The Action Potential 3.2

Learning Objectives

- Draw a picture of a motor neuron and identify soma, dendrites, axon, myelin sheath, and terminals
- Describe what is meant by depolarization and hyperpolarization and what currents achieve it
- Explain what is meant by the "all or none" law
- Define latency, absolute refractory period, relative refractory period, and overshoot in an action potential on a nerve
- Define rheobase and chronaxie and how they can be determined from a strength–duration curve
- Recognize the Weiss relation between strength and duration
- Draw a graph showing the conductance changes with time during an action potential on a nerve
- Explain what is meant by the "activation gate" and "inactivation gate" of the Na channel
- Describe the state of the activation gate and inactivation gate during an action potential
- Describe how the inactivation gate is reset
- Distinguish between unitary current and ensemble current
- Using the whole-cell i–v curve, explain the effect of small depolarizations below threshold and of depolarizations above threshold
- Explain why stronger stimuli need shorter durations to achieve an action potential

CELLS USE ACTION POTENTIALS AS FAST SIGNALS

Certain cells in the body are capable of initiating and propagating an **action potential** over their surface. These cells are called **excitable** cells and they include muscle and nerve cells. The action potential is a brief, pulse-like change in the membrane potential. Because it can be **propagated** rapidly over the surface of the cell, it conveys a fast signal from one place to another. We will use a motor neuron as an example of an excitable cell.

THE MOTOR NEURON HAS DENDRITES, A CELL BODY, AND AN AXON

Motor neurons are large cells in the ventral horn of the spinal cord as shown in Figure 3.2.1. They have a number of processes called **dendrites** that bring signals to the motor neuron. The motor neuron also has one large process, the **axon**, that connects the motor neuron on one end with a muscle fiber on the other. Action potentials move along the axon so that activity in the motor neuron alters activity in the muscle.

Axons from neurons can be **myelinated** or **unmyelinated**. Myelin refers to a sheath that covers the axon, but not entirely. In the peripheral nervous system, **Schwann cells** make the myelin by wrapping themselves around the axon, forming a multilayered structure of multiple cell membranes of the Schwann cell. In the central nervous system, **oligodendroglial cells** make the myelin. The sheath is not continuous in either the peripheral or central nervous system. At the end of each Schwann cell, there is a gap in the myelin. This gap is called the **Node of Ranvier** (see Figure 3.2.2).

Like all cells, the motor neuron has a nucleus located in its cell body or **soma**. The soma is also sometimes referred to as the **perikaryon** from the Greek root "peri" meaning "around" or "surrounding" and "karyon" meaning "nut" or "kernel," and referring to the nucleus. There is only one nucleus in the motor neuron and it is the site of mRNA transcription.

PASSING A CURRENT ACROSS THE MEMBRANE CHANGES THE MEMBRANE POTENTIAL

Figure 3.2.3 shows a highly schematic diagram of how the membrane potential can be measured by inserting a microelectrode through the membrane of the axon. The resting membrane potential in these axons is about -60 mV. Recall that the membrane potential is defined as $\psi_i - \psi_o$.

It is possible to pass current across the membrane through the arrangements shown in Figure 3.2.3. In one case, the battery is hooked up so that current will pass into the cell. Recall here that current is defined as the direction of positive charge flow and an outward flow is positive. An inward current, shown in the middle of Figure 3.2.3, will **depolarize** the cell. The cell is already polarized; an inward current would make the cell less polarized and thus it would depolarize it. If the battery was connected with the opposite polarity, the resulting current would be outward and this would make the membrane more polarized. This is a **hyperpolarizing** current.

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AN OUTWARD CURRENT HYPERPOLARIZES THE MEMBRANE POTENTIAL

When an outward current is passed across the membrane, the recorded membrane potential is a distorted version of the stimulus. As the magnitude of the current is increased (stimulus intensity is increased), the recorded hyperpolarization grows larger (see Figure 3.2.4). It takes some time for the current to reach a new steady-state membrane potential. In addition, the magnitude of the hyperpolarization depends on the distance from the stimulating electrode to the recording electrode. These phenomena are consequences of the electrical characteristics of the axon, its **cable properties**. In brief, the cable properties define a time constant and a length constant which describe how voltage builds up (or falls off) with time and with distance. The membrane parameters which influence these cable characteristics include the membrane resistance, the membrane capacitance, and the electrical resistance of the axoplasm.

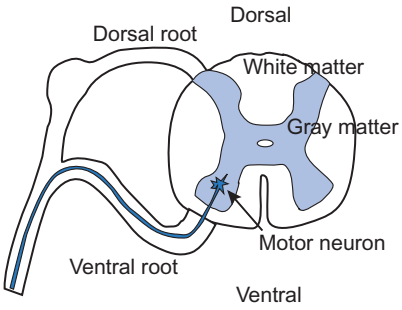

FIGURE 3.2.1 Location of the motor neuron in the spinal cord. The spinal cord is shown in cross-section. The dorsal aspect is toward the back; ventral is toward the front. The dorsal and ventral roots are paired, with one on each side of the cord, but only one side is shown here. The motor neuron is shown in dark blue. The motor neuron's cell body is located in gray matter in the ventral horn, and its long axon leaves the cord via the ventral root and continues on to a muscle where it makes a neuromuscular junction. These cells produce an action potential that propagates along the axon, excites the nerve at the neuromuscular junction, and conveys that excitation to the muscle in order to activate the muscle.

THE RESULT OF DEPOLARIZING STIMULUS OF ADEQUATE SIZE IS A NEW PHENOMENON—THE ACTION POTENTIAL

Depolarization to a small degree produces a change in E_m that mirrors hyperpolarization—the change is a distorted version of the stimulus. With larger depolarization, however, a new phenomenon arises. When E_m exceeds a threshold value, there is an abrupt rise in E_m, reaching positive values near +30 mV. Just as quickly, E_m returns to near normal values. This abrupt change in the membrane potential brought about by depolarization is the **action potential** (see Figure 3.2.5).

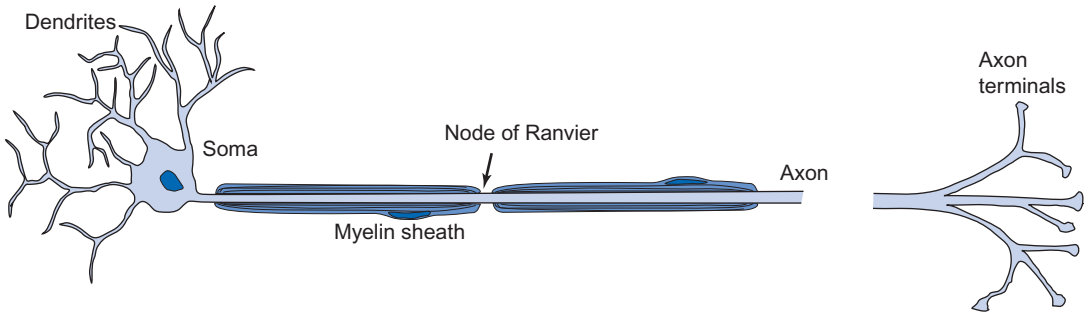

FIGURE 3.2.2 Parts of the motor neuron. Dendrites are multiple processes of the neuron that bring signals to the cell body, or soma. A single long axon exits the cell on one pole and reaches all the way to its target cell, the muscle fiber. The long axon is covered by a myelin sheath made by Schwann cells. The sheath is interrupted at regular intervals at the nodes of Ranvier.

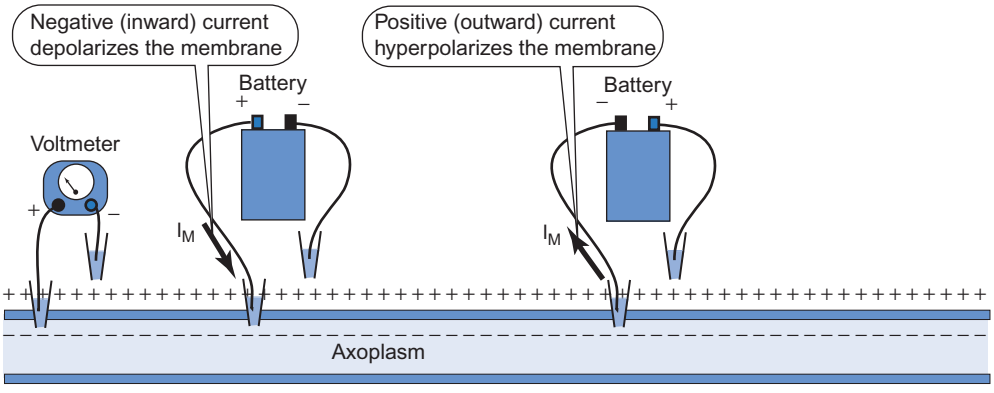

FIGURE 3.2.3 Arrangement of electrodes to record membrane potential (left); to inject a depolarizing current (middle) or to pass a hyperpolarizing current (right). I_M refers to "membrane current," or current across the membrane.

THE ACTION POTENTIAL IS ALL OR NONE

When the strength of depolarization is increased further, the resulting wave form in E_m remains remarkably constant. That is, if the depolarization is enough to trigger an action potential, the resulting action potential is independent of the stimulus. The action potential is "**all or none**." The action potential is not graded, meaning that it does not vary from a minimum to a maximum; rather, a given stimulus will either produce an action potential or it will not, and the resulting shape of the plot of E_m against time is approximately the same.

