8

Resting Potentials and Action Potentials in Excitable Cells

As discussed in Chapter 7, resting potentials are characteristic features of all cells in the body. But, nerve cells and other excitable cells, such as muscle cells, not only have resting potentials but are capable of altering these potentials for the purpose of communication, in the case of nerve cells, and for the purpose of initiating contraction, in the case of muscle cells. The material that follows introduces the ionic mechanisms that endow excitable membranes with this ability.

EXTRACELLULAR RECORDING OF THE NERVE ACTION POTENTIAL

The existence of "animal electricity" was known for well over 200 years, but the first direct experimental evidence for it was not provided until the development of electronic amplifiers and oscilloscopes. Figure 8.1 illustrates one of the earliest recordings that demonstrated the ability of nerve cells to alter their electrical activity for the purpose of coding and transmitting information. In this experiment, performed in 1934 by Hartline, extracellular recordings were made from the optic nerve of an invertebrate eye. Details of the techniques and interpretation of these extracellular recordings are described in Chapter 11, but for now it is sufficient to know that it is possible to place an electrode on the surface of a nerve axon and record electrical events that are associated with potential changes taking place across the axonal membrane. In the experiment illustrated in Fig. 8.1, light flashes of different intensities were delivered to the eye. With a very weak intensity light flash, there was no change in the baseline electrical activity. When the intensity of the light flash was increased, however, small spikelike transient events associated with the onset of the light were observed. Increasing the intensity of the light flash produced an increase in the rate of these spikelike events. These spikelike events are known as nerve action potentials, impulses, or, simply, spikes.

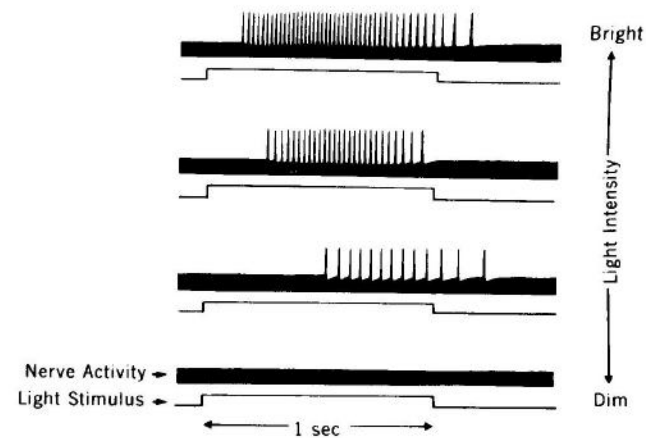

FIG. 8.1. Action potentials recorded from an invertebrate optic nerve in response to light flashes of different intensities. With dim illumination no action potentials are recorded, but with more intense illumination the number and frequency of action potentials increases. (Modified from H. K. Hartline, *J Cell Comp Physiol* 1934;5:229.)

Despite the fact that this experiment was performed more than 50 years ago, it nonetheless illustrates three basic properties of nerve action potentials and how they are utilized by the nervous system to encode information. First, nerve action potentials are very short, having a duration of only about 1 msec (1 msec $= 10^{-3}$ sec). Second, action potentials are initiated in an *all-or-nothing* manner. Note that the amplitude of the action potentials does not vary during a sustained light flash. Third, and related to the above, with increasing stimulus intensity, it is not the size of action potentials that varies but rather their number or frequency. This is the general means by which intensity information is coded in the nervous system, and it is true for a variety of peripheral receptors. The greater the intensity of a physical stimulus (whether it be a stimulus to a photoreceptor, a stimulus to a mechanoreceptor in the skin, or a stimulus to a muscle receptor), the greater is the frequency of nerve action potentials. This finding has given rise to the notion of the frequency code for stimulus intensity in the nervous system.

Most of the information transmitted to the central nervous system from the periphery is mediated by nerve action potentials. Moreover, all the motor commands initiated in the central nervous system are propagated to the periphery by nerve action potentials, and action potentials produced in muscle cells are the first step in the initiation of muscular contraction. Action potentials are therefore quite important, not only for the functioning of the nervous system, but also for the functioning of muscle cells, and for this reason it is important to understand the ionic mechanisms that underlie the action potential and its propagation.

INTRACELLULAR RECORDING OF THE RESTING POTENTIAL

The action potentials illustrated on Fig. 8.1 were recorded with extracellular electrodes. To examine the properties of action potentials in greater detail, it was necessary to move from these rather crude extracellular techniques to intracellular recording techniques.

Figure 8.2 illustrates in a schematic way how it is possible to record the membrane potential of a living cell. The upper left of Fig. 8.2A is an idealized nerve cell, composed of a cell body with a portion of its attached axonal process. Outside the nerve cell in the extracellular medium is a glass microelectrode that is connected to a suitable voltage recording device, such as a voltmeter, a pen recorder, or an oscilloscope. A glass microelectrode is nothing more than a piece of thin capillary tubing that is stretched under heat to produce a very fine tip having a diameter less than 1 μm. The electrode is then filled with an electrolyte solution such as KCl to conduct current. Initially, with the microelectrode in the extracellular medium, no potential difference is recorded, simply because the extracellular medium (the extracellular fluid) is isopotential. If, however, the microelectrode penetrates the cell membrane so that the tip of the microelectrode is inside the cell, a sharp deflection is obtained on the recording device (Fig. 8.2B). The potential suddenly shifts from its initial value of 0 mV to a new value of −60 mV. The inside of the cell is negative with respect to the outside. The potential difference that is recorded when a living cell is impaled with a microelectrode is known as the resting potential. The

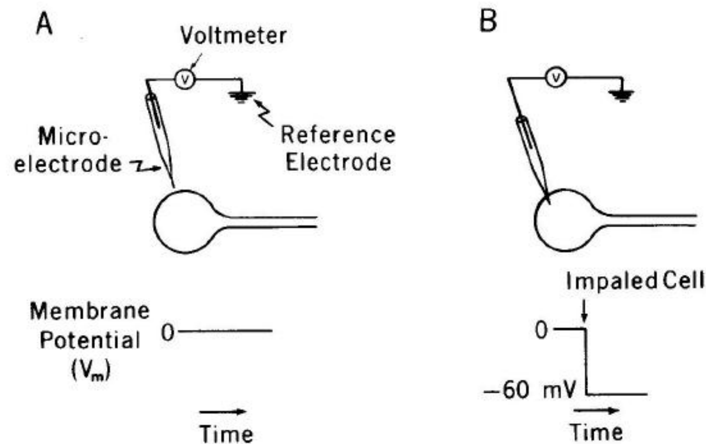

FIG. 8.2. Intracellular recording of the resting potential. **(A)** One input to a voltmeter is connected to a microelectrode, and the second input is connected to a reference electrode in the extracellular medium. No potential difference is recorded when the tip of the microelectrode is outside the cell. **(B)** When the tip of the microelectrode penetrates the cell a resting potential of −60 mV is recorded. (Modified from E. R. Kandel, *The cellular basis of behavior*. San Francisco: Freeman, 1976; Chapters 5 and 6.)

resting potential remains constant for indefinite periods of time as long as the cell is not stimulated or no damage occurs to the cell with impalement. The resting potential varies somewhat from nerve cell to nerve cell (−40 to −90 mV), but a typical value is about −60 mV.

INTRACELLULAR RECORDING OF THE NERVE ACTION POTENTIAL

The techniques for examining resting potentials can be extended to study the action potential. Although nerve action potentials are normally initiated by mechanical, chemical, or photic stimuli to classes of specialized receptors or by a process known as synaptic transmission (see Chapters 12 and 13), it is possible to artificially elicit action potentials in nerve cells and study their underlying ionic mechanisms in considerable detail and in a controlled fashion.

Figure 8.3 shows another idealized nerve cell with its cell body and attached

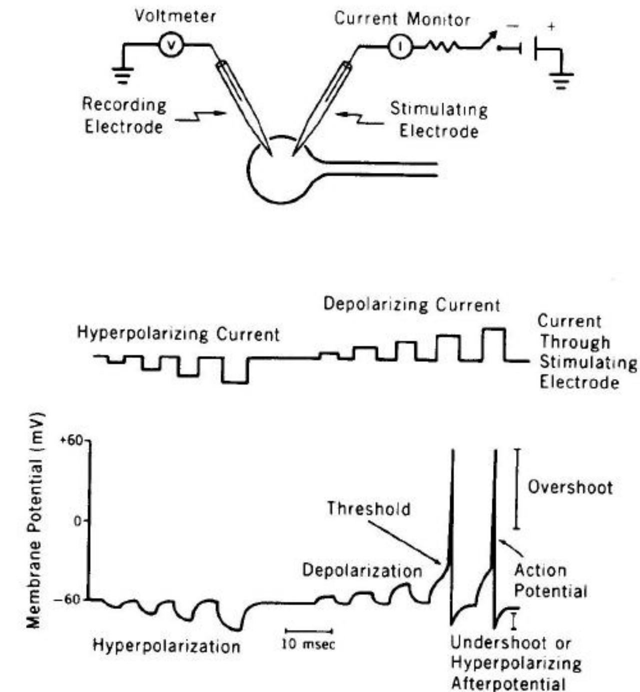

FIG. 8.3. Intracellular recording of the action potential. **Top:** A second intracellular microelectrode is used to hyperpolarize or depolarize the cell artificially. **Center:** Hyperpolarizing and depolarizing current pulses of increasing amplitudes were passed into the cell. **Bottom:** If the magnitude of the depolarizing current is sufficient to depolarize the membrane potential to threshold, an action potential is initiated. (Modified from E. R. Kandel, *The cellular basis of behavior*. San Francisco: Freeman, 1976; Chapters 5 and 6.)

axon. One microelectrode has penetrated the cell membrane so that the tip of the electrode is inside the cell. This electrode will be used to monitor the potential difference between the outside and inside of the cell. When this electrode penetrates the cell, a resting potential of about −60 mV is recorded. The cell is also impaled with a second microelectrode that will be used to alter the membrane potential artificially. This second electrode, called the stimulating electrode, is connected to a suitable current generator (in the simplest case, this current generator can be considered a battery). Obviously, there are two ways that a battery can be connected to any circuit. The battery can be inserted so that its positive pole is connected to the electrode, or the battery can be inserted so that its negative pole is connected to the electrode. A switch is placed in the circuit so that the battery can be connected and disconnected to the circuit. Assume that a small battery is inserted and its negative pole is connected to the stimulating electrode. With the switch open, a resting potential of −60 mV is recorded. As a result of closing the switch, however, the negative pole of the battery is connected to the stimulating electrode, which tends to artificially make the inside of the cell more negative relative to the external solution. There is a slight downward deflection of the recording trace.

If we repeat this experiment but instead use a slightly larger battery, more current flows into the cell, and a larger increase in the negativity of the cell is recorded. Larger batteries produce even greater increases in the potential. Any time the negativity of the cell interior is increased, the potential change is known as a hyperpolarization. The membrane is more polarized than normal.

This experiment can be repeated in a slightly different way by connecting the positive pole of the battery to the stimulating electrode. Turning on the switch now makes the inside of the cell artificially more positive relative to the external solution. The polarized state of the membrane is decreased. Increasing the size of the battery produces a greater decrease in the negativity of the cell, and over a limited range the resultant potential is a graded function of the size of the stimulus that is used to produce it. Any time the interior of the cell becomes more positive, the potential change is known as a depolarization.

These hyperpolarizations and depolarizations that are artificially produced are known as electrotonic, graded, or passive potentials. Some additional features of electrotonic potentials are discussed later. The point to note here, however, is that, within a limited range of stimulus intensities, hyperpolarizing and depolarizing electrotonic potentials are graded functions of the size of the stimulus used to produce them.

