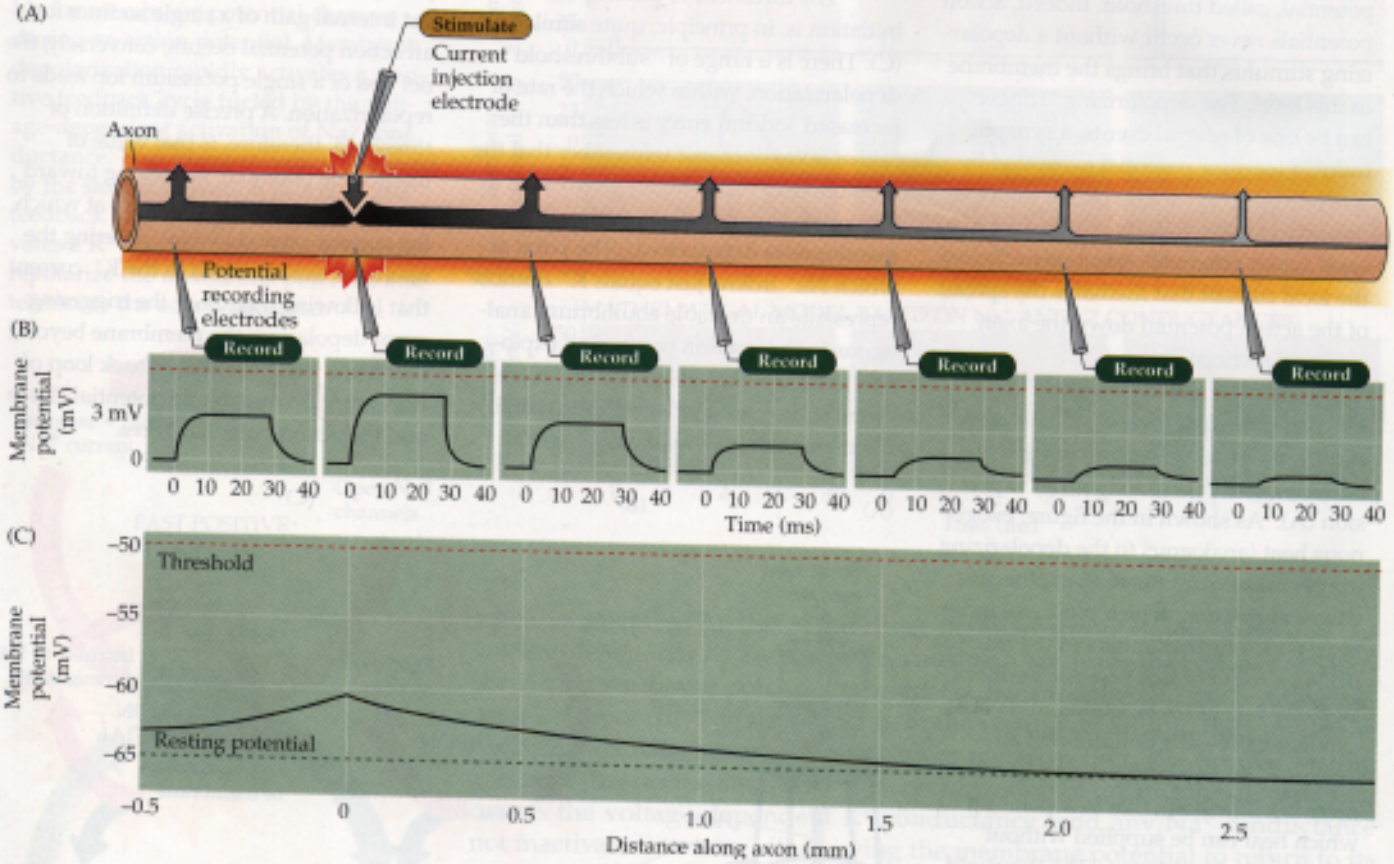


Figure 3.10 Passive current flow in an axon. (A) Experimental arrangement for examining the local flow of electrical current in an axon. A current-passing electrode produces a subthreshold change in membrane potential, which spreads passively along the axon. (B) Potential responses recorded at the positions 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. (After Hodgkin and Rushton, 1938.)

tial value at a distance 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 across the axonal membrane; accordingly, less current is available to change the membrane potential further along the axon. 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).

If the experiment shown in Figure 3.10 is repeated with a depolarizing current pulse sufficiently large to produce an action potential, the result is dramatically different (Figure 3.11). In this case, an action potential occurs without decrement along the entire length of the axon, which may be a distance of a meter or more. Thus, action potentials somehow circumvent the inherent leakiness of neurons.