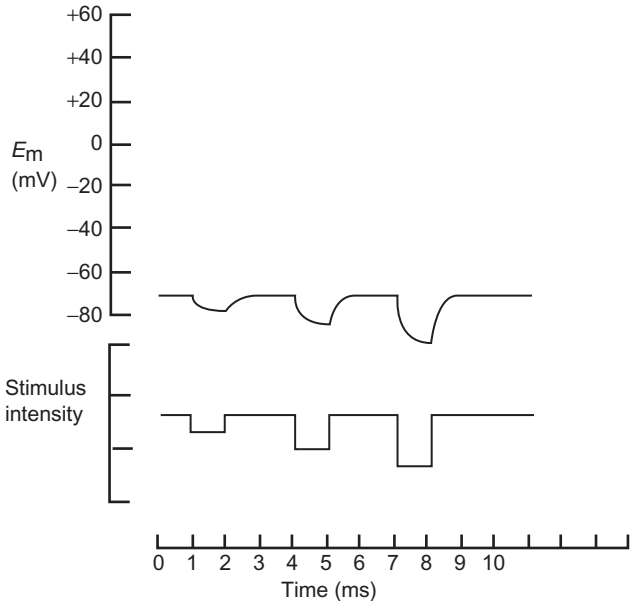

FIGURE 3.2.4 Results of hyperpolarizing stimuli of varying intensity on the axon membrane potential, E_m. The effect of hyperpolarizing stimuli on the membrane potential is a distorted version of the stimulus wave form. The rise time and fall time of E_m are part of the cable properties of the axon.

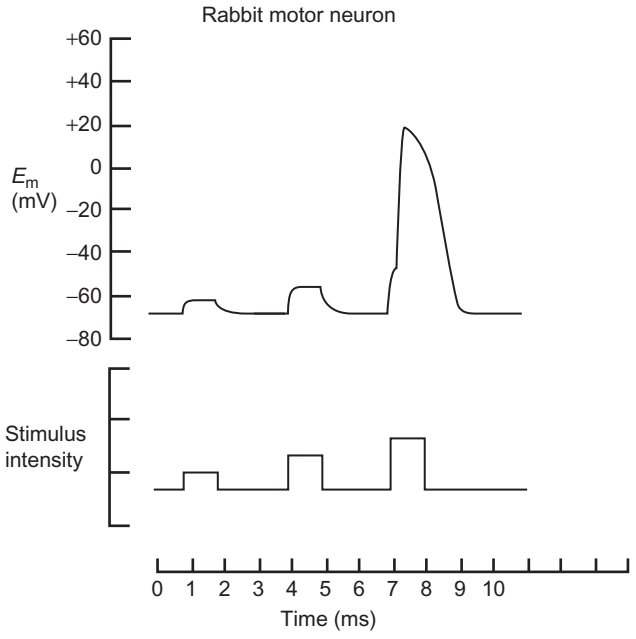

FIGURE 3.2.5 Results of depolarizing stimuli of varying intensity on the axon membrane potential. At low stimulus strength, E_m is a distorted version of the stimulus, as in the case of hyperpolarization. When the depolarization exceeds a threshold value, there is an abrupt rise in E_m, reaching positive values. This abrupt change in the membrane potential brought about by depolarization is the **action potential**.

THE LATENCY DECREASES WITH INCREASING STIMULUS STRENGTH

Once initiated, the action potential moves away from its point of origin, and this is called **propagation**. At some point away from its origin, the action potentials are indistinguishable. However, near the stimulus there is a slight difference, having to do with the time it takes to reach threshold, the **latency**. The membrane potential is not a completely faithful representation of the stimulus: the rise in E_m lags behind the stimulus rise and the fall in E_m lags behind the stimulus fall. The membrane distorts the stimulus. When the stimulus strength is increased above threshold, the time to reach threshold, the latency, decreases. The relationship between latency and stimulus strength is shown in Figure 3.2.6.

THRESHOLD IS THE MEMBRANE POTENTIAL AT WHICH AN ACTION POTENTIAL IS ELICITED 50% OF THE TIME

The **threshold** for a nerve is the membrane potential which must be reached in order to "fire" an action

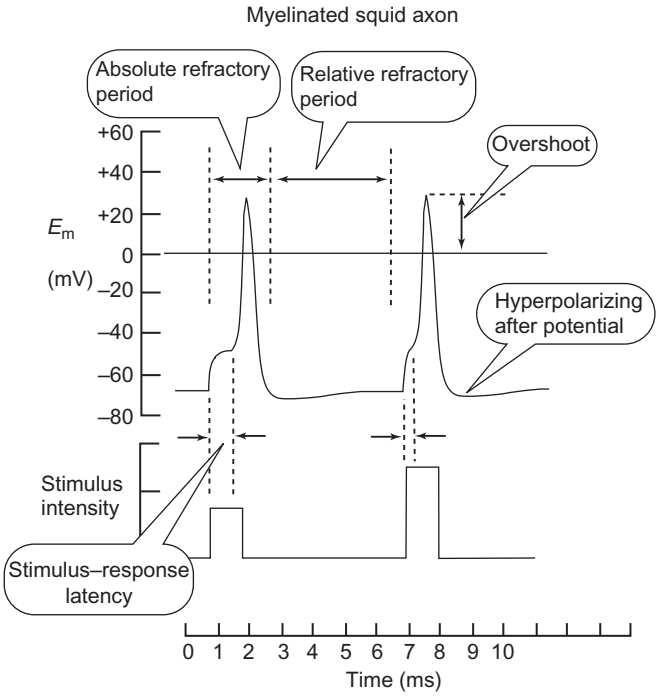

FIGURE 3.2.6 Effect of stimulus strength above threshold on resulting wave forms in a myelinated squid motor neuron axon. Note that the wave form here differs somewhat from that in mammalian motor neurons. It is shorter in the squid and shows a hyperpolarization after potential that is largely lacking in mammalian motor neurons.

potential. Operationally, it is defined as the membrane potential at which the nerve will fire an action potential 50% of the time. However, it is possible to initiate an action potential even when the membrane potential is below threshold, but the probability of this happening is much reduced. Similarly, it is possible to fail to initiate an action potential even when the membrane potential is above threshold, but with a much reduced probability. The probability of initiating an action potential is a steep function of the membrane potential.

THE NERVE CANNOT PRODUCE A SECOND EXCITATION DURING THE ABSOLUTE REFRACTORY PERIOD

While the first action potential is occurring, it is impossible to begin a second action potential, no matter how powerful the second stimulus. The time during which the nerve is **refractory** to a second stimulus is called the **absolute refractory period**. It typically lasts for 1–2 ms. Following the absolute refractory period is a second, **relative refractory period**. This typically lasts some 4 ms or so, and during this time it is possible to stimulate the nerve cell to make another action potential, but it is more difficult to do so than in the resting neuron. That is, the threshold is elevated during the relative refractory period.

THE ACTION POTENTIAL REVERSES TO POSITIVE VALUES, CALLED THE OVERSHOOT

The first recordings of action potentials with intracellular electrodes were accomplished in 1939 and 1940 by A.L. Hodgkin and Andrew Huxley and by K.S. Cole and H.J. Curtis, respectively, using the squid giant axon. They found results similar to those shown in Figure 3.2.6. In particular, they found that the membrane potential not only went to zero, but it took on positive values. This is called the **overshoot**. Given the fact that the equilibrium potentials for all known ions were negative except for Na^+ and Ca^{2+}, these pioneers suggested that the membrane could have a positive potential by becoming selectively permeable to Na^+.

THE STRENGTH–DURATION RELATIONSHIP IS HYPERBOLIC

The relationship between the current necessary to reach threshold and the duration of the current for a compound action potential is shown in Figure 3.2.7. This is related to the strength–latency relationship, as the latency is the time required to elicit an action potential for a given strength of stimulus. The strength–duration relationship shown in Figure 3.2.7 asks the question, for a given strength of stimulus, how long must it continue to produce an action potential? For some strengths of stimulus, no duration is sufficient—you never get an action potential. For a critical strength of stimulus, you get an action potential only if the stimulus lasts infinitely long. This strength of stimulus is

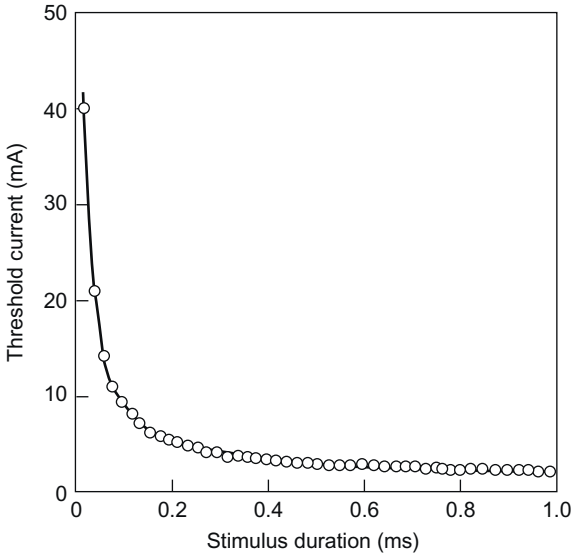

FIGURE. 3.2.7 Strength–duration relationship in human peripheral nerve. The median nerve was stimulated using surface electrodes 1 cm in diameter taped to the skin over the median nerve at the wrist, 4 cm apart oriented along the course of the nerve. Stimulus was a square wave with rise and fall times of 10 μs. The antidromic compound sensory action potential was recorded at the index finger using ring electrodes with 2–3 mm diameter set 2–3 cm apart on the finger. Stimulus intensity was reduced 2–5% until the amplitude of the compound action potential (the aggregate of a bundle of axons) was reduced to 30% of maximum (From Mogyoros, I, Kiernan, MC, and Burke, D. Strength–duration properties of human peripheral nerve. Brain **119**:439–447, 1996).

called the **rheobase** (see Figure 3.2.8). Weiss described this relationship by the equation:

$$[3.2.1] \qquad Q = I_{rh}(t + \tau_{SD})$$

where Q is the charge, I is the rheobase current, t is the time the current is on, and τ_{SD} is a strength–duration time constant, often called the **chronaxie**. Because the total charge is the current times the time, this equation becomes

$$[3.2.2] \qquad I = I_{rh}\frac{(t + \tau_{SD})}{t} = I_{rh} + I_{rh}\frac{\tau_{SD}}{t}$$

This last equation describes a rectangular hyperbole offset from the x-axis by I_{rh}. Also, when $t = \tau_{SD}$, the current $I = 2\ I_{rh}$; the current is twice the rheobase. These relationships are shown diagrammatically in Figure 3.2.8. We will seek to further understand this relationship after we establish how depolarization induces an action potential.