An interesting phenomenon occurs when the magnitude of the battery used to produce the depolarizing potentials is increased further. As the size of the battery and thus the amount of depolarization is increased, a critical level is reached known as the *threshold*, where a new type of potential is produced that is different in its amplitude, duration, and form from the depolarizing pulse used to produce it. This new type of potential change elicited when threshold is reached is known as the action potential. The action potential is elicited in an all-or-nothing fashion. Stimuli below threshold fail to elicit an action potential; stimuli at threshold or above threshold successfully elicit an action potential. Increasing the stimulus intensity beyond

threshold produces an action potential that is identical to the action potential produced at the threshold level. In this experiment, the duration of the depolarization is so short that only a single action potential could be initiated. If the duration is longer, multiple action potentials are initiated, and their frequency depends on the stimulus intensity. This is simply a restatement of the all-or-nothing law of action potentials presented earlier. Below threshold, no action potential is elicited; at or above threshold, an all-or-nothing action potential is initiated. Increasing the stimulus intensity still further produces the same amplitude action potential; only the frequency is increased.

Not only are action potentials elicited in an all-or-nothing fashion, but as is described in Chapter 10 they also propagate in an all-or-nothing fashion. If an action potential is initiated in the cell body, it will propagate along the nerve axon and eventually invade the synaptic terminals and initiate a process known as synaptic transmission (see Chapters 12 and 13). Unlike action potentials, electrotonic potentials do not propagate in an all-or-nothing fashion. Electrotonic potentials do spread but only for short distances (see Chapter 10).

There are several interesting features of the action potential. One is that the polarity of the cell completely reverses during the peak of the action potential. Initially, the inside of the cell is −60 mV with respect to the outside, but, during the peak of the action potential, the potential reverses and approaches a value +55 mV inside with respect to the outside. The region of the action potential that varies between the 0-mV level and its peak value is known as the *overshoot*. Another interesting characteristic of action potentials is their repolarization phase (the return to the resting level). The action potential does not immediately return to the resting potential of −60 mV; there is a period of time when the cell is actually more negative than the resting level. This phase of the action potential is known as the *undershoot* or the *hyperpolarizing* afterpotential.

As indicated earlier, nerve potentials are the vehicles by which peripheral information is coded and propagated to the central nervous system; motor commands initiated in the central nervous system are propagated to the periphery by nerve action potentials, and the action potential is the first step in the initiation of muscular contraction.

IONIC MECHANISMS OF THE RESTING POTENTIAL

Although the major focus of this chapter and that of Chapter 9 is to explain the ionic mechanisms that underlie the action potential, it is first necessary to review the ionic mechanisms that underlie the resting potential, since the two are intimately related. The basic principles have been introduced in Chapter 7.

Bernstein's Hypothesis (for the Resting Potential)

In 1902, Julius Bernstein proposed the first satisfactory hypothesis for the generation of the resting potential. Bernstein knew that the inside of cells have high K^+

and low Na^+ concentrations, and that the extracellular fluid has low K^+ and high Na^+ concentrations. In addition, there appeared to be large negatively charged molecules, presumably proteins, to which the cell was impermeable. Bernstein also knew (a critical piece of information) that cells were highly permeable to K^+ but not very permeable to other ions. Furthermore, Bernstein knew of the work of the physical chemist, Nernst. Bernstein therefore suggested that one could predict the resting potential simply by applying the Nernst equilibrium equation for potassium:

$$V_m \overset{?}{=} E_K = 60 \log \frac{[K^+]_o}{[K^+]_i} \text{ (mV)} \qquad [8.1]$$

where V_m is the membrane potential and E_K the potassium equilibrium potential (see also Chapter 4).

Although Bernstein's hypothesis was very interesting, it could not be directly tested at the time (hence, the question mark in the equation) because microelectrode recording techniques had not been developed. It was not until the 1930s and 1940s and the advent of microelectrode recording techniques that it became possible to test the hypothesis directly. The testing of Bernstein's hypothesis was done primarily by Hodgkin and Huxley and their colleagues in England. As a result of this work, a general theory was developed for the generation of the resting potential that appears to be applicable to most cells in the body.

Testing Bernstein's Hypothesis

How would one go about testing Bernstein's hypothesis? If the membrane potential (V_m) is equal to the K^+ equilibrium potential (E_K), one should be able to substitute the known outside and inside concentrations of K^+ (Table 8.1) into the Nernst equation and determine the equilibrium potential (E_K), which should equal the measured membrane potential (V_m). Furthermore, because of the logarithmic relationship in the Nernst equation, if the outside K^+ concentration is artificially manipulated by a factor of 10, then the equilibrium potential will change by a factor of 60 mV. If the membrane potential is governed by the K^+ equilibrium potential, then the membrane potential should also change by 60 mV.

TABLE 8.1. *Some common values of ion concentrations*

Ion	Extracellular concentration (mM)	Intracellular concentration (mM)	Nernst potential (mV)
Squid giant axon			
Na^+	440	50	+55
K^+	20	400	−75
Mammalian muscle fiber			
Na^+	145	12	+63
K^+	4	155	−92

Figure 8.4 illustrates one direct experimental test of Bernstein's hypothesis performed by Hodgkin and Horowicz. A cell was impaled with a microelectrode, and the resting potential was measured. The extracellular K^+ concentration was systematically varied, and the change in the resting potential was monitored. When the K^+ concentration was changed by a factor of 10, the resting potential changed by a factor of 60 mV. The straight line on the plot is the relationship predicted by the Nernst equation (note that it is a straight line because the data are plotted on a semilog scale).

The fit is not perfect, however, and the experimental data deviate from the predicted values when the extracellular K^+ concentration is reduced to low levels. If there is a deviation from the Nernst equation, the membrane must be permeable, not only to K^+, but to another ion as well. That other ion appears to be Na^+. As indicated earlier, Na^+ has a high concentration outside the cell and a low concentration inside the cell. If the cell has a slight permeability to Na^+, Na^+ will tend to diffuse into the cell and produce a charge distribution across the membrane so that the inside of the membrane will be positive with respect to the outside. This slight increase in the positivity on the inside surface of the membrane will tend to reduce

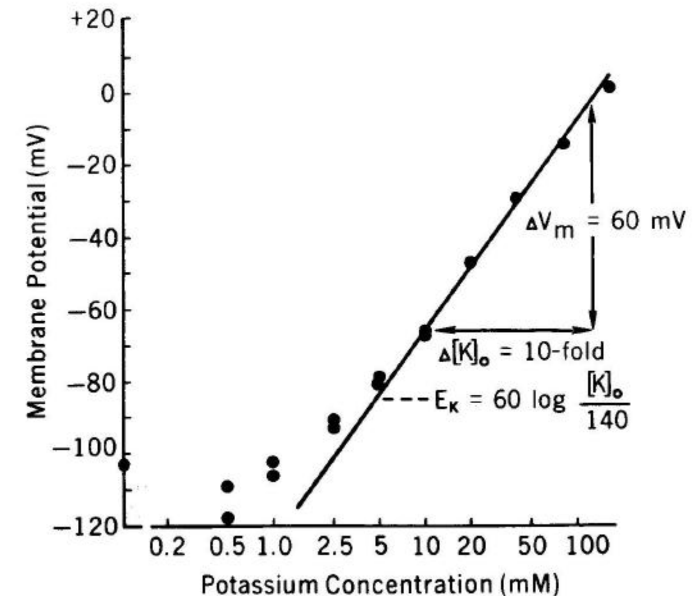

FIG. 8.4. Effects of altered extracellular concentrations of K^+ on the membrane potential: (•) measured membrane potential at each of a variety of different concentrations of K^+; (—) potential predicted by the Nernst equation. The value of 140 in the Nernst equation is the estimated intracellular concentration of K^+ for the cell used in this experiment. (Modified from A. L. Hodgkin and P. Horowicz, *J. Physiol.* 1959;148:127.)

the negative charge distribution produced by the diffusion of K^+ out of the cell. The slight permeability of the membrane to Na^+ will tend, therefore, to make the cell slightly less negative than would be expected were the membrane only permeable to K^+. If a membrane is permeable to more than one cation, the Nernst equation cannot be used to predict the resultant membrane potential. In such a case, however, the Goldman equation can be utilized.

GOLDMAN-HODGKIN-KATZ EQUATION

The Goldman equation is also known as the Goldman-Hodgkin-Katz (GHK) equation because Hodgkin and Katz applied it to biological membranes. As has already been seen in Chapter 7, the GHK equation can be used to determine the potential developed across a membrane permeable to Na^+ and K^+. Thus,

$$V_m = 60 \log \frac{[K^+]_o + \alpha[Na^+]_o}{[K^+]_i + \alpha[Na^+]_i} \text{ (mV)} \quad [8.2]$$

where V_m is the membrane potential in millivolts and α is equal to the ratio of the Na^+ and K^+ permeabilities (P_{Na}/P_K). This equation looks rather complex at first, but it can be pared down to size by examining two extreme cases. Consider the case when the Na^+ permeability is equal to zero. Then, α is equal to zero, and the GHK equation reduces to the Nernst equation for K^+. If the membrane is highly permeable to Na^+ and has a very low K^+ permeability, α will be a very large number, which causes the Na^+ terms to be very large so that the K^+ terms can be neglected and the GHK equation reduces to the Nernst equation for Na^+. Thus, the GHK equation has two extremes. In one case, when Na^+ permeability is zero, it reduces to the Nernst equation for K^+; in the other case, when Na^+ permeability is very high, it reduces to the Nernst equation for Na^+. The GHK equation allows one to predict membrane potentials between these two extreme levels, and these membrane potentials are determined by the ratio of K^+ and Na^+ permeabilities. If the permeabilities are equal, the membrane potential will be intermediate between the K^+ and the Na^+ equilibrium potentials.

Figure 8.5 illustrates a test of the ability of the GHK equation to fit the same experimental data shown in Fig. 8.4. The straight line is generated by the Nernst equation, whereas the curved trace is generated by the GHK equation. The value of α that gives the best fit is 0.01. Thus, although there is some Na^+ permeability at rest, it is only one hundredth that of the K^+ permeability. To a first approximation, the membrane potential is due to the fact that there is unequal distribution of K^+, and the membrane is selectively permeable to K^+ and to a large extent no other ion. Therefore, the membrane potential can be roughly predicted by the Nernst equilibrium potentials for K^+. However, there is a slight Na^+ permeability that tends to make the inside of the cell more positive than would be predicted, based on the assumption that the cell is only permeable to K^+. The GHK equation can be used to calculate or predict the membrane potential knowing the ratio of Na^+ and K^+

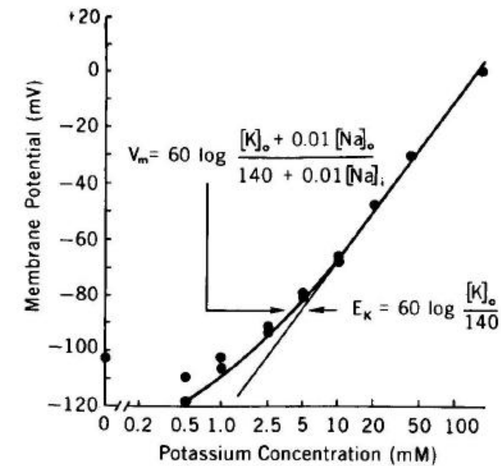

FIG. 8.5. Same experiment as Fig. 8.4, but the graph also contains the prediction of the change in membrane potential obtained with the GHK equation with a value of α equal to 0.01. (Modified from A. L. Hodgkin and P. Horowicz, *J. Physiol.* 1959;148:127.)

permeabilities and the individual extracellular and intracellular concentrations of Na^+ and K^+.

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9

Ionic Mechanisms Underlying the Action Potential

Having reviewed the ionic mechanisms that account for the generation of the resting potential, we can now examine the ionic mechanisms that account for the action potential.

