threshold.[1] Third, the action potential is conducted without decrement. It has a self-regenerative feature that keeps the amplitude constant, even when it is conducted over great distances. Fourth, the action potential is followed by a *refractory period*. For a brief time after an action potential is generated, the neuron's ability to fire a second action potential is suppressed. The refractory period limits the frequency at which a nerve can fire action potentials, and thus limits the information-carrying capacity of the axon.

These four properties of the action potential—initiation threshold, all-or-none amplitude, conduction without decrement, and refractory period—are unusual for biological processes, which typically respond in a graded fashion to changes in the environment. Biologists were puzzled by these properties for almost 100 years after the action potential was first recorded in the mid-1800s. Finally, in the late 1940s and early 1950s, studies of the membrane properties of the giant axon of the squid by Alan Hodgkin, Andrew Huxley, and Bernard Katz provided the first quantitative insight into the mechanisms underlying the action potential.

The Action Potential Is Generated by the Flow of Ions Through Voltage-Gated Channels

An important early insight into how action potentials are generated came from an experiment performed by Kenneth Cole and Howard Curtis that predated the studies by Hodgkin, Huxley, and Katz. While recording from the giant axon of the squid, they found that the conductance of the membrane increases dramatically during the action potential (Figure 10–1). This discovery provided evidence that the action potential results from a dramatic increase in the ion permeability of the cell membrane. It also raised two central questions: Which ions are responsible for the action potential, and how is the permeability of the membrane regulated?

Hodgkin and Katz provided a key insight into this problem by demonstrating that the amplitude of the action potential is reduced when the external Na^+ concentration is lowered, indicating that Na^+ influx is responsible for the rising phase of the action potential. They proposed that depolarization of the cell above the threshold for an action potential causes a brief increase in the cell membrane's Na^+ conductance, during which the Na^+ conductance overwhelms the K^+ conductance that predominates in the cell at rest, thereby driving the membrane potential towards E_{Na}. Their data also suggested that the falling phase of the action potential was caused by a later increase in K^+ permeability.

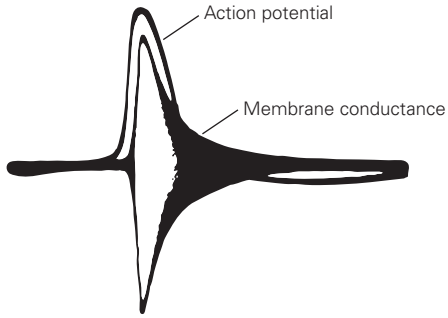


Figure 10–1 The action potential results from an increase in ion conductance of the axon membrane. This historic recording from an experiment conducted in 1939 by Kenneth Cole and Howard Curtis shows the oscilloscope record of an action potential superimposed on a simultaneous record of axonal membrane conductance.

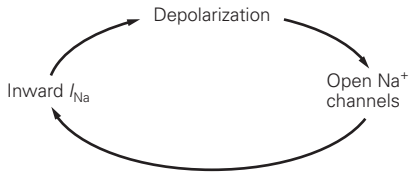
Sodium and Potassium Currents Through Voltage-Gated Channels Are Recorded With the Voltage Clamp

This insight of Hodgkin and Katz raised a further question. What mechanism is responsible for regulating the changes in the Na^+ and K^+ permeabilities of the membrane? Hodgkin and Andrew Huxley reasoned that the Na^+ and K^+ permeabilities were regulated directly by the membrane voltage. To test this hypothesis, they systematically varied the membrane potential in the squid giant axon and measured the resulting changes in the conductance of voltage-gated Na^+ and K^+ channels. To do this, they made use of a new apparatus, the voltage clamp, developed by Kenneth Cole.

Prior to the availability of the voltage-clamp technique, attempts to measure Na^+ and K^+ conductance as a function of membrane potential had been limited by the strong interdependence of the membrane potential and the gating of Na^+ and K^+ channels. For example, if the membrane is depolarized sufficiently to open some voltage-gated Na^+ channels, the influx of Na^+ through these channels causes further depolarization. The additional depolarization causes still more

[1]The all-or-none property describes an action potential that is generated under a specific set of conditions. The size and shape of the action potential *can* be affected by changes in membrane properties, ion concentrations, temperature, and other variables, as discussed later in the chapter. The shape can also be affected slightly by the current that is used to evoke it, if measured near the point of stimulation.

Na^+ channels to open and consequently induces more inward Na^+ current:



This positive feedback cycle drives the membrane potential to the peak of the action potential, making it impossible to achieve a stable membrane potential.

The voltage clamp interrupts the interaction between the membrane potential and the opening and closing of voltage-gated ion channels. It does so by adding or withdrawing a current from the axon that is equal to the current through the voltage-gated membrane channels. In this way, the voltage clamp prevents the membrane potential from changing. Thus, the amount of current that must be generated by the voltage clamp to keep the membrane potential constant provides a direct measure of the current through the voltage-gated channels (Box 10–1). Using the voltage-clamp technique, Hodgkin and Huxley were able to completely describe the ionic mechanisms underlying the action potential.