VOLTAGE-DEPENDENT CHANGES IN ION CONDUCTANCE CAUSE THE ACTION POTENTIAL

INCREASE IN ION CONDUCTANCE BEGINS AFTER THE MEMBRANE BEGINS TO DEPOLARIZE

K.S. Cole and H.J. Curtis in 1939 studied the impedance properties of the squid giant axon and discovered a marked decrease in the impedance (equivalently, an increase in the conductance) of the squid axon

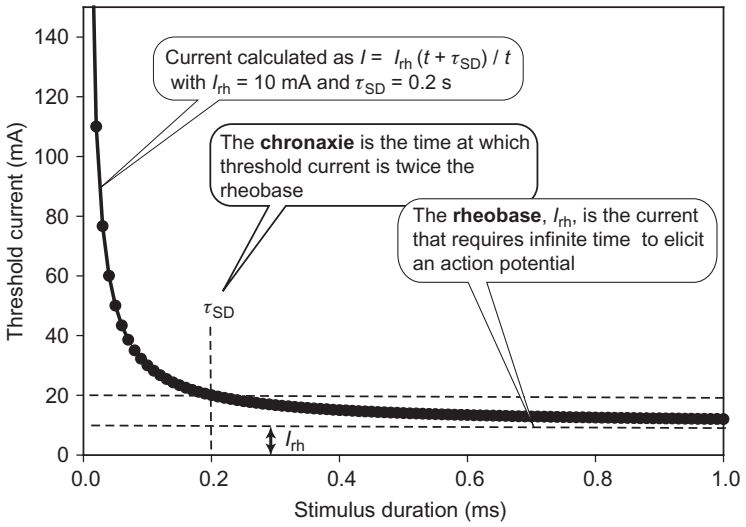

FIGURE 3.2.8 Definition of rheobase and chronaxie. The rheobase current is the current which takes an infinite time to elicit an action potential—it is obtained by extrapolation or curve fitting. The chronaxie is the time at which threshold current is twice the rheobase. These two parameters are parameters of Eqn [3.2.2] that can be obtained by fitting the equation to experimental data. In the figure, the current was calculated according to Eqn [3.2.2] using a rheobase of 10 mA and a chronaxie of 200 ms. Note that the calculated curve resembles the form of the experimental curve shown in Figure 3.2.7.

membrane during the upstroke of the action potential. This increase begins only after the membrane potential rises many millivolts above the resting membrane potential. They argued that the foot of the action potential resulted from the discharging of the membrane from local currents from elsewhere in the cell. At the inflection point on the rising phase of the action potential, the cell generated its own net inward current. Such a current must be carried by an ion, and the most likely candidate is Na^+, because of its high extracellular concentration.

THE ACTION POTENTIAL IS ACCOMPANIED BY NA$^+$ INFLUX

To test the idea that increases in Na^+ conductance might cause the action potential, Alan Hodgkin and Bernard Katz replaced some of the NaCl in seawater with choline chloride. Replacing the Na^+ reduced the upstroke of the action potential and markedly reduced the size of the action potential. Later experiments using radioactive tracers showed that Na^+ influx accompanies the action potential.

THE CHORD CONDUCTANCE EQUATION PREDICTS THAT CHANGES IN CONDUCTANCE WILL CHANGE THE MEMBRANE POTENTIAL

In Chapter 3.1, we developed the chord conductance equation that shows that the resting membrane potential is the conductance-weighted average of the equilibrium potentials for all ions (see Eqn [3.2.3]). At rest, the membrane potential is closer to the K^+ equilibrium potential because conductance K^+ is higher than the conductance of the other ions.

$$E_m = \frac{g_{Na}}{(g_{Na} + g_K + g_{Cl})} E_{Na} + \frac{g_K}{(g_{Na} + g_K + g_{Cl})} E_K + \frac{g_{Cl}}{(g_{Na} + g_K + g_{Cl})} E_{Cl} \quad [3.2.3]$$

During the rising phase of the action potential, the conductance to Na^+ increases, changing E_m from its resting, polarized value toward more positive values. If the conductance to Na^+ becomes large enough, relative to the conductances for K^+ and Cl^-, then E_m will be driven toward the equilibrium potential for Na^+, E_{Na}. Since E_{Na} is positive, E_m will be driven to positive values and will exhibit the overshoot.

G_{NA} INCREASES TRANSIENTLY DURING THE ACTION POTENTIAL; G_K INCREASES LATER AND STAYS ELEVATED LONGER

The results described earlier show that the rapid depolarization and overshoot in the action potential is due to a transient increase in membrane conductance, and this is accompanied by an Na^+ influx. The rapid repolarization of the membrane afterward is due to shutting off the increased Na^+ conductance and increasing the K^+ conductance. These results are consistent with the chord conductance equation. Hodgkin and Huxley succeeded in calculating g_{Na} and g_K during different parts of the action potential in the squid axon, and their results are summarized schematically in Figure 3.2.9. Although these results were determined in the squid, the principles remain the same for action potentials in mammalian excitable cells.

CONDUCTANCE AND EQUILIBRIUM POTENTIALS FOR NA$^+$ AND K$^+$ ACCOUNT FOR ALL OF THE FEATURES OF THE ACTION POTENTIAL

The origin of the action potential can be explained on the basis of the equilibrium potential for Na^+ and K^+ and the time course of their conductances. In the squid axon, the resting potential is on the order of −60 mV, with E_K about −75 mV. When the membrane is depolarized by some means, a conductance pathway for Na^+ begins to open. At this time, Na^+ is far away from its equilibrium

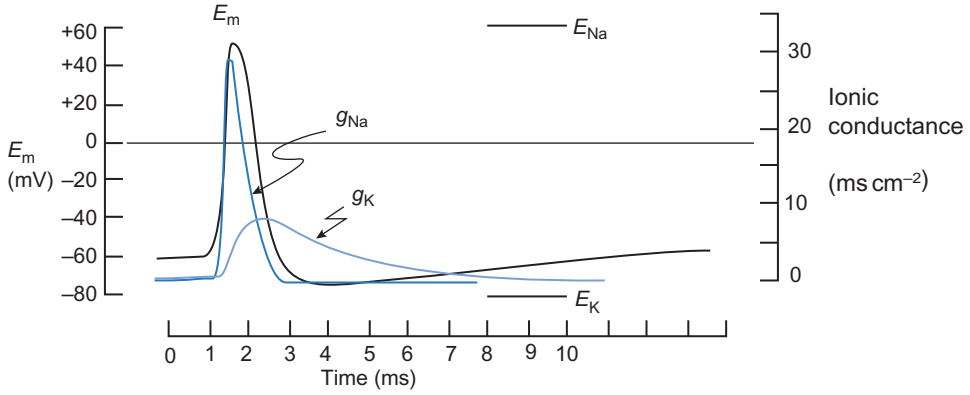

FIGURE 3.2.9 Changes in g_{Na} and g_K during the propagated action potential as calculated by Hodgkin and Huxley.

potential, so the driving force for Na^+ is large and the opening of the conductance pathway causes a Na^+ influx and a further depolarization of the membrane. This additional depolarization further opens additional conductance pathways. This positive feedback explains the explosive increase in Na^+ conductance and the rapid increase in the membrane potential toward E_{Na}.

The pathway for Na^+ conductance next undergoes a **time-dependent inactivation**. This by itself reduces the inward Na^+ current, except that the inactivation is accompanied by a reduction in E_m (i.e., a repolarization of the membrane) and a subsequent increase in the driving force. The Na^+ current is complicated, being the product of the conductance and the net driving force (see Eqn [3.1.18]).

Repolarization of the membrane would occur with a return of Na^+ conductance to normal, but it is hastened by a delayed increase in g_K followed by its gradual return to resting levels. The increased g_K increases an outward current carried by K^+, and this current more quickly repolarizes the cell. Because g_K remains elevated even when g_{Na} has returned to normal, the cell is hyperpolarized for several ms following the action potential until g_K returns to normal.

