9

Propagated Signaling: The Action Potential

NERVE CELLS ARE ABLE TO carry signals over long distances because of their ability to generate an action potential—a regenerative electrical signal whose amplitude does not attenuate as it moves down the axon. In Chapter 7 we saw how an action potential arises from sequential changes in the membrane's selective permeability to Na^+ and K^+ ions. In Chapter 8 we considered how the membrane's passive properties influence the speed at which action potentials are conducted. In this chapter we focus on the voltage-gated ion channels that are critical for generating and propagating action potentials and consider how these channels are responsible for many important features of a neuron's electrical excitability.

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

An important early clue about how action potentials are generated came from an experiment performed by Kenneth Cole and Howard Curtis. While recording from the giant axon of the squid they found that the ion conductance across the membrane increases dramatically during the action potential (Figure 9-1). This discovery provided the first evidence that the action potential results from changes in the flux of ions through the channels of

the membrane. It also raised the question: Which ions are responsible for the action potential?

A key to this problem was provided by Alan Hodgkin and Bernard Katz, who found 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. Their data also suggested that the falling phase of the action potential was caused by a later increase in K^+ permeability. Hodgkin and Katz proposed that depolarization of the cell above threshold causes a brief increase in the cell membrane's permeability to Na^+, during which Na^+ permeability overwhelms the dominant permeability of the resting cell membrane to K^+ ions.

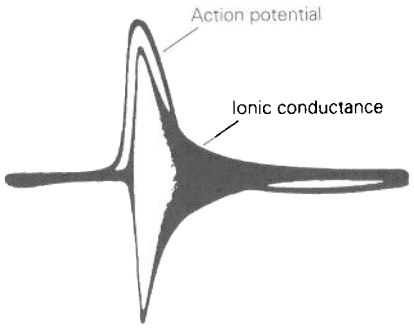
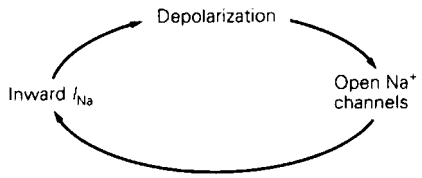


Figure 9-1 A net increase in ionic conductance in the membrane of the axon accompanies the action potential. This historic recording from an experiment conducted in 1938 by Kenneth Cole and Howard Curtis shows the oscilloscope record of an action potential superimposed on a simultaneous record of the ionic conductance.

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

To test this hypothesis, Hodgkin and Andrew Huxley conducted a second series of experiments. They systematically varied the membrane potential in the squid giant axon and measured the resulting changes in the membrane conductance to Na^+ and K^+ through voltage-gated Na^+ and K^+ channels. To do this they made use of a new apparatus, the voltage clamp. 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 of the voltage-gated Na^+ channels, inward Na^+ current flows through these channels and causes further depolarization. The additional depolarization causes still more Na^+ channels to open and consequently induces more inward Na^+ current:



This positive feedback cycle, which eventually drives the membrane potential to the peak of the action potential, makes it impossible to achieve a stable membrane potential. A similar coupling between current and membrane potential complicates the study of the voltage-gated K^+ channels.

The basic function of the voltage clamp is to interrupt the interaction between the membrane potential and the opening and closing of voltage-gated ion channels. The voltage clamp does so by injecting a current into the axon that is equal and opposite to the current flowing through the voltage-gated membrane channels. In this way the voltage clamp prevents the charge separation across the membrane from changing. 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 flowing across the membrane (Box 9-1). Using the voltage-clamp technique, Hodgkin and Huxley provided the first complete description of the ionic mechanisms underlying the action potential.

One advantage of the voltage clamp is that it readily allows the total membrane current to be separated into its ionic and capacitive components. As described in Chapter 8, 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 very rapid step of command potential, capacitive current flows only at the beginning and end of the step. Since the capacitive current is essentially instantaneous, the ionic currents that flow through the gated membrane channels can be analyzed separately.