One advantage of the voltage clamp is that it readily allows the ionic and capacitive components of membrane current to be analyzed separately. As described in Chapter 9, the membrane potential V_m is proportional to the charge Q_m on the membrane capacitance C_m. When V_m is not changing, Q_m is constant, and no capacitive current ($\Delta Q_m/\Delta t$) flows. Capacitive current flows *only* when V_m is changing. Therefore, when the membrane potential changes in response to a commanded depolarizing step, capacitive current flows only at the beginning and end of the step. Because the capacitive current is essentially instantaneous, the ionic currents that subsequently flow through the voltage-gated channels can be analyzed separately.

Measurements of these ionic currents can be used to calculate the voltage and time dependence of changes in membrane conductance caused by the opening and closing of Na^+ and K^+ channels. This information provides insights into the properties of these two types of channels.

A typical voltage-clamp experiment starts with the membrane potential clamped at its resting value. When a small (10 mV) depolarizing step is applied, a very brief outward current instantaneously discharges the membrane capacitance by the amount required for a 10 mV depolarization. This capacitive current (I_c) is followed by a smaller outward current that persists for the duration of the voltage step. This steady ionic current flows through the nongated resting ion channels of the membrane, which we refer to here as *leakage channels* (see Box 9–2). The current through these channels is called the *leakage current*, I_l, and the total conductance of this population of channels is called the *leakage conductance* (g_l). At the end of the step, a brief inward capacitive current repolarizes the membrane to its initial voltage and the total membrane current returns to zero (Figure 10–3A).

If a large depolarizing step is commanded, the current record is more complicated. The capacitive and leakage currents both increase in amplitude. In addition, shortly after the end of the capacitive current and the start of the leakage current, an inward (negative) current develops; it reaches a peak within a few milliseconds, declines, and gives way to an outward current. This outward current reaches a plateau that is maintained for the duration of the voltage step (Figure 10–3B).

A simple interpretation of these results is that the depolarizing voltage step sequentially turns on two types of voltage-gated channels, each selective for a distinct ion species. One type of channel conducts ions that generate a rapidly rising inward current, while the other conducts ions that generate a more slowly rising outward current. Because these two oppositely directed currents partially overlap in time, the most difficult task in analyzing voltage-clamp experiments is to determine their separate time courses.

Hodgkin and Huxley achieved this separation by changing ions in the bathing solution. By replacing Na^+ with a larger, impermeant cation (choline $\cdot$ H^+), they eliminated the inward Na^+ current. Later the task of separating inward and outward currents was made easier by the discovery of drugs or toxins that selectively block the different classes of voltage-gated channels. Tetrodotoxin, a poison from a certain Pacific puffer fish, blocks the voltage-gated Na^+ channel with a very high potency in the nanomolar range of concentration. (Ingestion of only a few milligrams of tetrodotoxin from improperly prepared puffer fish, consumed as the Japanese sashimi delicacy *fugu*, can be fatal.) The cation tetraethylammonium (TEA) specifically blocks some voltage-gated K^+ channels.

When TEA is applied to the axon to block the K^+ channels, the total membrane current (I_m) consists of I_c, I_l, and I_{Na}. The leakage conductance g_l is constant; it does not vary with V_m or with time. Therefore, the leakage current I_l can be readily calculated and subtracted from I_m, leaving I_{Na} and I_c. Because I_c occurs only briefly at the beginning and end of the pulse, it is easily isolated by visual inspection, revealing the pure I_{Na}.

Box 10–1 Voltage-Clamp Technique

The voltage clamp permits the experimenter to "clamp" the membrane potential at predetermined levels, preventing changes in membrane current from influencing the membrane potential. By controlling the membrane potential, one can measure the effect of changes in membrane potential on the membrane conductance of individual ion species.

The voltage clamp is connected to a pair of electrodes (one intracellular and one extracellular) used to measure the membrane potential and another pair of electrodes used to pass current across the membrane (Figure 10–2A). Through the use of a negative feedback amplifier, the voltage clamp is able to pass the correct amount of current across the cell membrane to rapidly step the membrane to a constant predetermined potential.

Depolarization opens voltage-gated Na^+ and K^+ channels, initiating movement of Na^+ and K^+ across the membrane. This change in membrane current ordinarily would change the membrane potential, but the voltage clamp maintains the membrane potential at the predetermined (commanded) level.

When Na^+ channels open in response to a moderate depolarizing voltage step, an inward ionic current