G_{NA} IS A FUNCTION OF A NA^+-SELECTIVE CHANNEL

The identification of two major components, I_{Na} and I_K, in the ionic currents by Hodgkin and Huxley, has allowed electrophysiologists to characterize the gating properties of the channels that pass these currents. The present view is that the Na^+ channel has at least four distinct components:

- **The Selectivity Filter Enables the Na^+ Channel to Pass Na^+ Preferentially**
  The relative permeabilities of ions in ion channels can be calculated by measuring the reversal potential when the ion composition is changed. The results show that the Na^+ channel actually conducts H^+ ions much more readily than it does Na^+ ions. The current through the Na^+ channel, however, is dominated by Na^+ because of its much higher concentration. The $[H^+]$ is around 10^{-7} M, whereas $[Na^+]$ is around 0.1 M, or 10^6-fold higher than $[H^+]$. It is believed that the selectivity relies on a combination of hydrated ion size and the free energy of hydration. Most other ions have a lower permeability through the Na^+ channel. Thus the Na^+ channel is **selective** for Na^+.
- **The Na^+ Channel Possesses an Activation Gate**
  At rest the membrane has a low g_{Na} because the Na^+ channel is blocked by its **activation gate**. This is a part of the Na^+ channel that can be moved to allow Na^+ to conduct through the channel. The opening of this gate is voltage dependent: the activation gate begins to open only when the membrane depolarizes. This voltage-dependent opening of the activation gates causes the explosive increase in Na^+ conductance in the rising phase of the action potential.
- **The Na^+ Channel Possesses an Inactivation Gate**
  Na^+ channels also inactivate. This is the function of a separate gate that closes according to time and voltage. When it closes, the Na^+ channel is nonconducting and, most importantly, it is **inactivatable**. Conductance through the Na^+ channel requires that both the inactivation gate and the activation gate are open. When the inactivation gate is closed, opening of the activation gate alone does not allow the channel to conduct ions.
- **Specific Toxins Bind to the Na^+ Channel**
  The puffer fish produces a potent toxin, **tetrodotoxin**, or TTX, that binds to Na^+ channels and blocks them. As a result, action potential conduction in nerve and muscle is blocked, with lethal consequences. Another natural toxin, **saxitoxin**, has similar properties. It derives its name from the Alaskan butter clam, *Saxidomus*. Eating a single contaminated shellfish can be fatal. The Na^+ channel contains regions that bind these toxins.

THE INACTIVATION GATES MUST BE RESET BEFORE ANOTHER ACTION POTENTIAL CAN BE FIRED

An action potential can occur in a nerve only when the Na^+ channels can open. If they are blocked, for example, by tetrodotoxin or saxitoxin, then no action potentials are possible. Similarly, if the channels are in the inactivatable state because the inactivation gate is

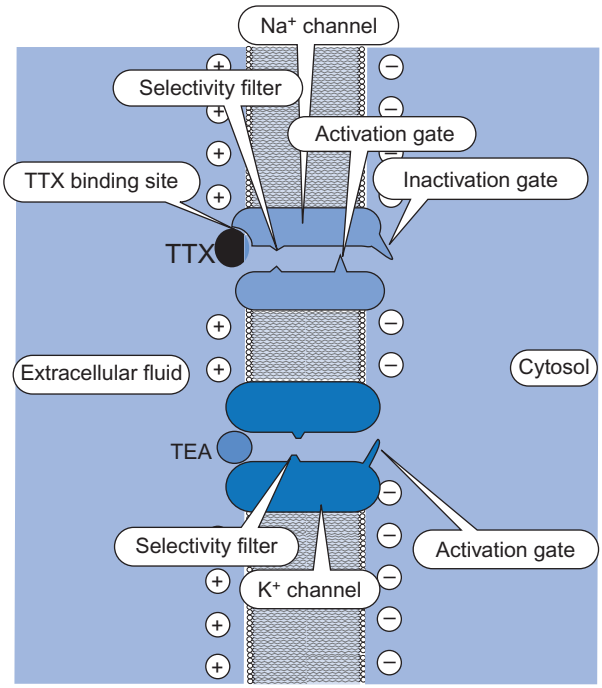

FIGURE 3.2.10 Hypothetical and conceptual model of the voltage-gated Na^+ channel and K^+ channel. The selectivity filter allows Na^+ to pass but not K^+ (top). When TTX (black circle) binds to its site, the channel is blocked. Closure of either the activation gate or inactivation gate will block Na^+ conductance. The K^+ channel also possesses an activation gate and is blocked by tetraethylammonium (TEA).

closed, they also cannot be activated by opening the activation gates. Following a normal action potential, the Na^+ channels are reset when the activation gates close and the inactivation gates open. This takes some time. Part of the refractoriness of the nerve cell immediately following the action potential is due to the lower conductance of the membrane to Na^+ because the inactivation gates are closed.

Figure 3.2.10 shows a conceptual model of the Na^+ and K^+ channels with their activation and inactivation gates and their binding sites for specific blockers. Figure 3.2.11 shows how these channels change during the action potential. Figure 3.2.12 shows the timing of opening of the activation and inactivation gates and that Na^+ entry is possible only when both are open.

CONDUCTANCE DEPENDS ON THE NUMBER AND STATE OF THE CHANNELS

Figure 3.2.11 shows a cartoon of the states of the Na^+ and K^+ channels in axon membranes that give rise to the action potential. Out of necessity, only representative channels are shown in the figure. They are meant to represent what a **population** of channels is doing. The overall current carried through a patch of membrane is given by

$$I_i = NP_o\mathbf{i} \quad [3.2.4]$$

where I is the current, N is the number of channels, P_o is the probability of a channel being open, and $\mathbf{i}$ is the **unitary current**, the current carried by the open channel under physiological conditions. Cells possess thousands of channels, each of whose behavior is **stochastic**, meaning that their opening and closing are not deterministic but probabilistic. The average behavior of many channels or the behavior of a large number of them is predictable, but the behavior of a single channel appears to be erratic and unpredictable. The sum of the currents of a population of channels is called the **ensemble current**.

PATCH CLAMP EXPERIMENTS MEASURE UNITARY CONDUCTANCES

It is possible to study the behavior of single channels using a patch clamp technique, shown in Figure 3.2.13. In this method, one clamps the voltage across the patch and measures current across it at that voltage. One can also step the potential from some holding value to a new value and measure the currents. Figure 3.2.14 shows the unitary Na^+ and K^+ currents in successive sweeps from patch clamp recordings in neuroblastoma cells (left) and squid giant axon (right). Distinction can be made between I_{Na} and I_K by judicious choice of ionic conditions and use of specific inhibitors. The Na^+ channels open briefly upon depolarization and then do not open later on. Note that the individual channels open and close rather erratically. It is not possible to predict exactly when a particular channel will either open or close. The presence of a large number of channels, however, will smooth out these discontinuities. The ensemble average is obtained by averaging many sweeps. This describes the average behavior of a single channel over many experiments, which is equivalent to the average behavior of a population of channels in a single sweep. The average behavior shows that Na^+ channels open upon depolarization and then close, or inactivate. K^+ channels, on the other hand, open after a slight delay and stay open during depolarization. Repolarization closes the K^+ channels.

THE CURRENT–VOLTAGE RELATIONSHIP FOR THE WHOLE CELL DETERMINES THE THRESHOLD FOR EXCITATION

Now that we know the ionic basis of both the resting membrane potential and the action potential, we are in a better position to understand why there is a critical level of depolarization. The whole-cell current–voltage relationship in a cell is approximated by the curve shown in Figure 3.2.15. This curve results mainly from the i–v relationship shown for the K^+ channels and for the Na^+ channels shown in Figure 3.1.3. These currents were for the open channels, and the whole-cell i–v relationship is not just a sum of these, but also reflects the probability of the channel opening at the particular membrane potential. The resulting curve has negative currents at hyperpolarized membrane potentials, positive current at slight depolarizations, and negative currents at further depolarizations. Recall our convention for current sign: a positive current is an outward current,