THE SODIUM HYPOTHESIS FOR THE NERVE ACTION POTENTIAL

Is it possible to specify ionic mechanisms that account for the action potential just as it was possible to do so for the resting potential? It is interesting to note that Julius Bernstein in 1902, when proposing his theory for the resting potential, also proposed a theory for the nerve action potential. Bernstein proposed that during a nerve action potential, the membrane suddenly became permeable to all ions. Bernstein predicted, based on this theory, that the membrane potential would shift from its resting level to a new value of about 0 mV. (Can you explain why?) We have already learned, however, that the potential changes during the action potential do not range from a value of −60 to 0 mV, but actually go well beyond 0 mV and approach a value of +55 mV. So while Bernstein's hypothesis for the resting potential was nearly correct, his hypothesis for the action potential clearly missed the mark.

At the same time that Bernstein proposed theories for resting potentials and action potentials, Overton, another physiologist, made some interesting observations about the critical role of Na^+. Overton observed that Na^+ in the extracellular medium was absolutely essential for cellular excitability. In general, in the absence of extracellular Na^+, nerve axons cannot propagate information, and *skeletal* muscle cells are unable to contract.

Overton, like Bernstein, could not test his hypothesis experimentally because microelectrodes were not available. Just as Hodgkin and his colleagues critically tested Bernstein's hypothesis for the resting potential, they also examined and extended Overton's observations. One of the earlier experiments performed by Hodgkin and Katz is illustrated in Fig. 9.1.

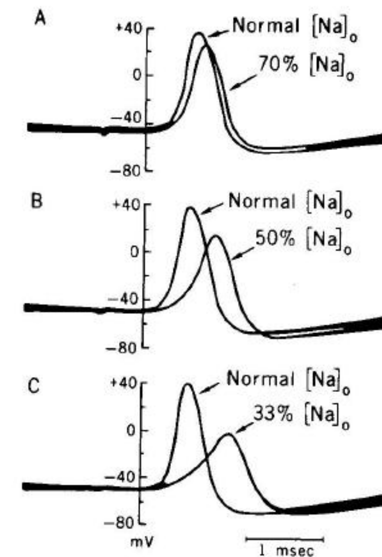

FIG. 9.1. Changes in the amplitude of the action potential in the squid giant axon as a function of the extracellular concentration of Na^+ reduced to 70% **(A)**, 50% **(B)**, and 33% **(C)** of its normal value. (Modified from A. L. Hodgkin and B. Katz, *J Physiol* 1949;108:37.)

Hodgkin and Katz repeatedly initiated action potentials in the squid giant axon while they artificially altered the extracellular Na^+ concentration. When the extracellular Na^+ concentration was reduced to 70% of its normal value (Fig. 9.1A), there was a slight reduction in the amplitude of the action potential. Reducing the Na^+ concentration to 50% and 33% of its normal value produced further reductions in the amplitude of the action potential. These experiments, therefore, directly confirmed Overton's initial observations that Na^+ is essential for the initiation of action potentials (exceptions to this are action potentials in cardiac and smooth muscle cells).

Hodgkin and his colleagues took these observations one step further. They suggested that during an action potential, the membrane behaved as though it was becoming selectively permeable to Na^+. In a sense, the membrane was "switching" from its state of being highly permeable to K^+ at rest to being highly permeable to Na^+ at the peak of the action potential. If a membrane is highly permeable to Na^+ at the peak of the action potential (for the sake of simplicity we assume that the membrane is solely permeable to Na^+ and no other ions), what potential difference would one predict across the cell membrane? If the membrane is only permeable to Na^+, the membrane potential should equal the Na^+ equilibrium potential (E_{Na}); and

$$V_m \overset{?}{=} E_{Na} = 60 \log \frac{[Na^+]_o}{[Na^+]_i} \text{ (mV)} \qquad [9.1]$$

Indeed, when the known values of extracellular and intracellular Na^+ concentrations for the squid giant axon are substituted, a value of +55 mV is calculated. This is approximately the peak amplitude of the action potential. Is this simply a coincidence? It is possible that the membrane is permeable to other ions as well. Perhaps the action potential is due to an increase in Ca^{2+} permeability; Ca^{2+} is in high concentration outside and low concentration inside the cell, so part of the action potential might be due to a selective increase in Ca^{2+} permeability. How can this issue be resolved? If the peak amplitude of the action potential is determined by E_{Na}, one would expect that as the extracellular levels of Na^+ are altered, the peak amplitude of the action potential would change according to the Nernst equation. Furthermore, because of the logarithmic relationship in the Nernst equation, if the extracellular Na^+ concentration is changed by a factor of 10, the Na^+ equilibrium potential and the peak amplitude of the action potential should change by a factor of 60 mV.

Figure 9.2 illustrates a test of this hypothesis. The peak amplitude of the action potential, shown on the vertical axis, is measured as a function of the extracellular Na^+ concentration. The dots on the graph represent the peak amplitude of the action potential recorded at various extracellular concentrations of Na^+. The straight line

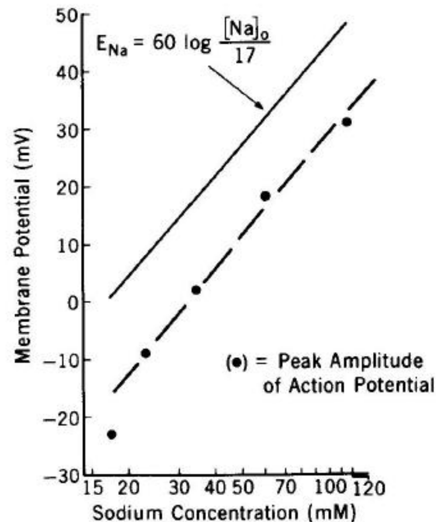

FIG. 9.2. Plot of the peak amplitude of the action potential vs. the extracellular concentration of Na^+. The *solid line* is a prediction of the Nernst equation with an estimated intracellular Na^+ concentration for this cell of 17 mM. (Modified from W. L. Nastuk and A. L. Hodgkin *J Cell Comp Physiol* 1950;35:39.)

is the relationship that describes the Na^+ equilibrium potential as a function of extracellular Na^+.

Although there are some deviations between the predicted Na^+ equilibrium potential and the peak amplitude of the action potential (the action potential never quite reaches the value of the Na^+ equilibrium potential), the critical observation is that the slopes of these two lines are nearly identical. For a tenfold change in the extracellular Na^+ concentration, there is approximately a 60-mV change in the peak amplitude of the action potential. These experiments, therefore, provide strong experimental support for the hypothesis that during the peak of the action potential, the membrane suddenly switches from a high permeability to K^+ to a high permeability to Na^+.

APPLYING THE GHK EQUATION TO THE ACTION POTENTIAL

There are two important positively charged ions (K^+ and Na^+), and the membrane potential appears to be governed by the relative permeabilities of these two ions. As a result, the GHK equation can be utilized:

$$V_m = 60 \log \frac{[K^+]_o + \alpha[Na^+]_o}{[K^+]_i + \alpha[Na^+]_i} \text{ (mV)} \qquad [9.2]$$

where $\alpha = P_{Na}/P_K$.

Figure 9.3 is a sketch of an action potential. One important observation is that the action potential traverses a region that is bounded by E_{Na} on one extreme and E_K on the other. Because the action potential traverses this bounded region, it is possible to utilize the GHK equation to predict any value of the action potential simply by adjusting the ratio of the Na^+ and K^+ permeabilities. For the resting level, we have already seen that the ratio of Na^+ and K^+ permeabilities is 0.01. Thus, we can

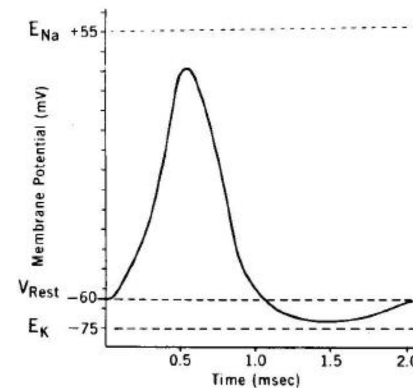

FIG. 9.3. Sketch of a nerve action potential.

substitute these values into the GHK equation and calculate a value of approximately −60 mV.

Assume that the Na^+ permeability is very high. Then α is a very large number, and the Na^+ terms dominate the GHK equation. In the limit, the GHK equation reduces to the Nernst equation for Na^+. So, when there is a high Na^+ permeability and a low K^+ permeability, we can calculate a potential that approximates the peak amplitude of the action potential. During the repolarization phase of the action potential, we can simply assume that the ratio of Na^+ and K^+ permeabilities returns back to normal, substitute this value into the GHK equation and calculate a membrane potential of −60 mV. The hyperpolarizing afterpotential could be accounted for by a slight decrease in Na^+ permeability to less than its resting level or by a K^+ permeability greater than its resting level.

The important point is that by adjusting the ratio of Na^+ and K^+ permeabilities, it is possible to predict the entire trajectory of the action potential.

THE CONCEPT OF VOLTAGE-DEPENDENT Na^+ PERMEABILITY

Despite the fact that the GHK equation gives a good qualitative fit to the trajectory of the action potential, it fails to provide any insight into the fundamental question of how the presumed switch in permeability takes place. How can a membrane at one instant in time be highly permeable to K^+ and a short time thereafter be highly permeable to Na^+? Hodgkin and Huxley proposed that there is a voltage-dependent change in Na^+ permeability; Na^+ permeability is low at rest, but as the cell is depolarized, Na^+ permeability increases (Fig. 9.4A).

Assume that the cell is depolarized by some stimulus. As a result of the depolarization, there will be an increase in Na^+ permeability. If Na^+ permeability is increased (assume for the moment that K^+ permeability remains unchanged), α in the GHK equation will be increased. If α is increased, the Na^+ terms are multiplied by a larger value, and they will tend to dominate the GHK equation. The membrane will become less negative (more depolarized); but as the cell depolarizes, Na^+ permeability increases further and α increases further. A positive feedback cycle is entered (Fig. 9.4B) such that once the cell is depolarized to a critical level, the cell will rapidly depolarize further in a regenerative fashion. Eventually, the membrane potential will approach the Na^+ equilibrium potential. Thus, a voltage-dependent relationship between membrane potential and Na^+ permeability can in principle completely account for the initiation of the action potential.

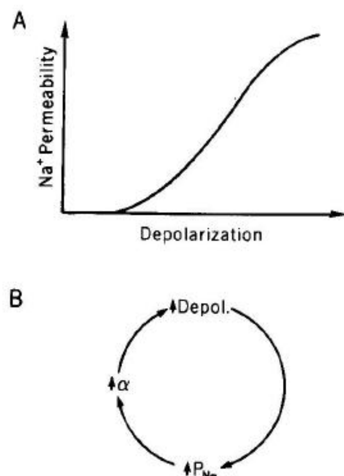

FIG. 9.4. Relationships between depolarization and Na^+ permeability critical for the initiation of an action potential (see text for explanation).

TESTING THE CONCEPT OF VOLTAGE-DEPENDENT Na^+ PERMEABILITY: THE VOLTAGE CLAMP TECHNIQUE

So far we have just a theory. The critical hypothesis is that Na^+ permeability is regulated by the membrane potential. The simple way of testing this hypothesis is to depolarize the cell to various levels and measure the corresponding Na^+ permeability. The problem, however, is that as soon as the cell is depolarized, Na^+ permeability changes, an action potential is initiated, and due to practical reasons, there is insufficient time to measure the permeability change. This was a major obstacle in the further analysis of the ionic mechanisms that govern the action potential.