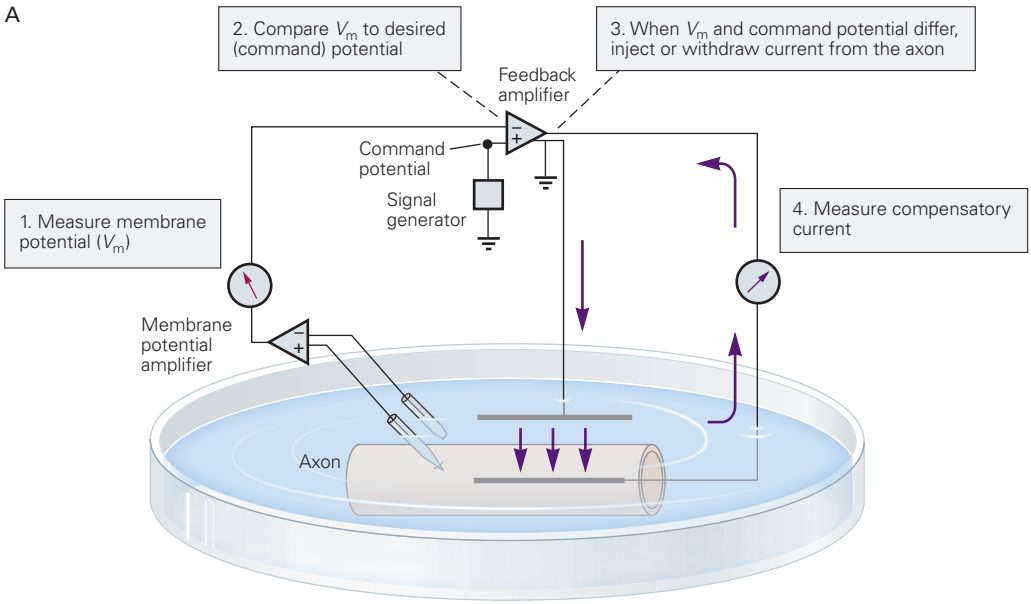


Figure 10–2 The negative feedback mechanism of the voltage clamp.

A. Membrane potential (V_m) is measured by two electrodes, one intracellular and one in the bath, connected to an amplifier. The membrane potential signal is displayed on an oscilloscope and also fed into the negative terminal of the feedback amplifier. The command potential, which is selected by the experimenter and can be of any desired amplitude and waveform, is fed into the positive terminal of the feedback amplifier. The feedback amplifier subtracts the membrane potential from the command potential and amplifies any difference between these two signals. The voltage output of the amplifier is connected to an internal current electrode, a thin wire that runs the length of the axon core. The negative feedback ensures that the voltage output of the amplifier will drive a current across the resistance of the current electrode that eliminates any difference between V_m and the command potential. To accurately measure the current–voltage relationship of the cell membrane, the membrane potential must be uniform along the entire surface of the axon. This is made possible by the highly conductive internal current electrode, which short-circuits the axoplasmic resistance, reducing axial resistance to near zero (see Chapter 9). This low-resistance pathway eliminates all variations in electrical potential along the axon core.

develops because Na^+ ions are driven through these channels by their electrochemical driving force. This Na^+ influx would normally depolarize the membrane by increasing the positive charge on the inside of the membrane and reducing the positive charge on the outside.

The voltage clamp intervenes in this process by simultaneously withdrawing positive charges from the cell and depositing them in the external solution. By generating a current that is equal and opposite to the ionic current through the membrane, the voltage-clamp circuit automatically prevents the ionic current from changing the membrane potential from the commanded value. As a result, the *net* amount of charge separated by the membrane does not change, and therefore, no significant change in V_m occurs.

The voltage clamp is a negative feedback system, a type of system in which the value of the output of the system (V_m in this case) is fed back as the input to the system and compared to a reference value (the command signal). Any difference between the command signal and the output signal activates a "controller" (the feedback amplifier in this case) that automatically reduces the difference. Thus, the actual membrane potential automatically and precisely follows the command potential.

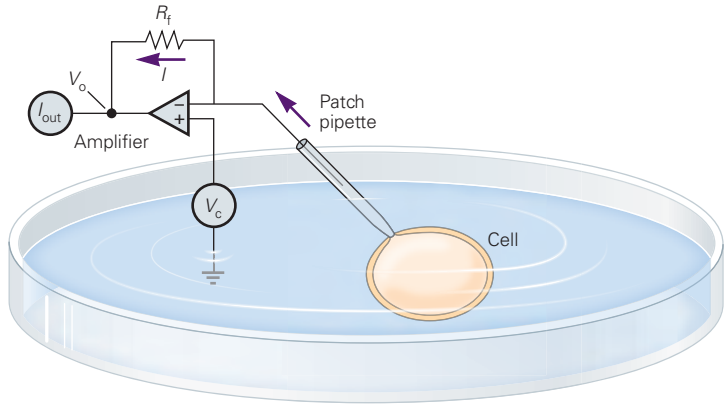
For example, assume that an inward Na^+ current through the voltage-gated Na^+ channels ordinarily causes the membrane potential to become more positive than the command potential. The input to the feedback amplifier is equal to ($V_{command} - V_m$). The amplifier generates an output voltage equal to this error signal multiplied by the gain of the amplifier. Thus, both the input and the resulting output voltage at the feedback amplifier will be negative.

This negative output voltage will make the internal current electrode negative, withdrawing net positive charge from the cell through the voltage-clamp circuit. As the current flows around the circuit, an equal amount of net positive charge will be deposited into the external solution through the other current electrode.

Today, most voltage-clamp experiments use a patch-clamp amplifier. The patch-clamp technique uses a feedback amplifier to control the voltage in a saline-filled micropipette and measures the current flowing through a patch of membrane to which the pipette is sealed. This allows the functional properties of single ion channels to be analyzed (see Box 8–1 and Figure 10–9).