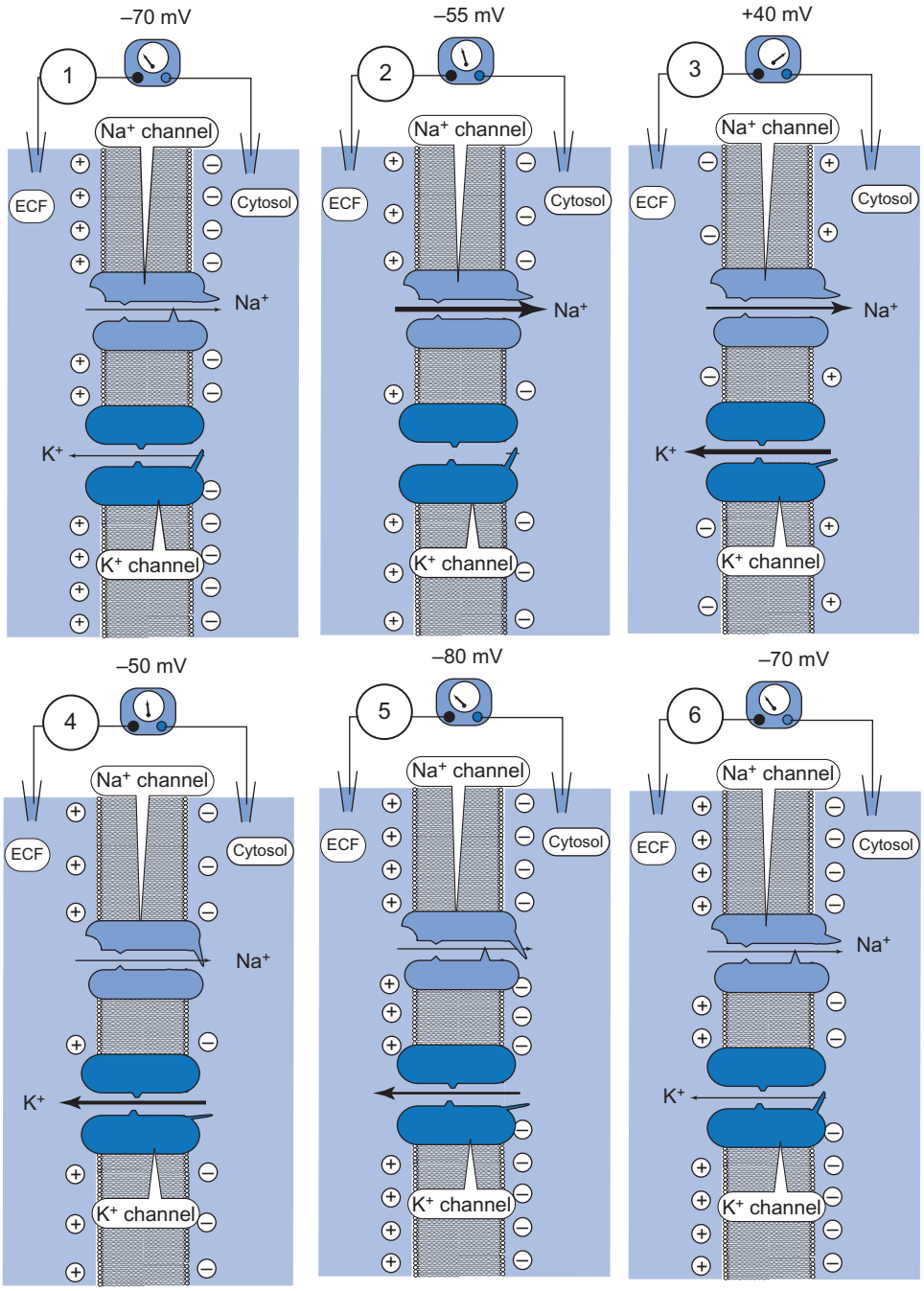

FIGURE 3.2.11 Proposed conceptual changes in the Na^+ and K^+ channels that give rise to the action potential. At rest (1), fluxes through both Na^+ and K^+ channels are small and the membrane is polarized. The Na^+ activation gate is closed and the inactivation gate is open. The K^+ activation gate is closed. During threshold depolarization (2), the Na^+ activation gate opens and inward Na^+ current further depolarizes the cell, leading to the upstroke of the action potential and the overshoot. Inward Na^+ slows as Na^+ approaches its equilibrium potential. Meanwhile, the K^+ activation gate opens, leading to an outward K^+ current (3). This outward K^+ current repolarizes the cell and the Na^+ inactivation gate closes (4). The continued outward K^+ current may lead to a hyperpolarization. The Na^+ activation gate closes (5). Upon repolarization, the K^+ activation gate closes and the Na^+ inactivation gate opens, resetting to the resting condition. The diagram here shows a single channel, but membrane conductance is governed by an ensemble of channels whose states are not necessarily identical.

and current is in the direction of positive ion flow. Thus a positive current removes positive ions from the cell, which hyperpolarizes the cell. Thus at membrane potentials lower than the resting membrane potential there is a negative total current carried by Na^+ ions into the cell, the result being a depolarization back toward the resting membrane potential. The total current is zero at the resting membrane potential. At the resting membrane potential, the total $i-v$ curve crosses the x-axis. At slight depolarizations, the current is positive, meaning that positive ions exit the cell and cause a return towards the resting membrane potential. At the uniform threshold, the $i-v$ curve again crosses the x-axis. At this point, the current shifts from positive to negative. A slightly more positive membrane potential shifts the current from positive, which returns the membrane to the resting membrane potential, to negative, caused by influx of Na^+, and the membrane depolarizes. This depolarization results in progressively more negative currents. This is the influx of Na^+ ions that constitutes the rising phase of the action potential. The point where the $i-v$ curve crosses the x-axis on the high potential side is the "uniform threshold," meaning that if the membrane was uniformly depolarized to this point, an action potential would ensue 50% of the time. It turns out that the membrane is seldom uniformly depolarized: the potential varies with distance from the point of excitation. As a consequence, the actual threshold is generally higher than the uniform threshold.

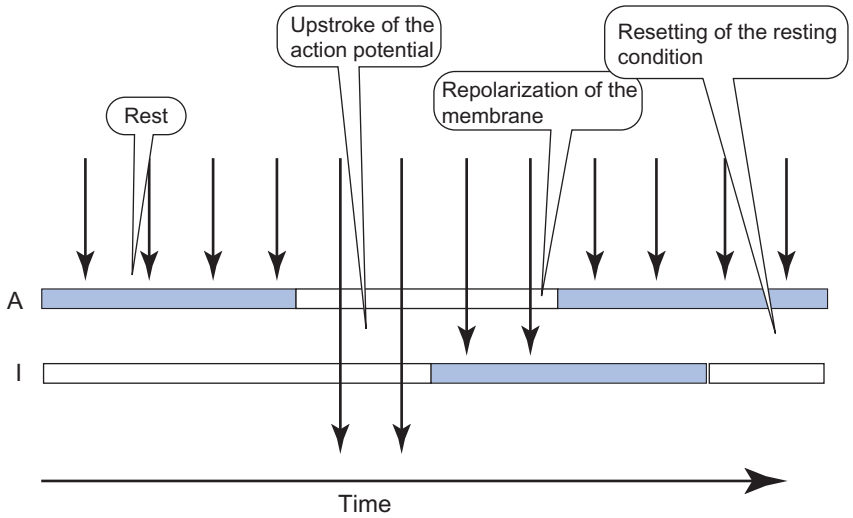

FIGURE 3.2.12 Timing of the opening of the Na^+ channel activation gate (A) and inactivation gate (I). The activation gate is closed at rest while the inactivation gate is open. The activation gate opens briefly during excitation, and Na^+ can cross the membrane because both gates are open. The inactivation gate closes, partly contributing to repolarization of the membrane. This first closes the activation gate, followed by a later resetting of the inactivation gate to the open state. Blue indicates closed state; white indicates open state.

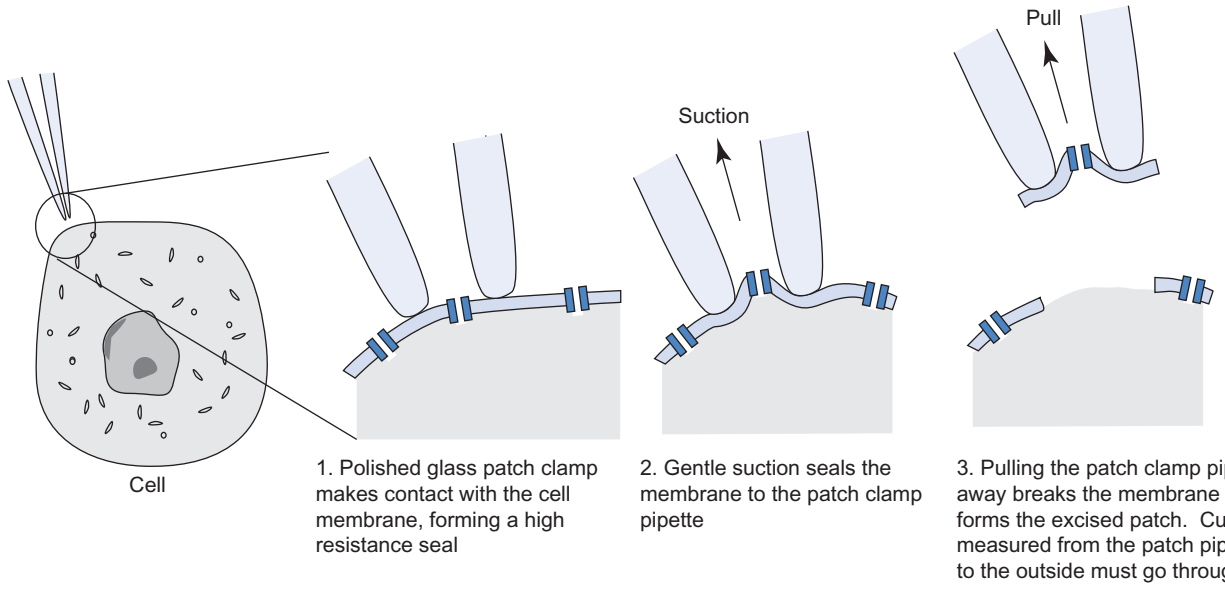

FIGURE 3.2.13 Method for making an excised cell patch. A polished glass microelectrode is brought near to a cell while positive pressure is applied to keep the microelectrode contents uncontaminated by the cell bathing solution. As the microelectrode approaches the cell, the resistance increases, indicating close approach. Positive pressure is turned off and the microelectrode advances to make contact with the membrane. Clean membranes will form a high-resistance seal. Suction is then applied and the microelectrode is reversed to pull off a small patch of membrane. In this configuration, any current that passes across the microelectrode tip must pass through the patch. In this way, single channel currents can be measured. The patch may have none, one, two, or several channels. Experiments can also be performed using cell-attached patches.

THRESHOLD DEPOLARIZATION REQUIRES A THRESHOLD CHARGE MOVEMENT, WHICH EXPLAINS THE STRENGTH–DURATION RELATIONSHIP

Depolarization to threshold requires enough charge to change the voltage across the membrane to the threshold voltage. When stimulation is at a point, the threshold voltage is actually higher because the negative current in the patch of membrane above threshold is partially offset by positive currents in nearby membrane that is below threshold. The condition for threshold is that the total current of the cable is inward. The area of membrane that supplies inward current must be large enough so that outward currents supplied by the rest of the membrane are counterbalanced. The minimum length of fiber that must be depolarized to threshold is called the **liminal length**. This idea is shown diagrammatically in Figure 3.2.16.