Hodgkin and Huxley and their colleagues devised a scheme that allowed them to stabilize the membrane potential at various levels for indefinite periods of time. They used an electronic feedback device known as a voltage clamp amplifier to hold the membrane potential at various levels (Fig. 9.5). The voltage clamp amplifier takes the difference between the actual recorded membrane potential and the desired

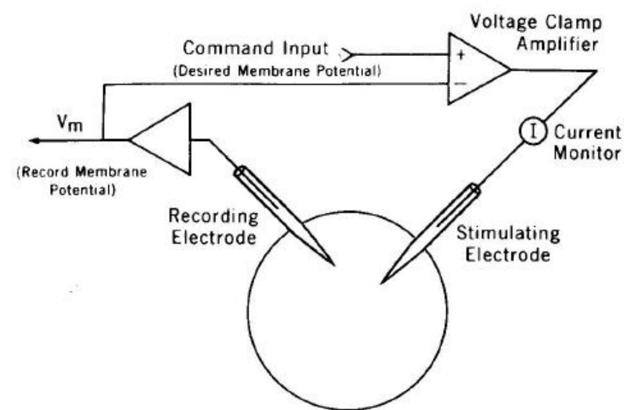

FIG. 9.5. Schematic diagram of the voltage clamp apparatus (see text for details).

level and generates sufficient hyperpolarizing or depolarizing current to minimize the difference. The amount of current necessary to hold the membrane potential fixed at the desired level provides an index of the membrane permeability, or conductance, at that particular voltage clamp level. For example, by measuring the ionic current as a function of time, $I(t)$, and knowing the potential difference (which is constant), the conductance as a function of time, $G(t)$, can be determined simply by using Ohm's law (conductance for our purpose can simply be considered an electrical measurement of permeability, so we will use permeability and conductance interchangeably):

$$G(t) = I(t)/\Delta V \quad [9.3]$$

By changing the potential difference with the voltage clamp amplifier, the corresponding conductances at a variety of different potentials can be determined.

Figure 9.6 illustrates some typical results. The procedure is as follows. Initially, the membrane potential is at its resting level of −60 mV. It is then artificially changed from the resting level to a new depolarized level (e.g., −35 mV) and held there for 5 msec or longer. The membrane potential is then returned back to its resting level, and the membrane is stepped or clamped to a new depolarized level of

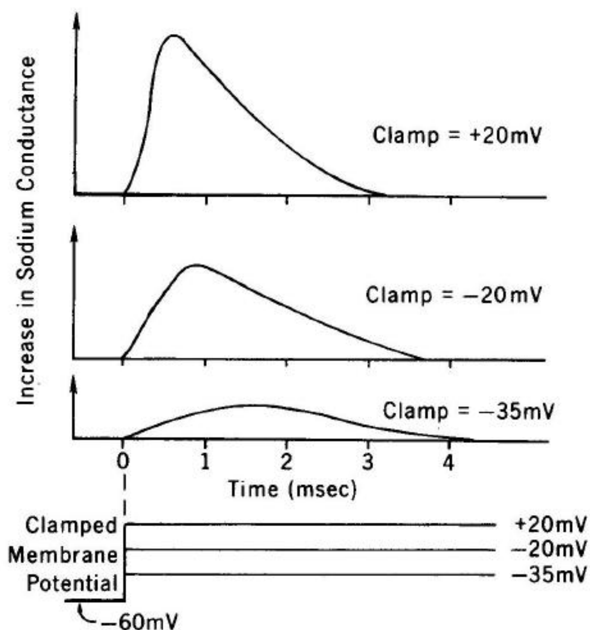

FIG. 9.6. Changes in Na^+ conductance produced by voltage steps to three depolarized levels. The greater the depolarization, the greater the amplitude of Na^+ conductance. (Modified from A. L. Hodgkin and A. F. Huxley, *J Physiol* 1952;117:500.)

−20 mV. By performing a sequence of these voltage clamp measurements, changes in Na^+ permeability as a function of both voltage and time can be determined.

In the upper part of Fig. 9.6, the horizontal axis shows the time and the vertical axis shows the measured Na^+ conductance. As the membrane potential is forced to various depolarized levels from the resting level, there is a graded increase in Na^+ permeability. The greater the level of depolarization, the greater the Na^+ permeability. This experiment therefore provides strong experimental support for the proposal that Na^+ permeability is voltage-dependent and demonstrates the existence of a mechanism that could explain the rising phase (initiation) of the action potential.

MOLECULAR BASIS FOR THE REGULATION OF Na^+ PERMEABILITY

At the molecular level, the relationship between the membrane potential and Na^+ permeability (Fig. 9.4A) is due to the existence of membrane channels that are selectively permeable to Na^+ and that are opened or gated by the membrane potential. This discovery was made possible by the patch clamp technique that allows the conductance of individual channels to be measured. With the patch clamp technique, a micropipette with a tip several microns in diameter is positioned so that the tip just touches the outer surface of the membrane (see Chapter 5). A high-resistance seal develops that allows the electrode and associated electronic circuitry to measure the current and thus the conductance of a small number of Na^+ channels or, indeed, a single Na^+ channel (Fig. 9.7). One of the major conclusions that has been derived from these studies is that, in response to membrane depolarization, single Na^+ channels open in all-or-nothing fashion. A single channel has at least two states—open and closed—and once opened it cannot open further in response to depolarization. The gating process is subserved by a membrane-bound protein that is charged, such that when the membrane is depolarized, a conformational change in the protein takes place that results in the channel becoming more permeable to Na^+ (see also below).

Individual channels open briefly and then close (Fig. 9.8). The opening of single channels is a probabilistic function of time and voltage; however, when the opening of many channels is averaged, the averaged conductance predicts the conductance change of the entire population of channels (Fig. 9.9). Thus, the time course of the changes of Na^+ permeability (Fig. 9.6) is a reflection of the average opening and closing times of many individual Na^+ channels. At the molecular level, the voltage dependence of the total membrane Na^+ permeability (Fig. 9.4A) can be viewed as the probability that a depolarization will open single Na^+ channels; the more the cell is depolarized, the greater the number of individual Na^+ channels that will be opened each in its characteristic all-or-nothing fashion.

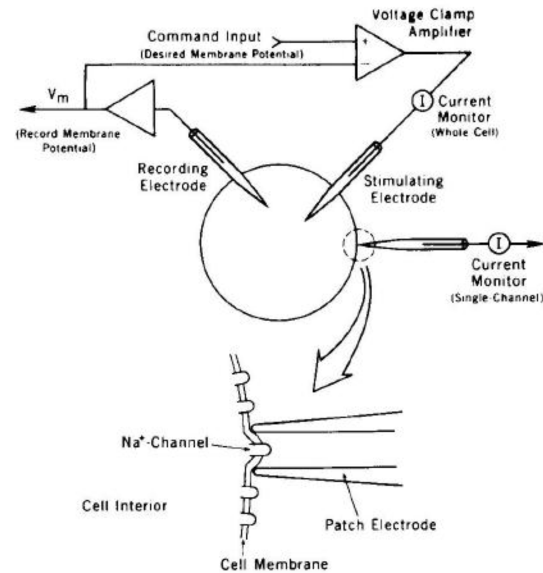

FIG. 9.7. Schematic diagram of the apparatus used for single-channel recording. A micropipette is positioned to touch the surface of the membrane. A tight seal develops, and the current flowing through individual channels can be monitored. Membrane potential can be altered by the conventional two-microelectrode technique.

STRUCTURE OF THE VOLTAGE-GATED Na^+ CHANNEL

The principal structural and functional unit of the voltage-gated Na^+ channel consists of a single polypeptide chain exhibiting four homologous domains (I to IV, Fig. 9.9), with each domain having six hydrophobic membrane-spanning regions designated S1 to S6. The functional significance of specific regions is now being elucidated. For example, region S4 contains a high density of positively charged residues and is believed to represent the channel's voltage sensor. The channel pore is believed to be formed by the four homologous regions between S5 and S6 (also designated the SS1 and SS2 regions, the H5 region or the "P" region). Channel inactivation (see below) seems to be associated with the region that links domains III and IV. In particular, a hinged-lid structure formed by the amino terminus of domain IV has properties consistent with an ability to move into the channel pore and cause its blockade. Finally, the Na^+ channel can be regulated by protein phosphorylation. Specifically, the region that links domains I and II contains phosphorylation sites for cAMP-dependent protein kinase, whereas the region that links domains III and IV contains a phosphorylation site for protein kinase C.

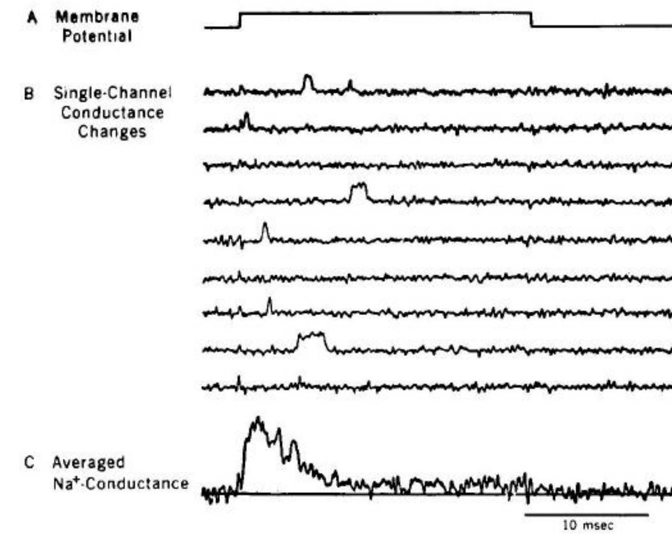

FIG. 9.8. Single-channel changes in Na^+ conductance. **(A)** In response to a pulse depolarization, the probability of single Na^+ channels opening is increased. **(B)** Successive traces obtained in response to multiple presentations of the voltage step in **(A)**. With any single trace the relationship is not particularly clear. **(C)** When the traces are averaged, a conductance change resembling that of the macroscopic conductance change is observed. (Modified from F. J. Sigworth and E. Neher, *Nature* 1980;287:447–449.)

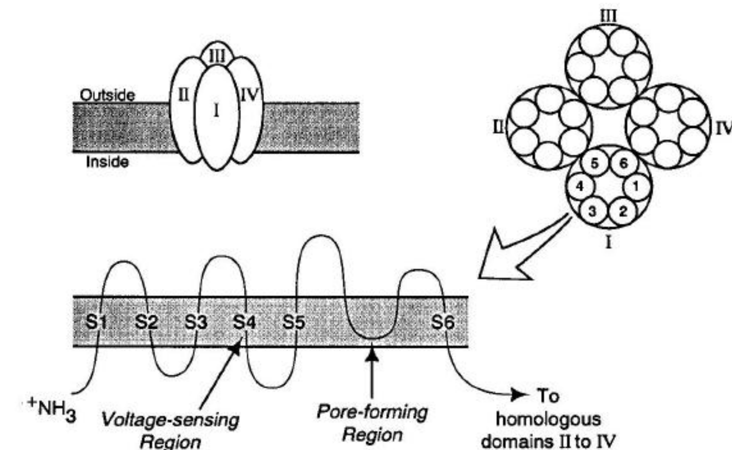

FIG. 9.9. Model of the principal structural and functional unit of the voltage-gated Na^+ channel.

Na^+ INACTIVATION

There is one other important aspect of the data illustrated in Fig. 9.6. Despite the fact that the membrane potential is depolarized throughout the duration of each trace, Na^+ permeability spontaneously falls back to its resting level. Thus, although Na^+ permeability is dependent on the level of depolarization, it does not remain elevated and is only transient. Once it reaches its maximum value, it spontaneously decays back to its resting level. The process by which Na^+ permeability spontaneously decays back to its resting level (despite the fact that the membrane is depolarized) is known as *inactivation*. At the molecular level, the process of inactivation can be considered to be a separate voltage- and time-dependent process regulating the Na^+ channel. In Fig. 9.10, the Na^+ channel is represented as having two regulatory components: an activation gate and an inactivation gate. For the channel to be open, both the activation and inactivation gates must be open. At the resting potential, the activation gate is closed, and despite the fact that the inactivation gate is open, channel permeability is zero (Fig. 9.10A). With depolarization, the activation gate opens rapidly (Fig. 9.10B), and the channel becomes permeable to Na^+. Depolarization also tends to close the inactivation gate, but the inactivation process is slower. With depolarization occurring over a longer time, the inactivation gate closes, and even though the activation gate is still open, channel permeability is zero (Fig. 9.10C).