If the pipette is sealed onto a cell and the patch under the membrane is ruptured by a pulse of suction, the result is a "whole-cell patch clamp" recording in which the intracellular voltage of the cell is controlled by the patch-clamp amplifier and the current flowing through the entire cell membrane is measured (Figure 10–2B). Whole-cell patch clamp recording allows voltage-clamp measurements in small cell bodies of neurons and is widely used to study the electrophysiological properties of neurons in cell culture, in brain slice preparations, and, recently, in vivo.

B



B. Voltage clamp of a neuronal cell body using the whole-cell mode of a patch-clamp amplifier. The patch pipette is sealed onto the cell and the membrane under the pipette is ruptured, providing electrical continuity between the inside of the cell and the pipette. An electrode in the pipette controls V_m, with an amplifier providing current (I) through a feedback resistor (R_f) to clamp the electrode (and therefore the pipette solution and the inside of the cell) to the command voltage (V_c), which is applied to the other amplifier input. The voltage on the output of the amplifier (V_o) is proportional to current flowing through the electrode and through the membrane.

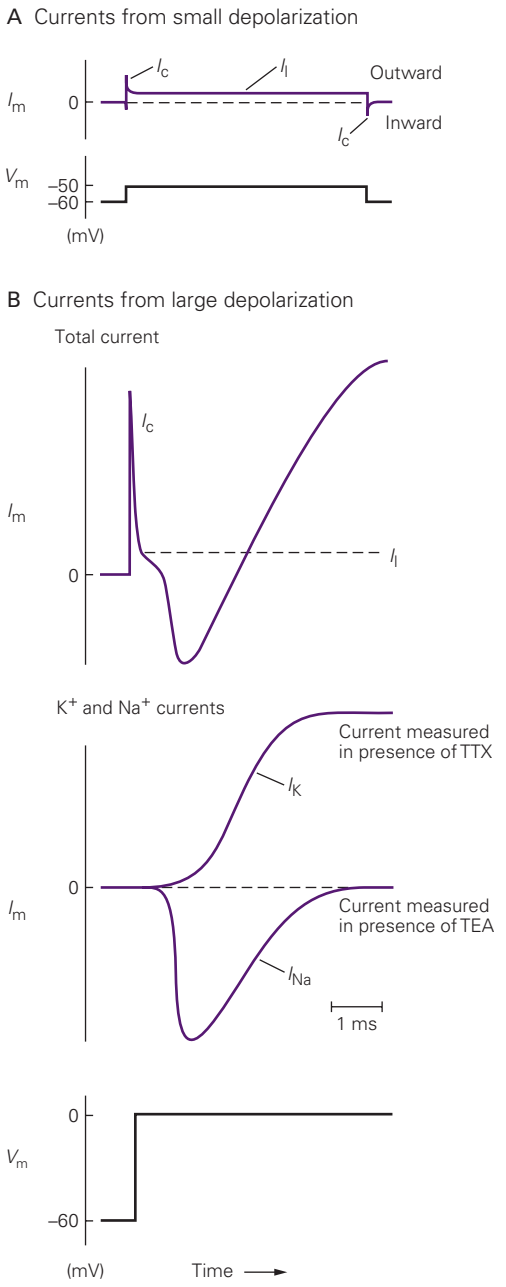


Figure 10–3 A voltage-clamp experiment demonstrates the sequential activation of voltage-gated sodium and potassium channels.

A. A small depolarization (10 mV) elicits capacitive and leakage currents (I_c and I_l, respectively), the components of the total membrane current (I_m).

B. A larger depolarization (60 mV) results in larger capacitive and leakage currents, plus a time-dependent inward ionic current followed by a time-dependent outward ionic current.

Top: Total (net) current in response to the depolarization. *Middle*: Individual Na^+ and K^+ currents. Depolarizing the cell in the presence of tetrodotoxin (**TTX**), which blocks the Na^+ current, or in the presence of tetraethylammonium (**TEA**), which blocks the K^+ current, reveals the pure K^+ and Na^+ currents (I_K and I_{Na}, respectively) after subtracting I_c and I_l. *Bottom*: Voltage step.

Similarly, I_K can be measured when the Na^+ channels are blocked by tetrodotoxin (Figure 10–3B).

By stepping the membrane through a wide range of potentials, Hodgkin and Huxley were able to measure the Na^+ and K^+ currents over the entire voltage extent of the action potential (Figure 10–4). They found that the Na^+ and K^+ currents vary as a graded function of the membrane potential. As the membrane voltage is made more positive, the outward K^+ current becomes larger. The inward Na^+ current also becomes larger with increases in depolarization, up to a certain extent. However, as the voltage becomes more and more positive, the Na^+ current eventually declines in amplitude. When the membrane potential is +55 mV, the Na^+ current is zero. Positive to +55 mV, the Na^+ current reverses direction and becomes outward.