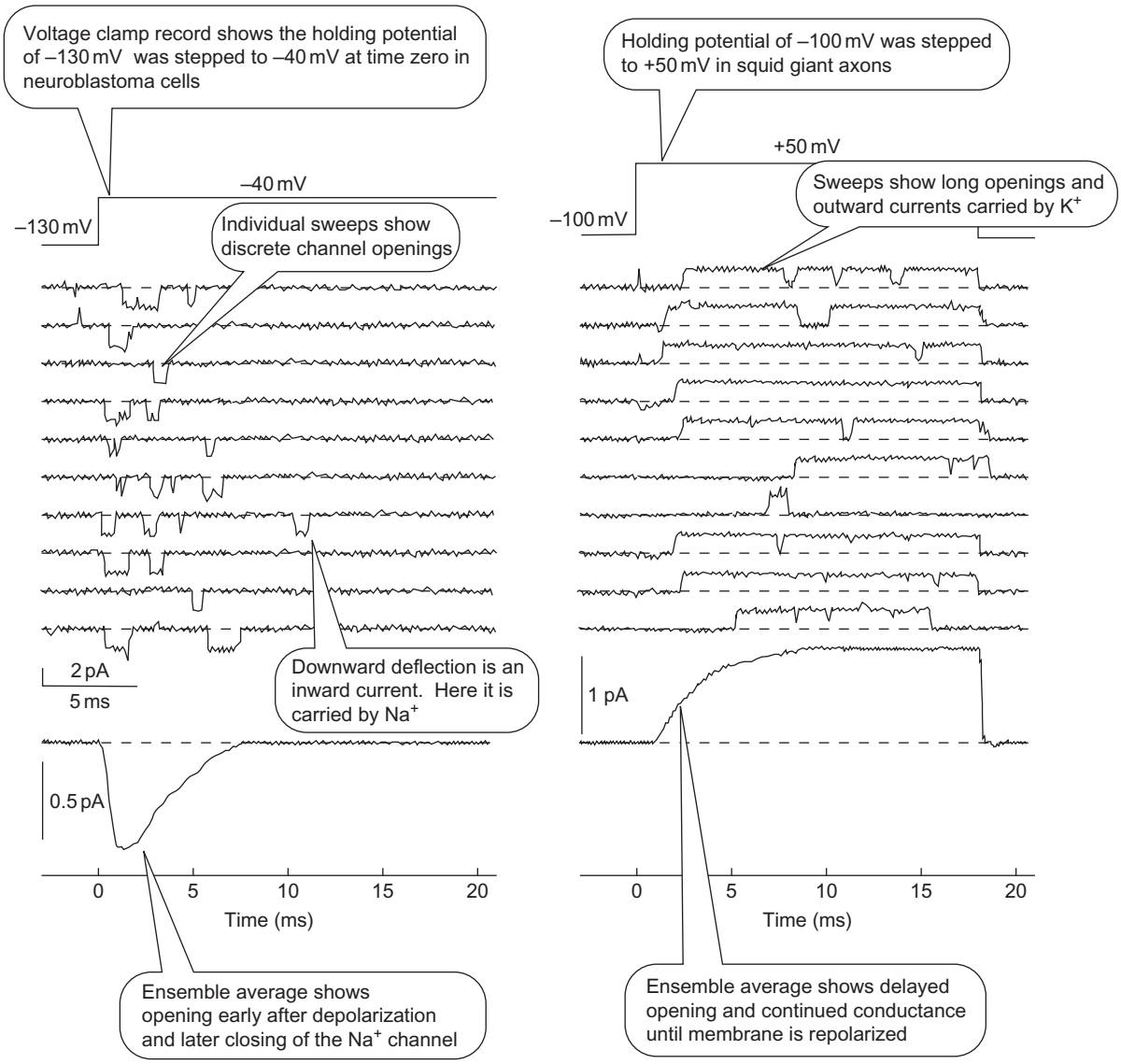

FIGURE 3.2.14 Patch clamp currents of Na^+ channels in neuroblastoma cells (left) and K^+ channels of squid giant axon (right). (Left) Adapted from data of C.M. Baumgarten, S.C. Dudley, R.B. Rogart and H.A. Fozzard, Unitary conductance of Na^+ channel isoforms in cardiac and NB2a neuroblastoma cells. *Am. J. Physiol.* **269**: C1356–C1363, 1995. (Right) Adapted from data of F. Bezanilla and C.K. Augustine cited in B. Hille, *Ionic Channels in Excitable Membranes*, Sinauer, 1992.

THE AMOUNT OF CHARGE NECESSARY TO REACH THRESHOLD EXPLAINS THE STRENGTH–DURATION RELATIONSHIP

Figure 3.2.16 indicates that depolarization to threshold requires movement of sufficient charge according to the capacitance of the membrane: $\Delta V = \Delta q/C$. Thus reaching threshold (a ΔV from the resting potential) requires a defined amount of charge movement. This explains the inverse relationship between stimulus strength (its current) and the duration, according to the Weiss Equation (see Eqn [3.2.1]).

SUMMARY

Resting nerve cells are polarized with a negative resting membrane potential caused by greater K^+ conductance in the resting cell. Application of an outward current further polarizes the membrane, and the recording membrane potential is a distorted version of the stimulus. Application of an inward current depolarizes the membrane. If depolarization reaches threshold, nerve cells fire an action potential. The action potential is a brief, pulse-like change in the membrane potential which can move from one area of the cell membrane to another and so it can be used to signal distant parts of the neuron.

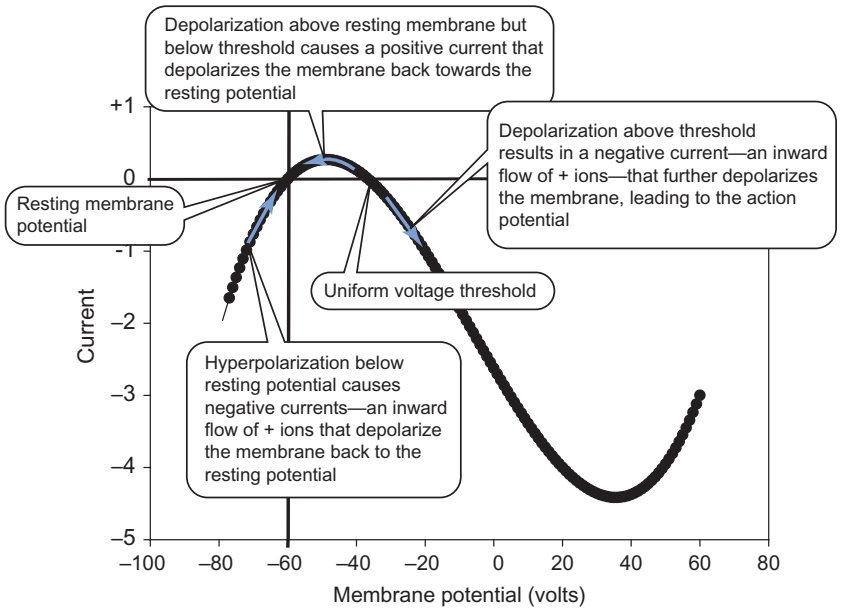

FIGURE 3.2.15 Whole-cell current–voltage relationship. Positive currents above the equilibrium potential for K^+ are mainly due to K^+ exit, mainly due to the increased driving force for K^+. Negative currents are mainly due to Na^+ entry, due to reduction in the driving force for K^+. Hyperpolarization below the resting membrane potential causes a negative current (inward flow of positive ions) that depolarizes the cell back towards the resting membrane potential. Slight depolarizations produce a positive current (outward flow of positive ions) that repolarizes the cell back towards the resting membrane potential. Thus slight hyperpolarization or depolarization returns the cell to rest. Larger depolarizations cause a negative current due to inward flow of Na^+ ions that further depolarize the cell in the action potential.

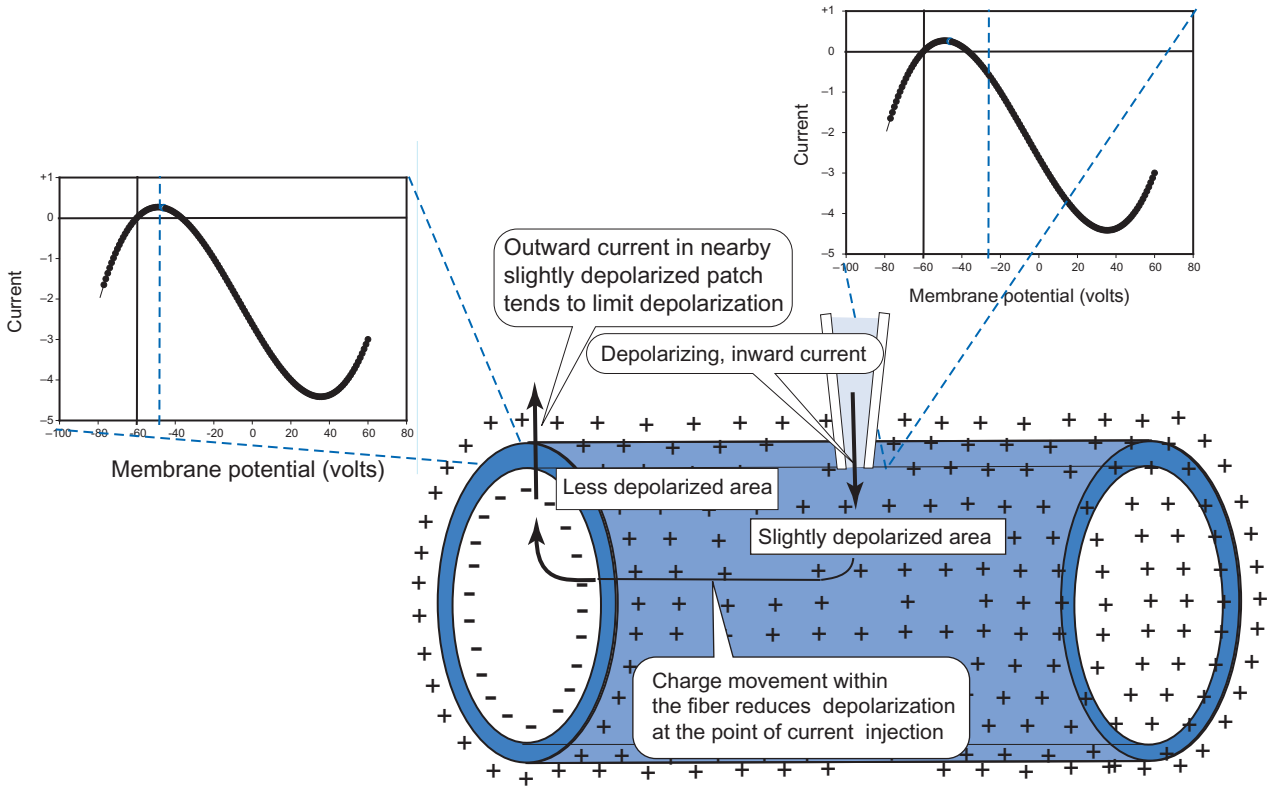

FIGURE 3.2.16 Consequence of spatial differences in membrane potential. Excitation occurs at a location in the membrane that causes a depolarization. This depolarization is sufficiently large to produce a negative or inward current that would further depolarize the cell. However, nearby patches of membrane are depolarized less and they are located on the part of the *i–v* curve that carries a positive current. This positive current lowers the depolarization at the point of excitation.