What is the physiological significance of Na^+ inactivation? Let us return to the positive feedback cycle once again. Depolarization increases Na^+ permeability, and the increase in Na^+ permeability depolarizes the cell. Eventually, as a result of this regenerative cycle, the cell is rapidly depolarized up to a value near E_{Na}. The problem with this mechanism is how to account for the repolarization phase of the action potential. Based on the relationship between Na^+ permeability and membrane potential, one would predict that once the membrane potential moves to E_{Na} it would stay there for an indefinite period of time. The steep relationship between voltage and Na^+ permeability is only transient, however. After approximately 1 msec, Na^+ permeability spontaneously decays. If Na^+ permeability decays, due to inactivation, the potential would move closer to E_K; or, stated in a slightly different way, it will become less depolarized. Depolarization would be reduced and the reduction in depolarization would produce a further reduction in Na^+ permeability because of the basic relationship between voltage and Na^+ permeability (Fig. 9.4A). As a result, a new feedback cycle is initiated that would tend to repolarize the cell.

It is therefore intriguing to think that simply by accounting for (a) the voltage-dependent increase in Na^+ permeability and (b) the process of Na^+ inactivation, both the initiation and the repolarization phases of the action potential could be explained fully. There are at least two problems with this hypothesis. First, the duration of the action potential is only about 1 msec, yet the Na^+ permeability change takes 4 msec or so to return to its resting level (fully inactivate). So by extrapolating these voltage clamp measurements, one might expect that the action potential would be somewhat longer in duration than 1 msec. Second, it is difficult

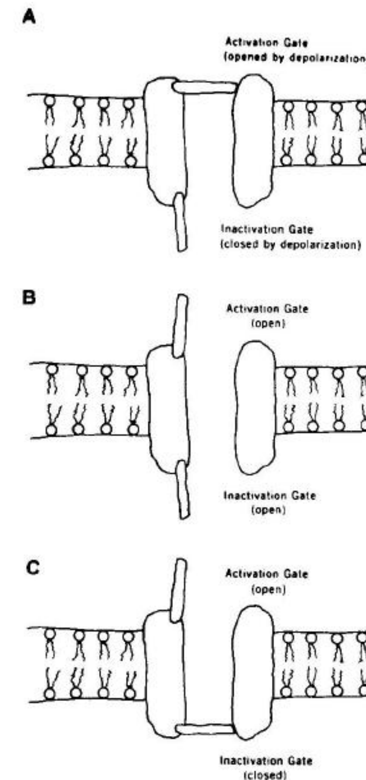

FIG. 9.10. Schematic diagram of three states of the Na^+ channel (see text for details). **(A)** Rest. **(B)** Peak g_{Na}. **(C)** Inactivated.

to explain the hyperpolarizing afterpotential. From the voltage clamp data (Fig. 9.6) it is clear that Na^+ permeability increases dramatically and then inactivates. To explain the hyperpolarizing afterpotential, Na^+ permeability would have to be less than its initial value (α would have to be less than 0.01). So, based on the observed changes in Na^+ permeability, it would be impossible to account for the hyperpolarizing afterpotential.

ROLE OF VOLTAGE-DEPENDENT K^+ CONDUCTANCE IN THE REPOLARIZATION OF THE ACTION POTENTIAL

Not only does Na^+ permeability change, but there is also a change in K^+ permeability during the course of an action potential. Figure 9.11 illustrates these results. The upper portion of the illustration simply reviews the experimental data of Fig. 9.6. When the membrane is depolarized and held fixed at various levels, there is an increase in Na^+ permeability that is proportional to the depolarization. Keep in

mind that during this entire sequence of events, the membrane potential is held depolarized by the voltage clamp at the levels indicated. The traces below (Fig. 9.11B) illustrate that in addition to changes of Na^+ permeability, there are also voltage-dependent changes in K^+ permeability. The greater the level of depolarization, the greater the increase in K^+ permeability. There are two important differences between these two permeability systems. First, the changes in K^+ permeability are rather slow. It takes some time for K^+ permeability to begin to increase, whereas the changes in Na^+ permeability begin to occur immediately after the depolarization is delivered. Second, whereas Na^+ permeability exhibits inactivation, K^+ permeability remains elevated as long as the membrane potential is held depolarized.

Now that it is clear that there are changes in both Na^+ and K^+ permeabilities, how can this information be utilized to better account for the entire sequence of events that underlies the action potential? Because of the slowness of K^+ permeability changes, the initial explanation for the rising phase of the action potential

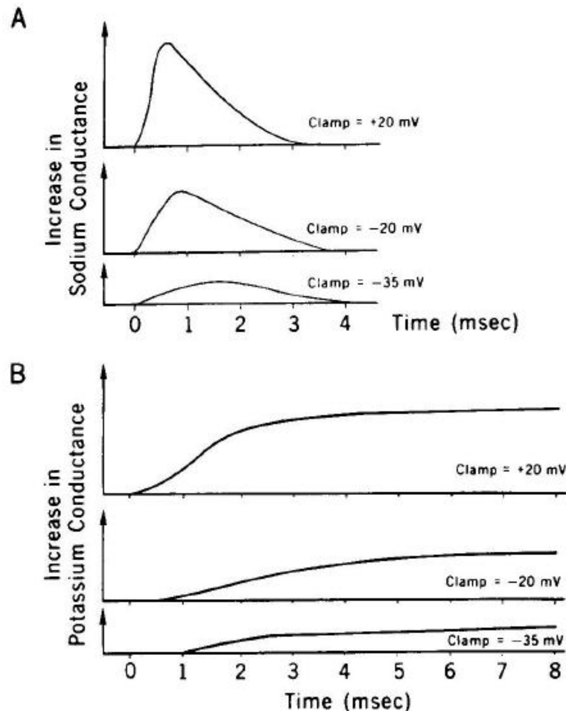

FIG. 9.11. Simultaneous changes in Na^+ **(A)** and K^+ **(B)** conductance produced by voltage steps to three depolarized levels. Note the marked differences between the changes in Na^+ and K^+ conductance. (Modified from A. L. Hodgkin and A. F. Huxley, *J Physiol* 1952;117:500.)

is unaltered, simply because for a period of time less than about 0.5 msec, there is no major change in K^+ permeability. In later phases of the action potential (at times greater than roughly 0.5–1 msec), we not only have to consider Na^+ permeability changes but also changes in K^+ permeability. What would be the consequences of not only having a fall in Na^+ permeability (due to inactivation) but also a simultaneous increase in K^+ permeability? Let us return to the GHK equation. At the peak of the action potential (about 0.5 to 1 msec from its initiation), there is very high Na^+ permeability. At this time, K^+ permeability begins to increase significantly. Thus, at any time after about 0.5 to 1 msec, not only will there be a certain increase in Na^+ permeability, but there will also be a K^+ permeability that is greater than its resting level. As a result, the value of α will be smaller than if only changes in Na^+ permeability were occurring. If α is smaller, then the Na^+ terms make less of a contribution to the GHK equation. Stated in a slightly different way, the K^+ terms make more of a contribution, and the membrane potential will be more negative. Thus, by incorporating the finding that there is a delayed increase in K^+ permeability, the membrane potential will be more negative for any given time (greater than about 0.5–1 msec) than it would have been without the changes in K^+ permeability. The delayed changes in K^+ permeability will tend to make the membrane potential repolarize faster because now there are two driving forces for repolarization. The first is Na^+ inactivation, and the second is the delayed increase in K^+ permeability. By incorporating the simultaneous changes in K^+ permeability, we can in principle account for a shorter duration action potential.

Can the changes in K^+ permeability help explain the hyperpolarizing afterpotential? The key is understanding the time course of the changes in K^+ permeability. You will note that the changes in K^+ permeability are very slow in turning on. They are also slow in turning off. As the action potential repolarizes to the resting level, Na^+ permeability returns back to its resting level. Because the K^+ permeability system is slow, however, K^+ permeability is still elevated. Therefore, α in the GHK equation will actually be less than its initial level of 0.01. If α is less than 0.01, the contributions of the Na^+ terms become even more negligible than at rest, and the membrane potential approaches E_K. Thus, because Na^+ permeability decays rapidly and K^+ permeability decays slowly, during the later phases of the action potential K^+ permeability is elevated, and the hyperpolarizing afterpotential is produced.

Up to this point, the arguments concerning the sequence of events underlying the action potential have been somewhat qualitative and are based on extrapolation of the voltage clamp data. This approach is somewhat unsatisfactory, and it was also unsatisfactory to Hodgkin and Huxley. Hodgkin and Huxley sought to test more rigorously this hypothesis, and they developed a quantitative mathematical model of the action potential based on the experimentally measured changes in Na^+ and K^+ permeabilities (for details, see the Appendix). They sought to determine whether it was possible to reconstruct an action potential from data based entirely on the changes in Na^+ and K^+ permeabilities that were measured with the voltage clamp technique. Their results are illustrated in Fig. 9.12. The solid line illustrates the

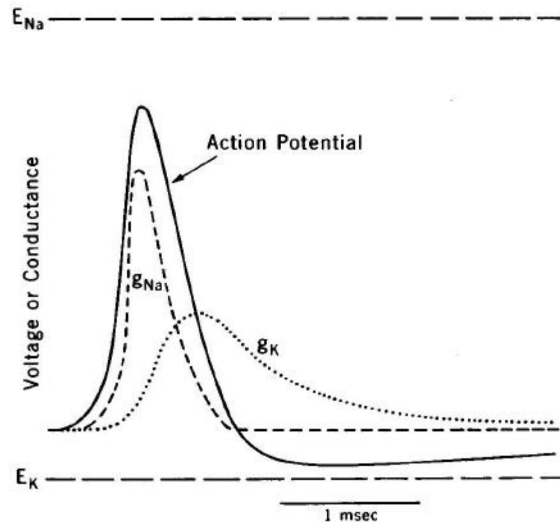

FIG. 9.12. Time course of the changes in Na^+ and K^+ conductance that underlie the nerve action potential.

computed action potential. This simulated action potential is identical to the experimentally recorded action potential. Since the simulated and experimental action potentials are identical, one can examine in detail the sequence of permeability changes that are necessary to generate the simulated action potential. The dashed line shows the underlying changes in Na^+ permeability, and the dotted line shows the underlying changes in K^+ permeability.

Assume that by some mechanism the cell is depolarized to threshold. The depolarization initiates the voltage-dependent increase in Na^+ permeability. That voltage-dependent increase in Na^+ permeability produces a further depolarization that produces further increases in Na^+ permeability. The positive-feedback cycle is entered, which leads to a rapid depolarization of the cell toward E_{Na}. At the peak of the spike, which occurs about $\frac{3}{4}$ msec from the initiation of the action potential, two important processes contribute to the repolarization. First, there is the process of Na^+ inactivation. As a result of the decay of Na^+ permeability, the membrane potential begins to return to the resting level. As the membrane potential returns to the resting level, the Na^+ permeability decreases further, which further speeds the repolarization process. A new feedback cycle is entered that moves the membrane potential in the reverse direction. Second, there is the delayed increase in K^+ permeability. As the action potential reaches its peak value, there is a rather dramatic change in K^+ permeability. This change in K^+ permeability tends to move the membrane potential toward E_K. Therefore, there are two independent processes that contribute to repolarization of the action potential. One is Na^+ inactivation, and the other is the

delayed increase in K^+ permeability. Note that when the action potential returns to its resting level of about -60 mV or so, the Na^+ permeability has reached its resting level; while Na^+ permeability has returned to its resting level, K^+ permeability remains elevated for a period of time. Thus, the ratio of the two permeabilities will be less than it was initially, and the membrane potential will move closer to E_K. Over a period of time, K^+ permeability gradually decays back to its resting level, and the action potential terminates.