Hodgkin and Huxley explained this behavior by a simple model in which the size of the Na^+ and K^+ currents is determined by two factors. The first is the magnitude of the Na^+ or K^+ conductance, g_{Na} or g_K, which reflects the number of Na^+ or K^+ channels open at any instant (Chapter 9). The second factor is the electrochemical driving force on Na^+ ions ($V_m - E_{Na}$) or K^+ ions ($V_m - E_K$). The model is thus expressed as:

$$I_{Na} = g_{Na} \times (V_m - E_{Na})$$

$$I_K = g_K \times (V_m - E_K).$$

According to this model, the amplitudes of I_{Na} and I_K change as the voltage is made more positive because there is an increase in g_{Na} and g_K. The conductances increase because the opening of the Na^+ and K^+ channels is voltage-dependent. The currents also change in response to changes in the electrochemical driving forces.

Both I_{Na} and I_K initially increase in amplitude as the membrane is made more positive because g_{Na} and g_K increase steeply with voltage. However, as the membrane potential approaches E_{Na} (+55 mV), I_{Na} declines because of the decrease in inward driving force, even though g_{Na} is large. That is, the positive membrane voltage now opposes the influx of Na^+ down its chemical concentration gradient. At +55 mV the chemical and electrical driving forces are in balance so there is no net I_{Na}, even though g_{Na} is quite large. As the membrane is made positive to E_{Na}, the driving force on Na^+ becomes positive. That is, the electrical driving force pushing Na^+ out is now greater than the chemical driving force pulling Na^+ in, so I_{Na} becomes outward. The behavior of I_K is simpler; E_K is quite negative (–75 mV), so in addition to an increase in g_K, the outward driving force on K^+ also becomes larger as the membrane