Action potentials are all or none: you either get one or you don't. Increasing stimulus strength shortens the delay before the start of the action potential, but it does not alter its peak or its duration. The threshold is the potential at which an action potential is triggered 50% of the time, but this depends on the rate of depolarization. For some period of time after the start of an action potential, nerve cells will not begin another action potential. This period is the absolute refractory period. The relative refractory period follows the absolute refractory period. During the relative refractory period, the threshold for initiating a second action potential is higher.

During the action potential, the membrane potential overshoots zero and becomes positive. Since only Na^+ and Ca^{2+} have positive equilibrium potentials, the membrane potential can become positive only if the permeability to one of these increases. The ionic currents giving rise to the action potential for squid giant axons were described by Alan Hodgkin and Andrew Huxley, who received the Nobel Prize for the work in 1963. They developed a kinetic model that explained the action potential in terms of a single voltage-gated K^+ channel and a Na^+ channel governed by voltage-dependent and time-dependent activation and inactivation gates. During rest, the Na^+ inactivation gate is open and the activation gate is closed. Upon depolarization, the activation gate opens and Na^+ conductance increases markedly. This causes an inward current (carried by Na^+) that further depolarizes the membrane and causes more Na^+ channels to open. The explosive increase in g_{Na} causes the rising phase of the action potential. With time, the Na^+ inactivation gate closes, the K^+ activation gate opens, and the Na^+ activation gate closes. Opening of the K^+ activation gate and closing of the Na^+ inactivation gate cause an outward current that repolarizes the membrane. With further time, the Na^+ inactivation gates reopen and this reestablishes the resting condition.

The squid axon is presented here as an example of how to think about excitable cells. Each excitable cell, and in fact each part of an excitable cell, can have different sets of channels with distinct activation and inactivation properties. The height of the action potential and its duration depend on these characteristics, as well as the electrical characteristics of the cell and the ionic conditions inside and outside the cell.

Channels have discrete states with discrete conductances. Each channel undergoes transitions between conducting and nonconducting or subconducting states. Such discontinuous conductance changes cannot be described by continuum mathematics but rely on probabilistic descriptions. Continuum mathematics can describe the average or ensemble behavior of a population of channels, provided the population is sufficiently large.

Depolarization to threshold requires the movement of sufficient charge to cause the depolarization. This charge can be delivered over a short time at high current or long time at low current. This is the basic nature of the strength–duration relationship. At lower currents there is more time for redistribution of charges within the nerve fiber so that the relationship is not purely reciprocal. The strength–duration relationship is adequately described by the Weiss equation: $I = I_{rh}(t + \tau_{SD})/t$, where I_{rh} is the rheobase and τ_{SD} is the chronaxie.

REVIEW QUESTIONS

1. If a Na-selective channel was to open on the membrane of a motor neuron at rest, which way would current flow? Would this depolarize or hyperpolarize the cell?
2. If a K-selective channel was to open on the membrane of a motor neuron at rest, which way would current flow? Would this depolarize or hyperpolarize the cell?
3. What is an action potential? Why does the membrane potential become positive during the action potential?
4. What do we mean when we say that action potentials are "all or none"? What is the absolute refractory period? Relative refractory period?
5. What is the "activation gate" of the Na^+ channel? When is it open? When does it close? What is the "inactivation gate" of the Na^+ channel? When is it open? When does it close?
6. What is tetrodotoxin? Why does it block action potentials?
7. What is a patch clamp? Why is it useful? What is an ensemble current?
8. How do g_{Na} and g_K change during the action potential? What causes these changes?
9. What is the total current across the membrane at the resting membrane potential? Why does a slight depolarization, below threshold, come back to the resting membrane potential? Why does a slight hyperpolarization return to the resting membrane potential?
10. Why is there an inverse relationship between current and time to reach threshold?

APPENDIX 3.2. A1 THE HODGKIN–HUXLEY MODEL OF THE ACTION POTENTIAL

ALAN HODGKIN AND ANDREW HUXLEY'S GOAL WAS TO ACCOUNT FOR THE ACTION POTENTIAL BY MOLECULAR MECHANISMS

Hodgkin and Huxley's goal was to explain the ionic fluxes and conductance changes during the action potential in terms of molecular mechanisms. After trying some different mechanisms, they concluded that not enough was known to determine a unique mechanism. Instead, they tried to develop an empirical kinetic description which would allow them to calculate electrical responses and which would correctly predict the shape of the action potential and its conduction velocity. Today this model is referred to as the Hodgkin–Huxley or HH model.

THE HH MODEL DIVIDES THE TOTAL CURRENT INTO SEPARATE NA^+, K^+, AND LEAK CURRENTS

Hodgkin and Huxley wrote the Na^+ and K^+ currents in terms of their maximum conductances which are multiplied by coefficients that vary continually between 0 and 1. The overall conductance, then, varies between 0 and the maximum conductance. All of the kinetic properties of the conductances are embedded in the characteristics of the coefficients. The conductances in the model vary with voltage and time but not with concentration of either Na^+ or K^+.

THE HH MODEL OF THE K^+ CONDUCTANCE INCORPORATES FOUR INDEPENDENT "PARTICLES"

On depolarization, the increase in g_K follows an S-shaped curve, whereas g_K decreases exponentially upon repolarization. Hodgkin and Huxley proposed that the gating of the K^+ channel could be modeled by four identical membrane "particles." The probability that each channel is in the position to allow K^+ conductance is n. All four particles must be correctly situated to allow conductance. The probability that all four are positioned for conductance is n^4. The K^+ current is given as

[3.2.A1.1] $$I_K = n^4 g_{K_{max}}(E_m - E_K)$$

Here the probability n depends on time and voltage. In the HH model, values of n are determined by a first-order reaction, written as

[3.2.A1.2] $$1 - n \underset{\beta_n}{\overset{\alpha_n}{\rightleftharpoons}} n$$

where α_n and β_n depend on voltage. The reaction in Eqn [3.2.A1.2] can be written in differential form as

[3.2.A1.3] $$\frac{dn}{dt} = \alpha_n(1 - n) - \beta_n n$$

Hodgkin and Huxley determined empirical relationships between the rate constants, α_n and β_n, and the membrane potential. For the squid axon at 6°C, these were:

[3.2.A1.4] $$\alpha_n = \frac{0.01(10 - (E_m - E_r))}{e^{(10-(E_m-E_r))/10} - 1}$$
$$\beta_n = 0.125e^{(-(E_m-E_r)/80)}$$

where the membrane potential is in millivolts and the rate constants have units of $M\,s^{-1}$. These are not the exact forms originally used by Hodgkin and Huxley, because their sign convention for membrane potential is the reverse of that used today. In addition, Hodgkin and Huxley derived their empirical equations based on voltage clamp experiments in which the degree of variation from resting membrane potential, here symbolized as E_r, was clamped. Equation [3.2.A1.4] is transformed to agree with today's conventions.

An alternative way of expressing Eqn [3.2.A1.3] is as follows:

[3.2.A1.5] $$\frac{dn}{dt} = \frac{(n_\infty - n)}{T_n}$$

where n_∞ is the steady-state value of n at any particular voltage and T_n is a time constant. The values of n_∞ and T_n are given by

[3.2.A1.6] $$n_\infty = \frac{\alpha_n}{\alpha_n + \beta_n}$$
$$T_n = \frac{1}{\alpha_n + \beta_n}$$

THE HH MODEL OF NA^+ CONDUCTANCES USES ACTIVATING AND INACTIVATING PARTICLES

Similar to the case with the K^+ conductance model, HH empirically modeled the Na^+ conductance with four hypothetical gating particles that make first-order transitions between conductive and nonconductive states. Because the Na^+ conductance has two opposing actions, activation and inactivation, Hodgkin and Huxley used two kinds of gating particles, called m and h. Here the probability of an open configuration is m and h, respectively. The probability that all four gates are open is m^3h. In this case, the Na^+ current is given as

[3.2.A1.7] $$I_{Na} = m^3 h g_{Na_{max}}(E_m - E_{Na})$$

The same formalism for transitions between open and closed states of the n gates for the K^+ channels also governs transitions between the open and closed states of the m and h gates of the Na^+ channel:

[3.2.A1.8] $$1 - m \underset{\beta_m}{\overset{\alpha_m}{\rightleftharpoons}} m$$
$$1 - h \underset{\beta_h}{\overset{\alpha_h}{\rightleftharpoons}} h$$

The rate constants all depend on voltage, according to the following empirical relations:

$$\alpha_m = \frac{0.1(25 - (E_m - E_r))}{e^{(25-(E_m-E_r))/10} - 1}$$

[3.2.A1.9] $$\beta_m = 4e^{(-(E_m-E_r)/18)}$$
$$\alpha_h = 0.07e^{(-(E_m-E_r)/20)}$$
$$\beta_h = \frac{1}{e^{((30-(E_m-E_r))/10)} + 1}$$

Relationships among m_∞, h_∞, T_m, and T_h are given in analogy to Eqn [3.2.A1.6].