Measurements of these ionic membrane currents can be used to calculate the voltage and time dependence of changes in membrane conductances 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. If a 10 mV depolarizing potential step is commanded, we

Box 9-1 Voltage-Clamp Technique

The voltage-clamp technique was developed by Kenneth Cole in 1949 to stabilize the membrane potential of neurons for experimental purposes. It was used by Alan Hodgkin and Andrew Huxley in the early 1950s in a series of experiments that revealed the ionic mechanisms underlying the action potential.

The voltage clamp permits the experimenter to "clamp" the membrane potential at predetermined levels. The voltage-gated ion channels continue to open or close in response to changes in membrane potential, but the voltage clamp prevents the resultant changes in membrane current from influencing the membrane potential. This technique thus permits measurement of the effect of changes in membrane potential on the conductance of the membrane to individual ion species.

The voltage clamp consists of a source of current connected to two electrodes, one inside and the other outside the cell (Figure 9-2A). By passing current across the cell membrane, the membrane potential can be stepped rapidly to a predetermined level of depolarization.

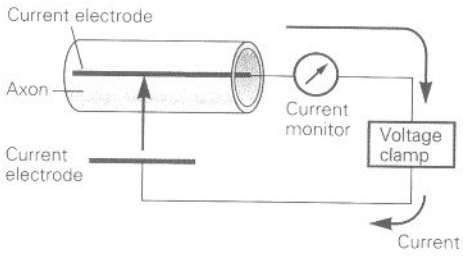


Figure 9-2 A

The voltage clamp is a current generator that is connected to a pair of electrodes. It is used to change the charge separation, and thus the electrical potential difference, across the membrane. Monitoring the additional current that is passed to clamp the membrane potential at its new value then provides a measure of the membrane current passing through the ion channels in the membrane.

These depolarizations open voltage-gated Na^+ and K^+ channels. The resulting movement of Na^+ and K^+ across the membrane would ordinarily change the membrane potential, but the voltage clamp maintains the membrane potential at its commanded level. When Na^+ channels open in response to a moderate depolarizing voltage step, an inward ionic current develops because Na^+ ions flow through these channels as a result of their electrochemical driving force. This Na^+ influx normally depolarizes 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, the voltage-clamp circuit automatically prevents the ionic current from changing the membrane potential from the commanded value (Figure 9-2A). As a result, the *net* amount of charge separated by the membrane does not change and therefore no significant change in V_m can occur.

The voltage clamp is a negative feedback system. A negative feedback system is one in which the value of the output of the system (V_m in this case) is "fed back" to the input of the system, where it is compared to a command signal for the desired output. Any difference between the command potential and the output signal activates a "controller" device that automatically reduces the difference. Thus the membrane potential *automatically* follows the command potential exactly (Figure 9-2B).

For example, assume that an inward Na^+ current through the voltage-gated Na^+ channels causes the membrane potential to become more positive than the command potential. The input to the feedback amplifier is equal to $(V_{command} - V_m)$. 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.

A refinement of the voltage clamp, the patch-clamp technique, allows the functional properties of *individual* ion channels to be analyzed (see Box 6-1).