In summary, the initiation of the action potential can be explained by the voltage-dependent increase in Na^+ permeability and the repolarization phase of the action potential by (a) the process of Na^+ inactivation and (b) by the delayed increase in K^+ permeability. Finally, the hyperpolarizing afterpotential can be explained by the fact that K^+ permeability remains elevated for a period of time after the Na^+ permeability has returned to its resting level.

Students frequently question the necessity for such an elaborate series of steps to generate short-duration action potentials. This question brings us back to a point raised at the beginning of Chapter 8. Recall that the nervous system codes information in terms of the number of action potentials elicited; the greater the stimulus intensity, the greater the frequency of action potentials. To encode and transmit more information per unit time, it is desirable to generate action potentials at a high frequency. With short-duration action potentials, a new action potential can be initiated soon after the first, and this requirement can be met.

The analysis of Hodgkin and Huxley, originally performed on the squid giant axon, has proved generally applicable to action potentials that are initiated in nerve axons and in skeletal muscle cells. The concept of voltage-dependent ion channels is now universal. What varies from cell to cell is the particular ion to which the channel is permeable. For example, a significant component of action potentials in smooth muscle and cardiac muscle cells is due to voltage-dependent Ca^{2+} channels. A number of different types of voltage-dependent K^+ channels have also been described.

The structure of voltage-gated Ca^{2+} channels is very similar to that of the Na^+ channel (see Fig. 9.9). Voltage-activated K^+ channels have similar six membrane spanning regions, but they differ in that the polypeptide chain does not contain multiply repeated domains. Rather, K^+ channels are formed by the functional association of four separate subunits to form an ionophore. A specific region of the *N*-terminal domain of the channel peptide appears to be essential for the proper aggregation of the subunits to form the tetrameric structures of the functional channel. Variations in the structure of individual subunits as well as different combinations of the subunits contribute to the great diversity of K^+ channel properties that has been observed in excitable membranes. For example, some K^+ channels exhibit inactivation like Na^+ channels. For these inactivating K^+ channels, the amino terminal sequence of the polypeptide appears to act as a plug to close the channel. Another important class of K^+ channels is activated by intracellular levels of Ca^{2+}. A potential Ca^{2+}-binding domain is found on the carboxy terminal end of the channel polypeptide.

SPECIFICITY OF ION CHANNELS UNDERLYING THE ACTION POTENTIAL

The voltage clamp analysis and mathematical reconstruction of the action potential leaves many students somewhat unsatisfied because of the seemingly obscure nature of the analysis. Fortunately, there are other types of experiments that confirm further the Hodgkin-Huxley hypothesis for the initiation and repolarization phases of the action potential. Compounds have been discovered that can be used selectively to block or inhibit these voltage-dependent permeability changes. One of these substances is known as tetrodotoxin (TTX), and the other is known as tetraethylammonium (TEA). TTX is a toxin that is isolated from the ovaries of the Japanese puffer fish. Every year in Japan this substance accounts for several hundred deaths due to improper food handling.

The reason why TTX has such devastating effects is illustrated in Fig. 9.13. Shown are the results of a voltage clamp experiment where Na^+ and K^+ permeabilities are measured in the presence of TTX. As a result of perfusing the extracellular medium with TTX, the voltage-dependent changes in Na^+ permeability are completely abolished. In contrast, the voltage-dependent changes in K^+ permeability are unaffected. Figure 9.14 illustrates the results of perfusing a squid giant axon preparation with TEA. TEA has absolutely no effect on the voltage-dependent changes in Na^+ permeability but completely abolishes the voltage-dependent changes in K^+ permeability. Thus, one substance (TTX) is capable of blocking the voltage-dependent Na^+ permeability, and another (TEA) is capable of blocking the voltage-dependent K^+ permeability.

Given that the effects of TEA and TTX on permeabilities measured with the voltage clamp are known, how would one expect these substances to affect the action potential? If the voltage-dependent change in Na^+ permeability is blocked, one would expect that no action potential could be initiated or propagated. If the voltage-dependent change in K^+ permeability is blocked, one would expect the action potential to be somewhat longer in duration, and, in addition, it should not

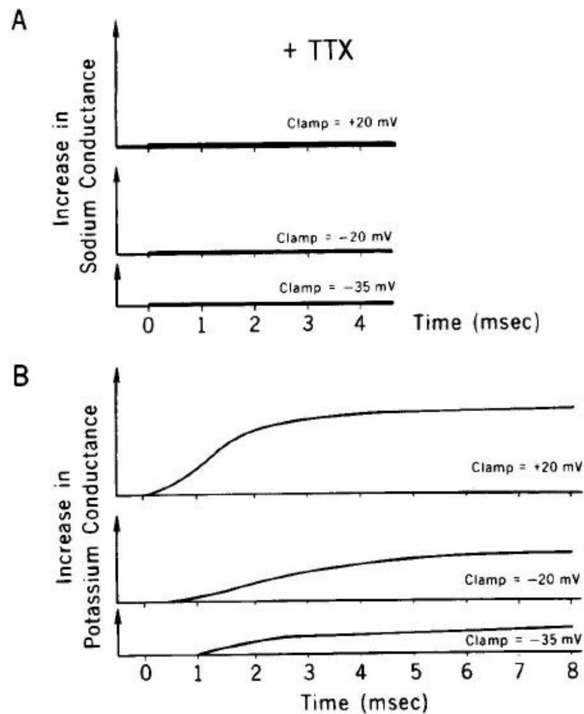

FIG. 9.13. Effects of tetrodotoxin (TTX) on voltage clamp responses from a squid giant axon. **(A)** Na^+ permeability. **(B)** K^+ permeability.

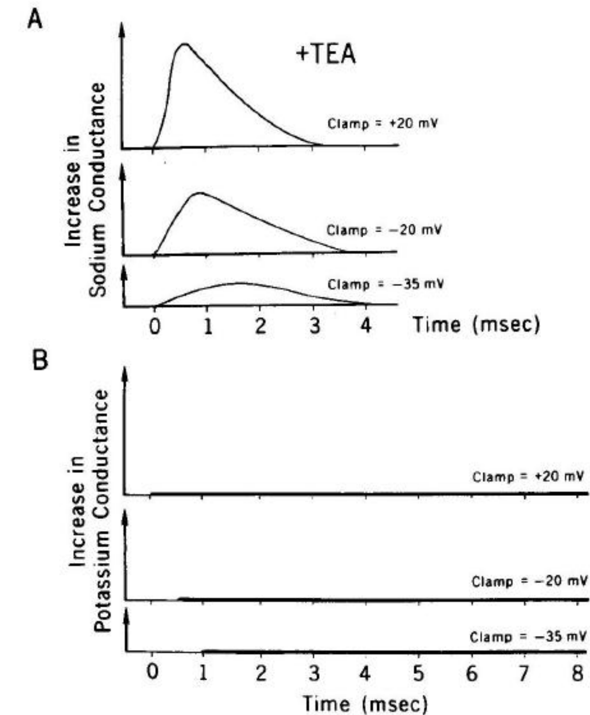

FIG. 9.14. Effects of tetraethylammonium (TEA) on voltage clamp responses from a squid giant axon. **(A)** Na^+ permeability. **(B)** K^+ permeability.

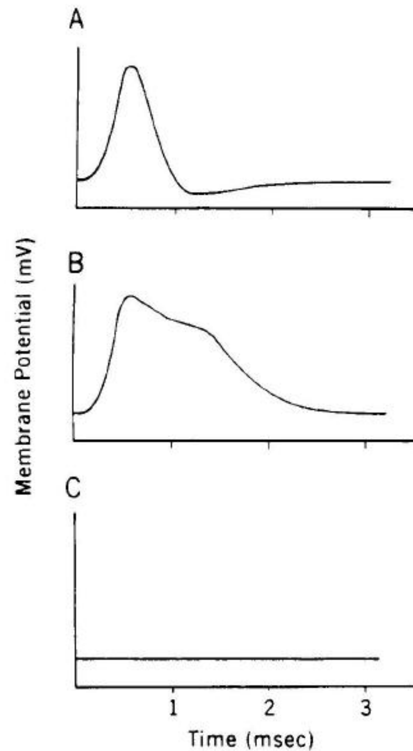

FIG. 9.15. Effects of TEA and TTX on action potentials. **(A)** Normal. **(B)** TEA. **(C)** TTX.

have a hyperpolarizing afterpotential. Figure 9.15 illustrates these results. Figure 9.15A is a normal action potential, and Fig. 9.15B is an action potential recorded in TEA. In TEA, the initiation and the rising phase of the action potential as well as its peak value are unaffected, but there is a dramatic increase in the spike duration and an absence of the hyperpolarizing afterpotential. Thus, the use of TEA confirms the Hodgkin-Huxley theory that the delayed increase in K^+ permeability contributes to the repolarization phase of the action potential and to the undershoot. In the presence of TEA, the process of Na^+ inactivation accounts entirely for the repolarization. Figure 9.15C illustrates the effects of perfusing the preparation with TTX. First, it should be mentioned that in this particular experiment an action potential is elicited in one portion of the axon and is allowed to propagate along the axon to a more distant point where the action potential is recorded. When one perfuses the axon with TTX, one finds that no action potential can be elicited and no action potential can be propagated and monitored at the recording site (Fig. 9.15C). Thus,

the use of TTX confirms the Hodgkin-Huxley theory for the critical role that the increase in Na^+ permeability plays in initiating the action potential in nerve axons.

These experiments with TTX and TEA are also interesting in another respect because they demonstrate that the voltage-dependent changes in Na^+ permeability are mediated by completely different membrane channels from the voltage-dependent change in K^+ permeability simply because it is possible to selectively block one but not the other.

DO CHANGES IN Na^+ AND K^+ CONCENTRATIONS OCCUR DURING ACTION POTENTIALS?

It is clear that, as a result of the voltage-dependent increase in Na^+ permeability, some Na^+ ions will flow from the outside of the cell to the inside of the cell. A frequently asked question is whether the flux of Na^+ that occurs during the action potential produces a concentration change on the inside of the cell. Alternatively, since there is an increase in K^+ permeability during an action potential, there is a tendency for some K^+ to flow out of the cell. Does that flux of K^+ cause a change in the intracellular K^+ concentration? Although some Na^+ does indeed enter the cell with each action potential and some K^+ leaves the cell with each action potential, for cells with small surface area-to-volume ratios, these fluxes are generally minute compared to the normal intracellular concentrations. For example, as a result of an action potential, the change in Na^+ concentration for a 1-cm^2 surface area of the membrane is only equal to approximately 1 pM (1×10^{-12} M) and that concentration change is restricted to the inner surface of the membrane. Therefore, despite the fact that some Na^+ does enter the cell with each action potential, the concentration change is minute compared to the normal millimolar concentration of Na^+ within the cell. There is also some K^+ that leaves the cell during an action potential, but the concentration change again is minute compared to the normal K^+ concentration. Indeed, if the (Na^+–K^+) exchange pump is blocked in the squid giant axon, it is possible to initiate more than 500,000 action potentials without any noticeable change in either the resting potential or the amplitude of the action potential.

The role of the membrane (Na^+–K^+) pump is to provide long-term maintenance of the Na^+ and K^+ concentration differences. Eventually, if one were to generate more than 500,000 action potentials, there would be a change in ionic distribution, but this is a long-term phenomenon, and in the short range the (Na^+–K^+) pump is not essential. Even with the (Na^+–K^+) exchange pump blocked, a cell is capable of initiating a large number of action potentials without any major change in either the resting potential or the peak amplitude of the action potential. If the resting potential is unchanged, it can be inferred that the K^+ equilibrium potential is also unchanged. Similarly, if the peak amplitude of the action potential is unchanged, the Na^+ equilibrium potential is also unchanged.