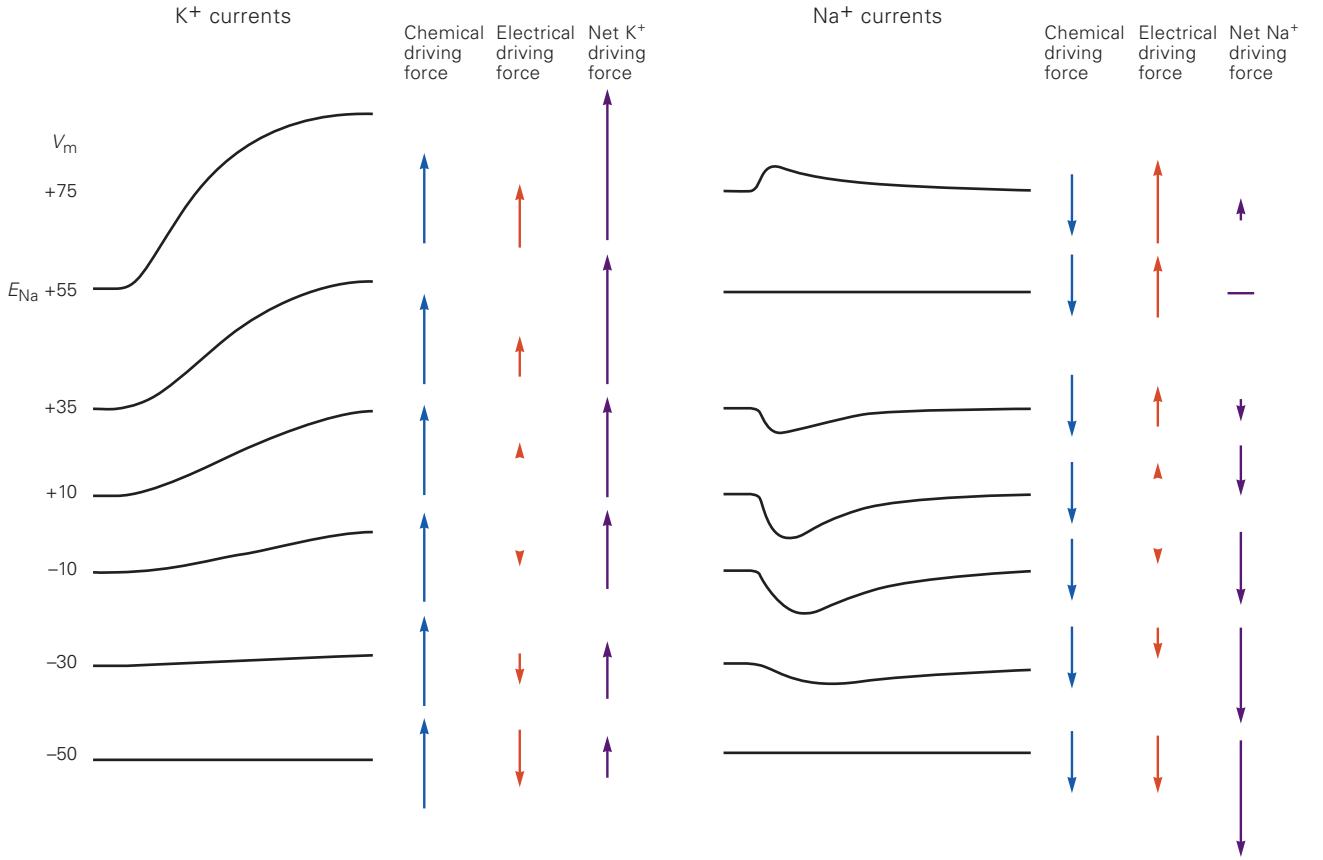


Figure 10–4 The magnitude and polarity of the sodium and potassium membrane currents vary with the amplitude of membrane depolarization. *Left*: With progressive depolarization, the voltage-clamped membrane K^+ current increases monotonically, because both g_K and $(V_m - E_K)$, the driving force for K^+, increase with increasing depolarization. The voltage during the depolarization is indicated at left. The direction and magnitude of the chemical (E_K) and electrical driving force on K^+, as well as the net driving force, are given by arrows at the right of each trace. (**Up arrows** = outward force; **down arrows** = inward force.) *Right*: At first, the Na^+ current becomes increasingly inward with greater depolarization due to the increase in g_{Na}. However, as the membrane potential approaches E_{Na} (+55 mV), the magnitude of the inward Na^+ current begins to decrease due to the decrease in inward driving force $(V_m - E_{Na})$. Eventually, I_{Na} goes to zero when the membrane potential reaches E_{Na}. At depolarizations positive to E_{Na}, the sign of $(V_m - E_{Na})$ reverses and I_{Na} becomes outward.

is made more positive, thereby increasing the outward K^+ current.

Voltage-Gated Sodium and Potassium Conductances Are Calculated From Their Currents

From the two preceding equations, Hodgkin and Huxley were able to calculate g_{Na} and g_K by dividing measured Na^+ and K^+ currents by the known Na^+ and K^+ electrochemical driving forces. Their results provided direct insight into how membrane voltage controls channel opening because the values of g_{Na} and g_K reflect the number of open Na^+ and K^+ channels (Box 10–2).

Measurements of g_{Na} and g_K at various levels of membrane potential reveal two functional similarities and two differences between the Na^+ and K^+ channels. Both types of channels open in response to depolarization. Also, as the size of the depolarization increases, the extent and rate of opening increase for both types of channels. The Na^+ and K^+ channels differ, however, in the rate at which they open and in their responses to prolonged depolarization. At all levels of depolarization, the Na^+ channels open more rapidly than K^+ channels (Figure 10–6). When the depolarization is maintained for some time, the Na^+ channels begin to close, leading to a decrease of inward current. The process by which Na^+ channels close during a prolonged depolarization is termed *inactivation*.

Thus, depolarization causes Na^+ channels to switch between three different states—resting, activated, or inactivated—which represent three different conformations of the Na^+ channel protein (see Figure 8–6).

Box 10–2 Calculation of Membrane Conductances From Voltage-Clamp Data

Membrane conductances can be calculated from voltage-clamp currents using equations derived from an equivalent circuit (Figure 10–5) that includes the membrane capacitance (C_m); the leakage conductance (g_l), representing the conductance of all of the resting (nongated) K^+, Na^+, and Cl^- channels (Chapter 9); and g_{Na} and g_K, the conductances of the voltage-gated Na^+ and K^+ channels.

In the equivalent circuit, the ionic battery of the leakage channels, E_l, is equal to the resting membrane potential, and g_{Na} and g_K are in series with their appropriate ionic batteries.

The current through each class of voltage-gated channel can be calculated from a modified version of Ohm's law that takes into account the electrical driving force (V_m) and chemical driving forces (E_{Na} or E_K) on Na^+ and K^+ (Chapter 9):

$$I_K = g_K \times (V_m - E_K)$$
$$I_{Na} = g_{Na} \times (V_m - E_{Na}).$$

Rearranging and solving for g gives two equations that can be used to compute the conductances of the active Na^+ and K^+ channel populations:

$$g_K = \frac{I_K}{(V_m - E_K)}$$

$$g_{Na} = \frac{I_{Na}}{(V_m - E_{Na})}.$$

In these equations, the independent variable V_m is set by the experimenter. The dependent variables I_K and I_{Na} can be calculated from the records of voltage-clamp experiments (see Figure 10–4). The parameters E_K and E_{Na} can be determined empirically by finding the values of V_m at which I_K and I_{Na} reverse their polarities, that is, their *reversal potentials*.

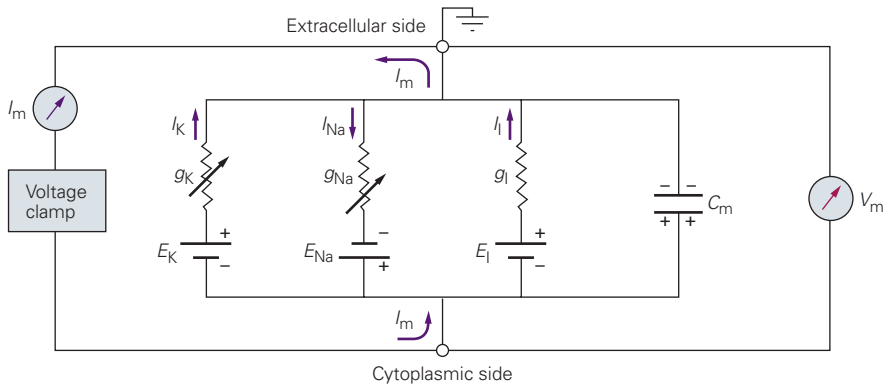


Figure 10–5 Equivalent circuit of a voltage-clamped neuron. The voltage-gated conductance pathways (g_K and g_{Na}) are represented by the symbol for a variable conductance—a conductor (resistor) with an arrow through it. The conductance is variable because of its dependence on time and voltage. These conductances are in series with batteries representing the chemical gradients for Na^+ and K^+. In addition, there are parallel pathways for leakage current (g_l and E_l) and capacitive current (C_m). Arrows indicate current flow during a depolarizing step that has activated g_K and g_{Na}.