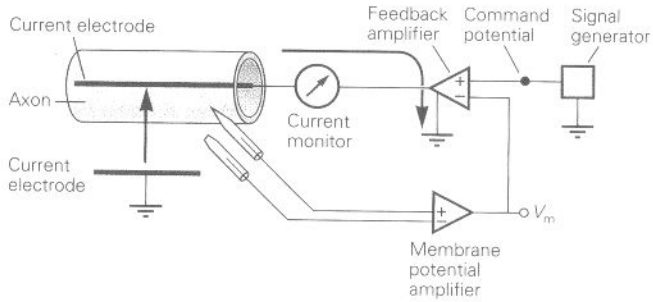


Figure 9-2 B

The negative feedback mechanism by which the voltage clamp operates. Membrane potential is measured by one amplifier connected to an intracellular electrode and to an extracellular electrode in the bath. The membrane potential signal is displayed on an oscilloscope and is also fed into one terminal of the "feedback" amplifier. This amplifier has two inputs, one for membrane potential (V_m) and the other for the command potential. The command potential, which comes from a signal generator, is selected by the experimenter and can be of any desired amplitude and waveform. The feedback amplifier subtracts the membrane potential from the command potential. Any difference between these two signals is amplified several thousand times at the feedback amplifier. The output of this amplifier is connected to a current electrode, a thin wire that runs the length of the axon. 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 achieved by using a highly conductive current electrode, which short circuits the axoplasmic resistance, reducing the axial resistance to zero (see Chapter 8). This low-resistance pathway within the axon eliminates all potential differences along the axon core.

observe that an initial, 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 ionic current that persists for the duration of this pulse. At the end of the pulse there is a brief inward capacitive current, and the total membrane current returns to zero (Figure 9-3A). The steady ionic current that persists throughout the depolarization is the current that flows through the resting ion channels of the membrane (see Chapter 6) and is called the *leakage current*, I_l. The total conductance of this population of channels is called the *leakage conductance* (g_l). These resting channels, which are always open, are responsible for generating the resting membrane potential (see Chapter 7). In a typical neuron most of the resting channels are permeable to K^+ ions; the remaining channels are permeable to Cl^- or Na^+ ions.

If a larger depolarizing step is commanded, the current record becomes 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 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 pulse (Figure 9-3B).

A simple interpretation of these results is that the depolarizing voltage step sequentially turns on active conductance channels for two separate ions: one type of channel for inward current and another for 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 substituting a larger, impermeant cation (choline·H^+) for Na^+, they eliminated the inward Na^+ current. Subsequently, the task of separating inward and outward currents was made easier by selectively blocking the voltage-sensitive conductance channels with drugs or toxins. Tetrodotoxin, a poison from 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 sushi delicacy fugu, can be fatal.) The cation tetraethylammonium specifically blocks the voltage-gated K^+ channel (Figure 9-4).

When tetraethylammonium 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,

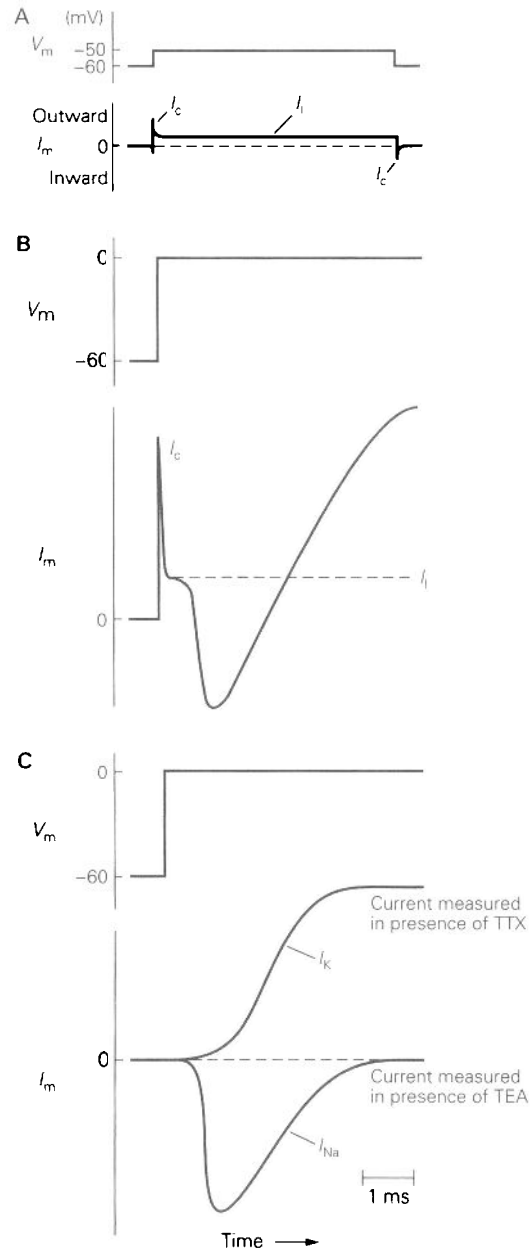


Figure 9-3 A voltage-clamp experiment demonstrates the sequential activation of two types of voltage-gated channels.