THRESHOLD, ACCOMMODATION, AND ABSOLUTE AND RELATIVE REFRACTORY PERIODS

The Hodgkin-Huxley analysis not only described quantitatively the mechanisms that account for the initiation and the repolarization of the action potential but also provided the explanation for some phenomena that had been known for some time but were poorly understood. Four of these phenomena are *threshold*, *accommodation*, and the *absolute* and *relative refractory periods*.

Threshold

The voltage dependence of Na^+ permeability explains the initiation of the action potential but by itself does not explain completely the threshold phenomenon, since the relationship between depolarization and Na^+ permeability, although steep, is a continuous function of membrane depolarization (e.g., Fig. 9.4A). Threshold can be explained by taking into account the fact that K^+ permeability (both resting and voltage-dependent) tends to oppose the effects of increasing Na^+ conductance in depolarizing the cell and initiating an action potential. Threshold is the point where the depolarizing effects of the increased Na^+ permeability just exceed the counter (hyperpolarizing) effects of K^+ permeability. Once the inward flow of Na^+ exceeds the outward flow of K^+, threshold is reached, and an action potential occurs through the positive feedback cycle.

Absolute and Relative Refractory Periods

The absolute refractory period refers to that period of time after the initiation of one action potential when it is impossible to initiate another action potential despite the stimulus intensity utilized. The relative refractory period refers to that period of time after the initiation of one action potential when it is possible to initiate another action potential but only with a stimulus intensity greater than that utilized to produce the first action potential.

At least part of the relative refractory period can be explained by the hyperpolarizing afterpotential. Assume that a cell has a resting potential of −60 mV and a threshold of −45 mV. If the cell is depolarized by 15 mV to reach threshold, an all-or-nothing action potential will be initiated, followed by the associated repolarization phase and the hyperpolarizing afterpotential. What happens if one attempts to initiate a second action potential during the undershoot? Initially the cell was depolarized by 15 mV (from −60 to −45 mV). If the same depolarization (15 mV) is delivered during some phase of the hyperpolarizing afterpotential, the 15 mV depolarization would fail to reach threshold (−45 mV) and initiate an action potential. If, however, the cell is depolarized by more than 15 mV, threshold can again be reached, and another action potential initiated. Eventually, the hyperpolarizing afterpotential would terminate, and the original 15-mV stimulus would again be sufficient to reach threshold. The process of Na^+ inactivation also contributes to the relative refractory period (see below).

The absolute refractory period refers to that period of time after an action potential when it is impossible to initiate a new action potential no matter how large the stimulus. This is a relatively short period of time that varies from cell to cell but roughly occurs approximately ½ to 1 msec after the peak of the action potential. To understand the absolute refractory period, it is necessary to understand Na^+ inactivation in greater detail. In Fig. 9.16, a membrane initially at a potential of −60 mV is voltage clamped to a new value of 0 mV (pulse 1). With depolarization, there is a rapid increase in Na^+ permeability, followed by its spontaneous decay. When this first pulse is followed by an identical pulse (pulse 2) to the same level of membrane potential soon thereafter (Fig. 9.16B), there is still an increase in Na^+ permeability, but the increase is much smaller than it was for the first stimulus. Indeed, when the separation between these pulses is reduced further, a point is reached where there is absolutely no change in Na^+ permeability produced by the second depolarization (Fig. 9.16C). The two pulses must be separated by several milliseconds before the change in Na^+ permeability is equal to that obtained initially (Fig. 9.16A). How do we explain these results, and what do they have to do with the absolute refractory period? Just as it takes a certain amount of time for the Na^+ channels to inactivate, it also takes some time for these channels to recover from the inactivation and be able to respond again to a second depolarization. Therefore, as a result of initiating one action potential, there is an increase in Na^+

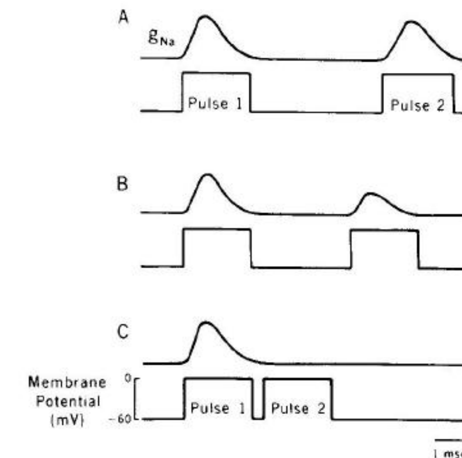

FIG. 9.16. Recovery from inactivation. The second of two depolarizing pulses activates less Na^+ conductance as the interval between the pulses decreases. Once the Na^+ channels become inactivated by the first pulse, several milliseconds are required before they recover completely. (See text for explanation.)

permeability that spontaneously inactivates. As a result, if one attempts to initiate a second action potential soon after the first, the Na^+ permeability will not have recovered from inactivation, making a second depolarization ineffective in initiating a voltage-dependent change in Na^+ permeability. If there is no voltage-dependent change in Na^+ permeability, no action potential will be produced. Thus, the absolute refractory period is most simply understood in terms of this process of recovery from Na^+ inactivation. The recovery from Na^+ inactivation may contribute to the relative refractory period as well. During this recovery time, the threshold for an action potential will be higher because greater depolarization will be required to activate sufficient Na^+ influx to exceed the K^+ efflux.

Accommodation

Accommodation is defined as a change in the threshold of an excitable membrane when slow depolarization is applied. In the previous examples, rapid depolarization is applied, and the threshold occurs at a relatively fixed membrane potential (e.g., Fig. 8.3). When a slowly developing depolarization is applied, however, the threshold is frequently at a more depolarized level, and indeed, if the depolarization is slow enough, no action potential will be initiated despite the level of depolarization. The process of Na^+ inactivation contributes to the phenomenon of accommodation. Essentially, slow depolarization provides sufficient time for the Na^+ channels to inactivate before they can be sufficiently activated. In terms of the molecular model of Fig. 9.10, there are an insufficient number of Na^+ channels in state B because they are already in state C.

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10

Propagation of the Action Potentials

Up to this point, we have been considering the ionic mechanisms that underlie the action potential in a dimensionless nerve cell. One of the interesting features of action potentials, however, is that not only are they elicited in an all-or-nothing fashion, but they also are propagated in an all-or-nothing fashion. If an action potential is initiated in the cell body, it will propagate without decrement along the axon and eventually invade the synaptic terminal. An action potential recorded in the nerve axon has an amplitude and time course identical to the action potential that was initiated in the cell body. It is the ability of action potentials to propagate in this all-or-nothing fashion that endows the nervous system with the capability to transmit information over long distances.

BASIC PRINCIPLES

To begin to consider the mechanisms that account for the propagation of the action potential, it is useful to examine the charge distribution that is found in an isolated section of a nerve axon. When an axon is at rest, the potential of the inside is negative with respect to the outside (Fig. 10.1A). The distribution of charge is simply due to the tendency of K^+ to diffuse from its region of high concentration inside the axon to its region of low concentration outside the axon. Consider what will happen if at some point along the axon an action potential is initiated. At the peak of the action potential the inside of the cell will be positive with respect to the outside (Fig. 10.1B). At this point there is a new charge distribution at a localized portion of the membrane. Adjacent regions of the axon, however, still have their initial charge distribution (inside negative). Unlike charges attract each other, so the positive charge produced by the action potential will tend to move toward the adjacent region of membrane (still at rest), which has a negative charge (Fig. 10.1C). As a result of this positive charge movement the adjacent region of the axon will become depolarized. If sufficient charge moves to depolarize the adjacent portion of the membrane to threshold and elicit voltage-dependent changes in Na^+ per-

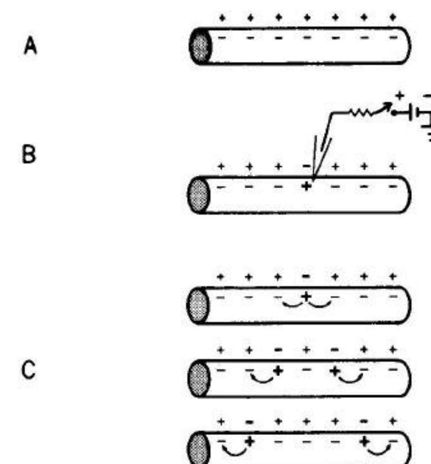

FIG. 10.1. Schematic diagram of sequence of steps underlying propagation of the action potential. **(A)** Rest. **(B)** Initiation. **(C)** Propagation.

meability, a new action potential at this adjacent region will be initiated. So as a result of an action potential in one portion of an axon and the subsequent charge transfer along the surface of the membrane, a "new" action potential will be generated. This new action potential then will cause charge transfer to its adjacent region causing, in a sense, another new action potential to be initiated. It should be clear that this process once initiated will propagate all the way to the end of the axon.

DETERMINANTS OF PROPAGATION VELOCITY

What are the factors that determine the rate of propagation of the action potential? To address this question, some details of the passive properties of axonal membranes must be examined. Two important passive properties that are directly related to the rate at which an axon can propagate action potentials are the space (or length) constant and the time constant. The space and time constants are known as passive properties because they are not *directly* dependent on metabolism or any voltage-dependent permeability changes such as those that underlie the action potential. These are intrinsic properties that are reflections of the physical properties of the neuronal membrane. Indeed, they are properties of all membranes.

Time Constant

To consider the time constant, first consider a very simple thermal analog. Take the case where a block of metal that is initially at 25°C is placed on a hot plate that is

at 50°C. Assume that the hot-plate temperature is constant. What will be the consequences of placing the block on the hot plate? It is obvious that over a period of time, the temperature of the block will change from its initial value of 25°C to a final value of 50°C, but it will not do so instantaneously. It will take a certain period of time for the heat transfer to occur. If the temperature of the block is measured as a function of time, the temperature will change as an exponential function of time, approaching a final value of 50°C.

A similar phenomenon is observed in membranes when one applies an artificial depolarizing or hyperpolarizing stimulus. This is illustrated in Fig. 10.2. A nerve cell is impaled with one electrode to record the membrane potential and another electrode to depolarize or hyperpolarize the cell artificially (Fig. 10.2A). The cell is initially at its resting potential of −60 mV. At time zero, the stimulating electrode is connected to a battery. The size of the battery is such that the stimulus will eventually depolarize the cell by 10 mV (Fig. 10.2B). As a result, the membrane potential will change from its initial value of −60 mV to a final value of −50 mV. Note that even though the current flow is instantaneous (and constant), the membrane potential does not change instantaneously. There is a period of time during which the membrane potential charges to its new final level of −50 mV. This charging process is an exponential function of time. Just as the temperature in the block changed exponentially as a function of time, the change in potential in the neuron produced by an applied depolarization (or hyperpolarization) also follows an exponential time course. For such exponential functions of time it is possible to

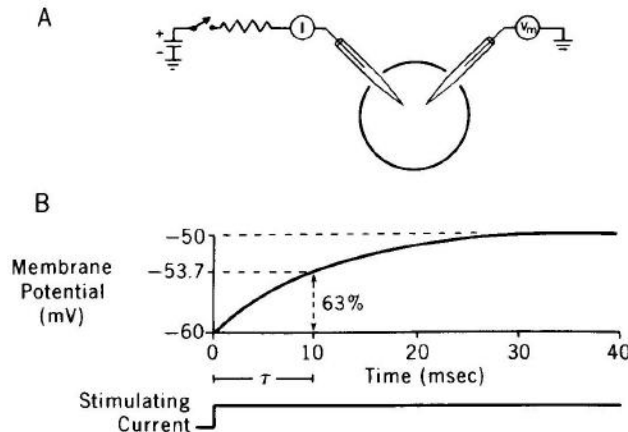

FIG. 10.2. Measurement of the membrane time constant. **(A)** Experimental setup. **(B)** Change in membrane potential as a function of time after the delivery of a constant step of depolarizing current. (Modified from E. R. Kandel, *The cellular basis of behavior*. San Francisco; Freeman, 1976: Chapters 5 and 6.)

define what is known as a time constant. The time constant refers to the time it takes for the potential change to reach 63% of its final value.