In contrast, squid axon K^+ channels do not inactivate; they remain open as long as the membrane is depolarized, at least for voltage-clamp depolarizations lasting up to tens of milliseconds (Figure 10–6).

In the inactivated state, the Na^+ channel cannot be opened by further membrane depolarization. The inactivation can be reversed only by repolarizing the membrane to its negative resting potential, whereupon the channel switches to the resting state. This switch takes some time.

These variable, time-dependent effects of depolarization on g_{Na} are determined by the kinetics of two gating mechanisms in Na^+ channels. Each Na^+ channel has an *activation gate* that is closed while the membrane is at the resting potential and opened by depolarization. An *inactivation gate* is open at the resting potential

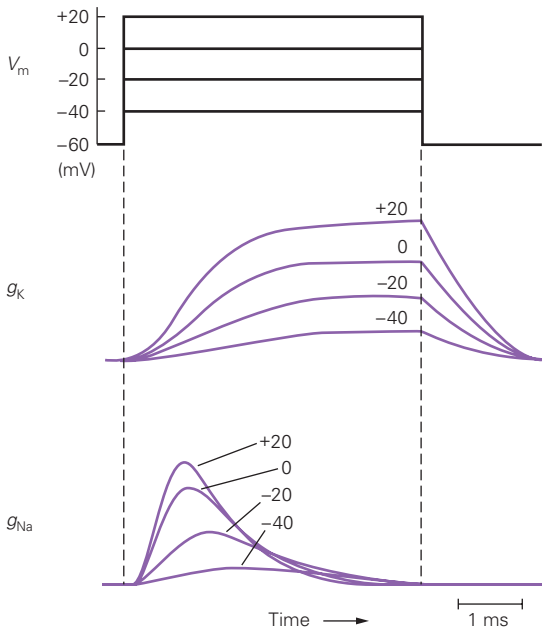


Figure 10–6 The responses of potassium and sodium ion channels to prolonged depolarization. Increasing depolarizations elicit graded increases in K^+ and Na^+ conductance (g_{Na} and g_K), which reflect the proportional opening of thousands of voltage-gated K^+ and Na^+ channels. The Na^+ channels open more rapidly than the K^+ channels. During a maintained depolarization, the Na^+ channels close after opening because of the closure of an inactivation gate. The K^+ channels remain open because they lack a fast inactivation process. At very positive V_m, the K^+ and Na^+ conductances approach a maximal value because the depolarization is sufficient to open nearly all available channels.

and closes after the channel opens in response to depolarization. The channel conducts Na^+ only for the brief period during depolarization when *both* gates are open.

The Action Potential Can Be Reconstructed From the Properties of Sodium and Potassium Channels

Hodgkin and Huxley were able to fit their measurements of membrane conductance to a set of empirical equations that completely describe the Na^+ and K^+ conductances as a function of membrane potential and time. Using these equations and measured values for the passive properties of the axon, they computed the shape and conduction velocity of the action potential. Remarkably, these equations also provided insights into the biophysical bases for voltage-gating that were confirmed over 50 years later when the structure of certain voltage-gated channels was elucidated through X-ray crystallography.

The calculated waveform of the action potential matched the waveform recorded in the unclamped axon almost perfectly. This close agreement indicates that the mathematical model developed by Hodgkin and Huxley accurately describes the properties of the channels that are responsible for generating and propagating the action potential. More than a half-century later, the Hodgkin-Huxley model stands as the most successful quantitative model in neural science if not in all of biology.

According to the model, an action potential involves the following sequence of events. Depolarization of the membrane causes Na^+ channels to open rapidly (an increase in g_{Na}), resulting in an inward Na^+ current. This current, by discharging the membrane capacitance, causes further depolarization, thereby opening more Na^+ channels, resulting in a further increase in inward current. This regenerative process drives the membrane potential toward E_{Na}, causing the rising phase of the action potential. The depolarization limits the duration of the action potential in two ways: (1) It gradually inactivates the voltage-gated Na^+ channels, thus reducing g_{Na}, and (2) it opens, with some delay, the voltage-gated K^+ channels, thereby increasing g_K. Consequently, the inward Na^+ current is followed by an outward K^+ current that tends to repolarize the membrane (Figure 10–7).

Two features of the action potential predicted by the Hodgkin-Huxley model are its threshold and all-or-none behavior. A fraction of a millivolt can be the difference between a subthreshold stimulus and a stimulus that generates a full-sized action potential.