CALCULATION OF $G_{NA(T)}$ AND $G_{K(T)}$ FOR A VOLTAGE CLAMP EXPERIMENT

To calculate the time dependence of Na^+ and K^+ conductances, we need to know the set of rate constants

which describe the transitions between states of the conductances in the HH model: (α_n, β_n, α_m, β_m, α_h, β_h). Further, we need to know this set at the two voltages. In the voltage clamp experiment, the nerve is held at some voltage and then is rapidly switched to another voltage. As a result, the conductances go through a transition from one state to another. Because the gates have different kinetics, the overall behavior can be complex. Each of the gates relaxes between the steady-state value before the voltage jump, and the second steady-state value at the new voltage. The equation describing the time course from an initial value of n, m, and h (n_0, m_0, and h_0) and a final value of n, m, and h (n_∞, m_∞, and h_∞) can be derived from integrating Eqn [3.2.A1.3]:

[3.2.A1.10]

$$\frac{dn}{dt} = \alpha_n(1-n) - \beta_n n$$

$$= \left(\frac{\alpha_n}{\alpha_n+\beta_n} - n\right)(\alpha_n+\beta_n)$$

$$\frac{dn}{(\alpha_n/\alpha_n+\beta_n)-n} = (\alpha_n+\beta_n)dt$$

$$\int_{n_0}^{n} \frac{dn}{(\alpha_n/\alpha_n+\beta_n)-n} = \int_0^t (\alpha_n+\beta_n)dt$$

Integrating, we obtain

[3.2.A1.11]

$$\ln\left[\frac{\alpha_n}{\alpha_n+\beta_n} - n\right]_{n_0}^{n} = -\frac{t}{T_n}$$

$$\ln\left[\frac{n_\infty - n}{n_\infty - n_0}\right] = -\frac{t}{T_n}$$

$$n = n_\infty - (n_\infty - n_0)e^{-(t/T_n)}$$

Here we have identified T_n and n_∞ according to Eqn [3.2.A1.6]. It is easy to justify the assignment of n_∞: simply let dn/dt in Eqn [3.2.A1.3] go to zero at infinite time (at which point the steady-state value of n should have been reached). From that constraint, we find $n_\infty = \alpha_n/(\alpha_n+\beta_n)$.

The last equation tells us that at $t=0$, $n=n_0$ and at $t=\infty$, $n=n_\infty$. In between, n relaxes between n_0 and n_∞ with an exponential time course. If we know n_0, n_∞, and T_n, we can calculate the $n(t)$. All of these values depend on the α and β for the given gates. The procedure is entirely analogous for m and h.

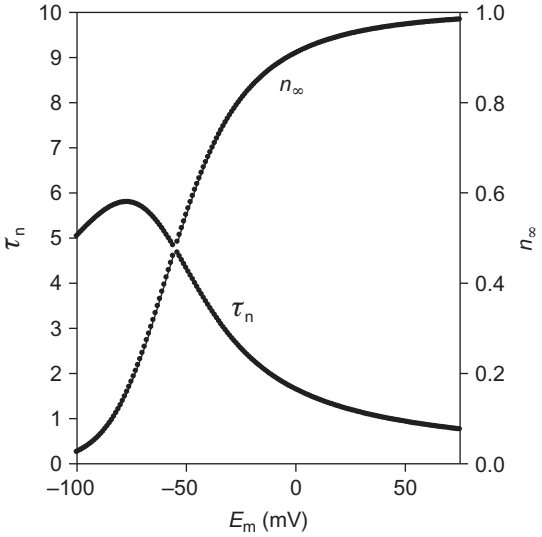

FIGURE 3.2.A1.1 The dependence of τ_n and the steady-state value of n (n_∞) as a function of the membrane potential, E_m. These values of n control the conductance through the K^+ channel in the HH formalism.

RESULTS OF THE CALCULATIONS

Figure 3.2.A1.1 shows the voltage dependence of n_∞ and T_n.

The voltage clamp experiments performed by Hodgkin and Huxley involved changing the membrane potential essentially instantaneously from the resting potential (−65 mV) to some set potential and clamping it there. What happens is that the resting values of n, m, and h (called n_0, m_0, and h_0) relax to their new values at the clamped voltage, which we will call n_∞, m_∞, and h_∞. The resulting time course of n, m, and h generates a time course for the conductances, calculated according to Eqns [3.2.A1.1] and [3.2.A1.7]. For a +88-mV clamp from −65 to +23 mV, the relevant values are given in Table 3.2.A1.1 (see Figure 3.2.A1.2).

These values are substituted into equations of the form of Eqn [3.2.A1.11] to derive $n(t)$, $m(t)$, and $h(t)$, from which the instantaneous conductances can be calculated

TABLE 3.2.A1.1 Values of n, m, and h for Voltage Clamp from −65 to +23 mV

K Channel	Na Channel	
$n_0 = 0.3177$	$m_0 = 0.0529$	$h_0 = 0.5961$
$n_\infty = 0.9494$	$m_\infty = 0.9953$	$h_\infty = 0.0009$
$T_n = 1.2028$ ms	$T_m = 0.1577$ ms	$T_h = 1.0022$ ms
$g_{Kmax} = 36$ ms cm^{-2}	$g_{Namax} = 120$ ms cm^{-2}	

Derived from Figures 3.2.A1.1 and 3.2.A1.2.

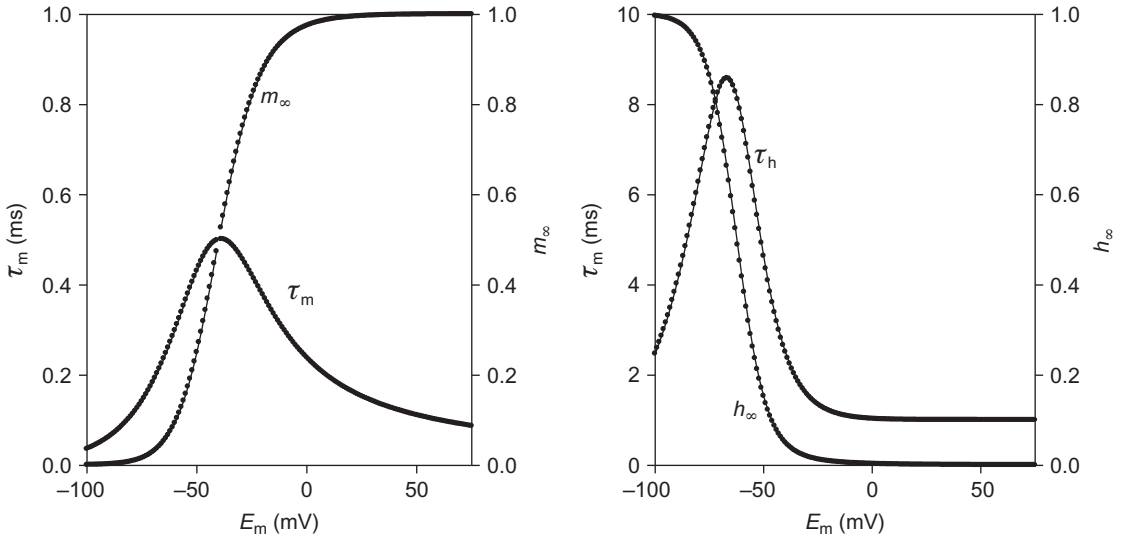

FIGURE 3.2.A1.2 The dependence of τ_m and m_∞ on the membrane potential. These values control the opening of the activation gate of the Na^+ channel in the HH fomalism.

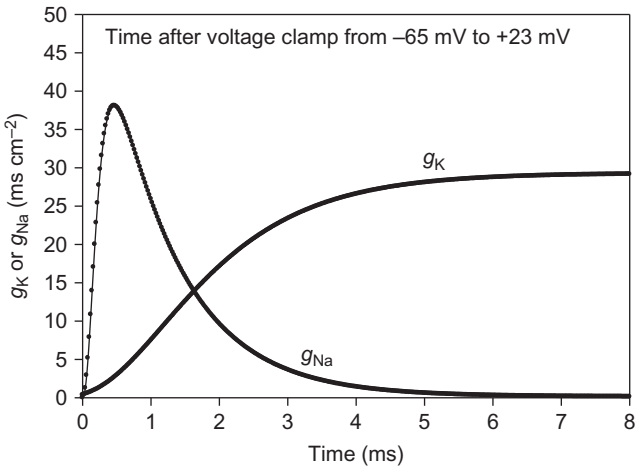

FIGURE 3.2.A1.3 Calculated changes in g_{Na} and g_K during a voltage clamp from −65 mV to +23 mV using the HH formalism.

according to Eqns [3.2.A1.1] and [3.2.A1.7]. The results of these calculations for the given voltage clamp are shown in Figure 3.2.A1.3.

Of course, Hodgkin and Huxley had it much more difficult than this, because they had to find the original equations and parameters to fit their voltage clamp results, whereas here we are simply confirming that the equations and parameters they found do, indeed, look like their voltage clamp records. It is important to remember that the Hodgkin–Huxley formalism is an empirical model, designed to fit the data. Although there are deficiencies in the model, it succeeds admirably well in predicting the wave form of the action potential. It is now generally accepted that the basis of the action potential is a rapid switching on of the Na^+ conductance, followed by its inactivation and more slowly turning on of the K^+ conductance.