The general equation for a response that changes as an exponential function of time is

$$\% \text{ Response} = 100(1 - e^{-t/\tau}) \quad [10.1]$$

where t is time and τ is the time constant. At time 0, e^{-0} is equal to 1, and the % response is zero. At infinite time, $e^{-\infty}$ is equal to zero, so the % response is 100. A special case occurs when $t = \tau$. Then, $e^{-1} = 0.37$, and the % response = 63%. A detailed understanding of the mathematics is not important. What is important is that the time constant is a simple index of how rapidly a membrane will respond to a stimulus.

The time constant for the cell illustrated in Fig. 10.2B is 10 msec. Thus, in 10 msec the potential has changed from −60 mV to −53.7 mV (63% of its final displacement). The smaller the time constant of a cell, the more rapidly the cell will respond to an applied stimulus. If the time constant were 1 msec, the potential change would occur very rapidly, and the cell would reach a value of −53.7 mV in just 1 msec. If the time constant were 40 msec, the potential change would occur very slowly, and the potential would reach −53.7 mV in 40 msec.

A simple formula that describes the time constant in terms of the physical properties of the membrane is

$$\tau = R_m C_m \quad [10.2]$$

Here, R_m simply reflects the resistive properties of the membrane and is equivalent to the inverse of the permeability, since the less permeable the membrane the higher the resistance. C_m represents the membrane capacitance. This is a physical parameter that describes the ability of a membrane to store charge. It is equivalent to the ability of the metal block to store heat. The larger the size of a block, the better able it is to store heat. Similarly, the larger the membrane capacitance, the better able it is to store charge.

Space Constant

Before discussing how the time constant is related to propagation velocity, the other passive membrane property, the space (or length) constant, will be discussed. To introduce this phenomenon, it is useful to turn again to a thermal analog. Instead of considering a small block on a hot plate, consider what might happen when one end of a long metal rod touches the hot plate. The hot plate is at 50°C, and the rod is initially at 25°C. If the rod is placed in contact with the hot plate and a sufficient period of time elapses for the temperature changes to stabilize, what will be the temperature gradient along the rod? It is obvious that the temperature at the end of the rod in contact with the hot plate will be 50°C (the same temperature as the hot plate). The temperature of the rod, however, will not be 50°C along its length. The

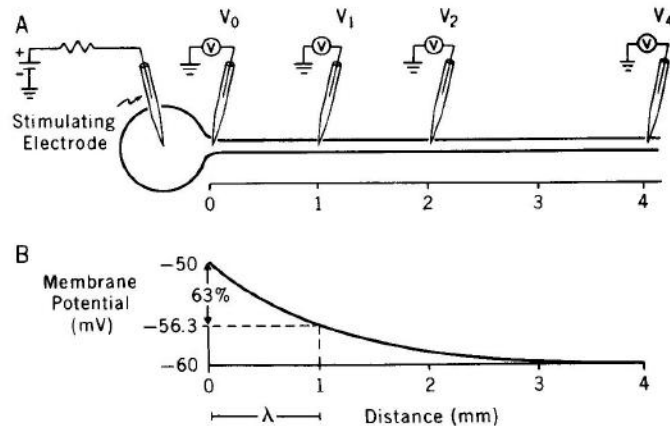

FIG. 10.3. Measurement of the space constant. **(A)** Experimental setup. **(B)** Changes in membrane potential as a function of distance along the axon. A constant depolarizing current is applied to the cell body to depolarize the cell body from rest (−60 mV) to −50 mV. (Modified from E. R. Kandel, *The cellular basis of behavior*. San Francisco; Freeman, 1976: Chapters 5 and 6.)

temperature near the hot plate will be 50°C, but along the rod the temperature will gradually fall, and if the rod is long enough the temperature may still be 25°C at its end. If the temperature of the rod at various distances from the hot plate is measured, the temperature will be found to decay as an exponential function of distance.

Just as there is a spatial degradation of temperature in a long rod, there is also a spatial degradation of potential along a nerve axon, which is referred to as electrotonic conduction. Figure 10.3A illustrates how it is possible to demonstrate this. One electrode is in the cell body and will be used to depolarize the cell artificially. A number of other electrodes are placed at various distances along the axon to record the potential gradient as a function of distance from the cell body. Initially, the cell body and all regions of its axon are at the resting potential of −60 mV. A sufficient subthreshold depolarization is then applied to the cell body to depolarize the cell body to −50 mV. Just as one end of the rod was placed on a 50°C heat source, the cell body is forced to a potential of −50 mV that is different from its resting level. After waiting a sufficient period of time for the potential changes to stabilize, the measurements are made. Very near the cell body the potential is −50 mV (Fig. 10.3B). Because the membrane potential is sampled at points away from the cell body, however, there is a change in the electronic potential from its value or −50 mV in the cell body to more negative values. Measurements made a great enough distance from the cell body reveal that the potential recorded is the resting potential (−60 mV). The potential profile is an exponential function of distance and a space constant (denoted by the symbol λ) can be defined. The space constant is the distance it takes for the depolarizing displacement (i.e., 10 mV) to decay by 63% of

its initial value. In this particular cell the space constant is 1 mm. This means that 1 mm away from the cell body the potential would have changed from its value of −50 mV in the cell body to a value of −56.3 mV in the axon. The greater the space constant, the greater will be the extent of the propagation of this electrotonic potential. If the space constant were 2 mm, this potential profile would decay less so that at 2 mm the potential would be at −56.3 mV.

Just as it is possible to provide a formula for the time constant in terms of the physical properties of the membrane, it is also possible to derive a formula for the space constant. The space constant is equal to

$$\lambda = \sqrt{\frac{dR_m}{4R_i}} \qquad [10.3]$$

where R_m once again refers to the resistive properties of the membrane (the inverse of the membrane permeability); R_i is a term that refers to the resistive properties of the intracellular medium (the resistance of the axoplasm to the flow of ions); and d is the diameter of the axon.

Relationship Between Propagation Velocity and the Time and Space Constants

It is possible to make some qualitative predictions about the way in which the space and time constants affect propagation velocity. If the space constant is large, a potential produced at one portion of an axon will spread greater distances along the axon. Since the potential will spread a greater distance along the axon, it will bring distant regions to threshold sooner. Thus, the greater the space constant, the greater will be the propagation velocity. The time constant is a reflection of the rate that a membrane can respond to an applied stimulus current. The smaller the time constant, the greater will be the ability of a membrane to respond rapidly to stimulus currents. Action potentials will be initiated sooner, and the propagation velocity will be greater. Therefore, the smaller the time constant, the greater will be the propagation velocity.

Thus, the propagation velocity is directly proportional to the space constant but inversely proportional to the time constant. Since relationships for both the space and time constants are known, it is possible to derive a new formula that describes the propagation velocity:

$$\text{Velocity} \propto \frac{\sqrt{dR_m/4R_i}}{R_m C_m} \qquad [10.4]$$

Thus,

$$\text{Velocity} \propto \frac{1}{C_m}\sqrt{\frac{d}{4R_m R_i}} \qquad [10.5]$$

It is desirable to have axons that have high propagation velocities, since there is great survival value to rapid information transmission. For example, to initiate a motor response to some noxious stimulus, such as touching a hot stove, action potentials must propagate rapidly along sensory and motor axons.

Given that the propagation velocity can be described in terms of the physical properties of nerve axons, we can begin to examine strategies utilized by the nervous system to endow axons with high propagation velocities. One of the simplest and most obvious ways of doing this is to increase the diameter of the axon. By increasing the diameter, the propagation velocity is increased. This is exactly the strategy that has been used extensively by many invertebrate axons, of which the squid giant axon is the prime example. The giant squid axon has a diameter of about 1 mm, which endows it with perhaps the highest propagation velocity of any invertebrate axon. There is one severe price that is paid, however, when the propagation velocity is increased in this way. The key to understanding this problem is the square root relationship in the formula for propagation velocity. The square root relationship requires that to double the propagation velocity the fiber diameter must be quadrupled.

Therefore, to get moderate increases in propagation velocity one has to increase axons to very large diameters. Although this is frequently observed in invertebrates, it is not generally utilized in the vertebrate central nervous system. For example, it is known that the propagation velocity of axons in the optic tract is about the same as that of the squid giant axon. If all the axons in the optic tract were the size of the squid giant axon, however, the optic tract by itself would take up the space of the entire brain.

Conduction in Myelinated Axons

Clearly, there must be another means available by which axons can increase their propagation velocity without drastic changes in fiber diameter. You will note from the relationship for propagation velocity that by changing the membrane capacitance, velocity can be affected directly without involving the square-root relationship. It is possible to decrease the membrane capacitance simply by coating the axonal membrane with a thick insulating sheath. This is exactly the strategy used by the vertebrates. Many vertebrate axons are coated with a thick lipid layer known as myelin. As a result of myelin, the capacitance is greatly reduced, and propagation velocity is greatly increased. In principle, there is one severe problem with increasing the propagation velocity with this technique. Coating the axon with a lipid layer would tend to cover the channels or pores in the membrane that endow the axon with the ability to initiate and propagate action potentials. The nervous system has solved this problem by only coating portions of the axon with myelin. Certain regions called nodes are not covered. At these bare regions, voltage-dependent changes in membrane permeability take place that generate action potentials.

The process of conduction in myelinated fibers is illustrated in Fig. 10.4. The dashed lines show a nerve axon that is covered with a layer of myelin. Note that the myelin does not cover the entire axon; there are bare regions or nodes where voltage-dependent changes in permeability can take place and action potentials can be elicited. Assume there is an action potential elicited at the node to the left. As a result of the action potential, there is a large depolarization. The inside of the cell becomes positive with respect to the outside. The action potential cannot propagate along the myelinated region via the active process that was described earlier, simply because the voltage-dependent changes in permeability cannot take place; however, the action potential can conduct passively. That conduction will occur very rapidly because the membrane capacitance is reduced. Because of the small amount of decrement, the potential that emerges at the next node will be of a sufficient level to depolarize the next node to threshold. A new action potential will be initiated, and the process will be repeated.

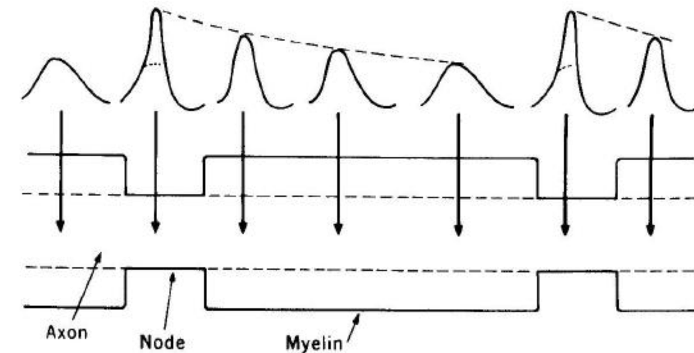

FIG. 10.4. Saltatory conduction in myelinated axon. The electrical activity "jumps" from node to node. Between the nodes the action potential is propagated electrotonically with little decrement. At the nodes, where ionic current can flow, the electronic potential reaches threshold and triggers an action potential. The process is then repeated. *Arrows* indicate regions of the axon where the potentials were recorded.

The type of propagation that occurs in myelinated fibers is known as *saltatory conduction* because the action potential appears to "jump" from node to node. At the nodes, there are voltage-dependent changes in membrane permeability, whereas in the internodal regions, the potential is conducted in a passive fashion. No voltage-dependent changes in permeability take place in the internodal region.

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