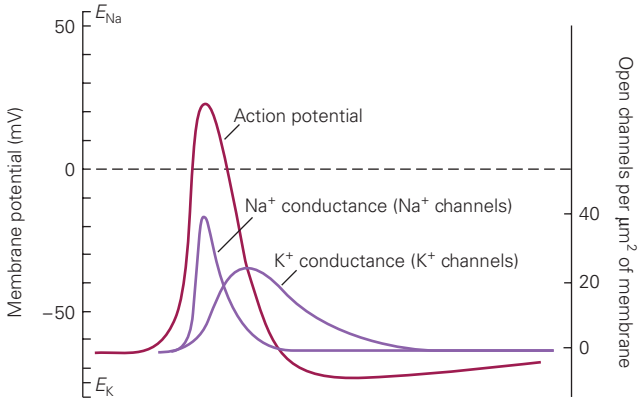


Figure 10–7 The sequential opening of voltage-gated Na^+ and K^+ channels generates the action potential. One of Hodgkin and Huxley's great achievements was to dissect the change in conductance during an action potential into separate components attributable to the opening of Na^+ and K^+ channels. The shape of the action potential and the underlying conductance changes can be calculated from the properties of the voltage-gated Na^+ and K^+ channels. (Adapted, with permission, from Hille 2001.)

This all-or-none phenomenon may seem surprising when one considers that Na^+ conductance increases in a strictly *graded* manner as depolarization increases (Figure 10–6). Each increment of depolarization increases the number of voltage-gated Na^+ channels that open, thereby gradually increasing Na^+ current. How then can there be a discrete threshold for generating an action potential?

Although a small subthreshold depolarization increases the inward I_{Na}, it also increases two *outward* currents, I_K and I_l, by increasing the electrochemical driving forces acting on K^+ and Cl^-. In addition, the depolarization augments K^+ conductance by gradually opening more voltage-gated K^+ channels (Figure 10–6). As the outward K^+ and leakage currents increase with depolarization, they tend to repolarize the membrane and thereby resist the depolarizing action of the Na^+ influx. However, because of the high voltage sensitivity and more rapid kinetics of activation of the Na^+ channels, the depolarization eventually reaches the point at which the increase in inward I_{Na} exceeds the increase in outward I_K and I_l. At this point, there is a net inward ionic current. This produces a further depolarization, opening even more Na^+ channels, so that the depolarization becomes regenerative, rapidly driving the membrane potential V_m all the way to the peak of the action potential. The specific value of V_m at which the net ionic current ($I_{Na} + I_K + I_l$) changes from outward to inward, depositing a positive charge on the inside of the membrane capacitance, is the threshold.

Early experiments with extracellular stimulation of nerve fibers showed that, for a short time after an action potential (typically a few milliseconds), it is impossible to generate another action potential. This *absolute refractory period* is followed by a period when it is possible to stimulate another action potential, but only with a stimulus larger than what was needed for the first. This *relative refractory period* typically lasts 5 to 10 ms.

The Hodgkin-Huxley analysis provided a mechanistic explanation of two factors underlying the refractory period. In the immediate aftermath of an action potential, it is impossible to evoke another one, even with a very strong stimulus, because the Na^+ channels remain inactivated. After repolarization, Na^+ channels recover from inactivation and reenter the resting state, a transition that takes several milliseconds (Figure 10–8). The relative refractory period corresponds to partial recovery from inactivation.

The relative refractory period is also influenced by a residual increase in K^+ conductance that follows the action potential. It takes several milliseconds for all of the K^+ channels that open during the action potential to return to their closed state. During this period, when the K^+ conductance remains somewhat elevated, V_m is slightly more negative than its normal resting value, as V_m approaches E_K (Figure 10–7, Equation 9–4). This *afterhyperpolarization* and residual increase in g_K contribute to the increase in depolarizing current required to drive V_m to threshold during the relative refractory period.

The Mechanisms of Voltage Gating Have Been Inferred From Electrophysiological Measurements

The empirical equations derived by Hodgkin and Huxley are quite successful in describing how the flow of ions through the Na^+ and K^+ channels generates the action potential. However, these equations describe the process of excitation in terms of changes in membrane conductance and current. They tell little about the mechanisms that activate or inactivate channels in response to changes in membrane potential or about channel selectivity for specific ions.

We now know that the voltage-dependent conductances described by Hodgkin and Huxley are generated by ion channels that open in a voltage- and time-dependent manner. Patch-clamp recordings from a variety of nerve and muscle cells have provided detailed information about the properties of the voltage-dependent Na^+ channels that generate the action potential. Recordings of single voltage-gated Na^+ channels show that, in response to a depolarizing step, each channel opens in an all-or-none fashion, conducting brief current pulses of constant amplitude but variable duration.

Each channel opening is associated with a current of about 1 pA (at voltages near −30 mV), and the open state is rapidly terminated by inactivation. Each channel behaves stochastically, opening after a variable time and staying open for a variable time before inactivating. If the openings of all the channels in a cell membrane in response to a step depolarization are summed or the openings of a single channel to multiple trials of the same depolarization are summed (Figure 10–9), the result is an averaged current with the same time course as the macroscopic Na^+ current recorded in voltage-clamp experiments (see Figure 10–4B).

To explain how changes in membrane potential lead to an increase in Na^+ conductance, Hodgkin and Huxley deduced from basic thermodynamic considerations that a conformational change in some membrane component that regulates the conductance must move charged particles through the membrane electric field. As a result, membrane depolarization would exert a force causing the charged particles to move, thereby opening the channel. For a channel with positively charged mobile particles, the depolarization