How are action potentials capable of traversing great distances along such a poor passive conductor? The answer is in part 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 (Figure 3.11B). Thus, the action potential has a measurable rate of transmission,

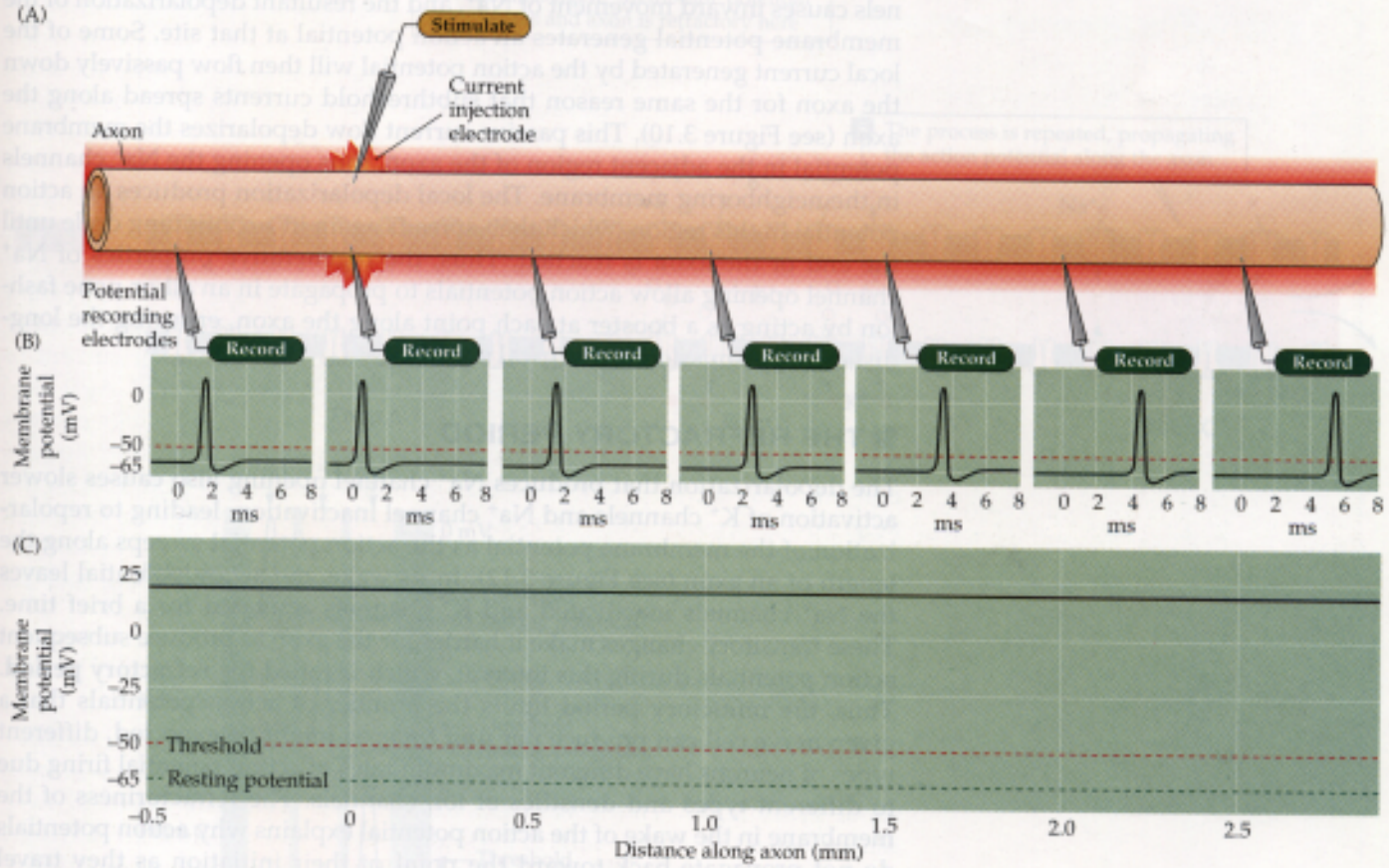


Figure 3.11 Propagation of an action potential produces the same voltage changes at all intervals along the length of an axon, albeit with an increasing delay.

Figure 3.12 Action potential conduction requires both active and passive current flow. Depolarization at one point along an axon opens Na^+ channels locally (1) and produces an action potential in this region (A) of the axon (time point 1). The resulting inward current flows passively along the axon (2), depolarizing the adjacent region (B) of the axon. At a later time (time point 2), the depolarization of the adjacent membrane has opened Na^+ channels in region B, resulting in the initiation of the action potential at this site and additional inward current that again spreads passively to an adjacent region (C) farther along the axon (3). At a still later time (time point 3), the action potential has propagated even farther. This cycle continues along the full length of the axon (5). 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 (4). ▶

called the **conduction velocity**. The delay in the arrival of the action potential at successively more distant points 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 the 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—usually a synaptic signal or a receptor potential in an intact neuron, or an injected current pulse in an experiment—locally depolarizes the axon, thus 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 will then flow passively down the axon for the same reason that subthreshold currents spread along the axon (see Figure 3.10). 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 produces an action potential in this region, which then spreads again in a continuing cycle until the end of the axon is reached. Thus, the regenerative properties of Na^+ channel opening allow action potentials to propagate in an all-or-none fashion by acting as a booster at each point along the axon, ensuring the long-distance transmission of electrical signals.

■ THE REFRACTORY PERIOD

The depolarization that produces Na^+ channel opening also causes slower activation of K^+ channels and Na^+ channel inactivation, leading to repolarization of the membrane potential as the action potential sweeps along the length of an axon (see Figure 3.12). In its wake, the action potential leaves the Na^+ channels inactivated and K^+ channels activated for a brief time. These transitory changes make it harder for the axon to produce subsequent action potentials during this interval, which is called the **refractory period**. Thus, the refractory period limits the number of action potentials that a given nerve cell can produce per unit time; as might be expected, different types of neurons have different maximum rates of action potential firing due to different types and densities of ion channels. The refractoriness of the membrane in the wake of the action potential explains why action potentials do not propagate back toward the point of their initiation as they travel along an axon.