A. A small depolarization is accompanied by capacitive and leakage currents (I_c and I_l, respectively).

B. A larger depolarization results in larger capacitive and leakage currents, plus an inward current followed by an outward current.

C. Depolarizing the cell in the presence of tetrodotoxin (which blocks the Na^+ current) and again in the presence of tetraethylammonium (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.

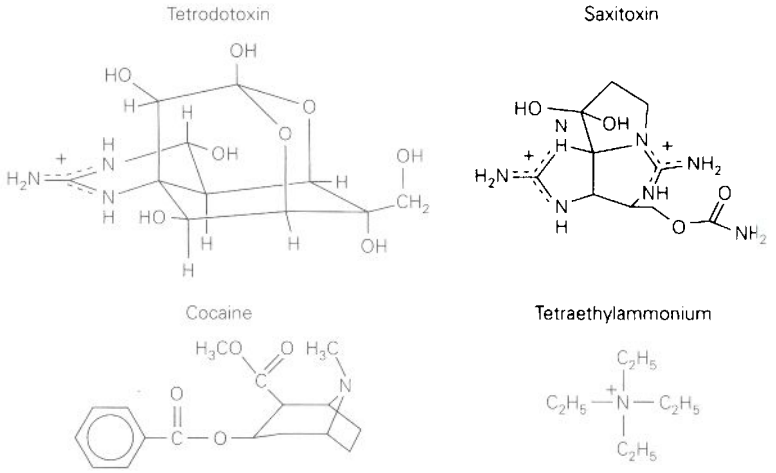


Figure 9-4 Drugs that block voltage-gated Na^+ and K^+ channels. Tetrodotoxin and saxitoxin both bind to Na^+ channels with a very high affinity. **Tetrodotoxin** is produced by certain puffer fish, newts, and frogs. **Saxitoxin** is synthesized by the dinoflagellates *Gonyaulax* that are responsible for red tides. Consumption of clams or other shellfish that have fed on the dinoflagellates during a red tide causes paralytic shellfish poisoning. **Cocaine,** the active substance isolated from coca leaves, was the first substance to be used as a local anesthetic. It also blocks Na^+ channels but with a lower affinity and specificity than tetrodotoxin. **Tetraethylammonium** is a cation that blocks certain voltage-gated K^+ channels with a relatively low affinity. The red plus signs represent positive charge.

g_l, is constant; it does not vary with V_m or with time. Therefore, I_l, the leakage current, 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 can be easily isolated by visual inspection, leaving the pure I_{Na}. The full range of current flow through the voltage-gated Na^+ channels (I_{Na}) is measured by repeating this analysis after stepping V_m to many different levels. With a similar process, I_K can be measured when the Na^+ channels are blocked by tetrodotoxin (Figure 9-3C).

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

The Na^+ and K^+ currents depend on two factors: the conductance for each ion and the electrochemical driving force acting on the ion. Since the Na^+ and K^+ membrane conductance is directly proportional to the number of open Na^+ and K^+ channels, we can gain insight into how membrane voltage controls channel opening by calculating the amplitudes and time courses of the Na^+ and K^+ conductance changes in response to voltage-clamp depolarizations (Box 9-2).

Measurements of Na^+ and K^+ conductances 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 depolarizing steps of membrane potential. Moreover, as the size of the depolarization increases, the probability and rate of opening increase for both types of channels. The Na^+ and K^+ channels differ, however, in their rates of opening and in their responses to prolonged depolarization. At all levels of depolarization the Na^+ channels open more rapidly than do the K^+ channels (Figure 9-6). 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 maintained depolarization is termed *inactivation*. In contrast, the K^+ channels in the squid axon do not inactivate; they remain open as long as the membrane is depolarized (Figure 9-7).

Thus, depolarization causes Na^+ channels to undergo transitions among three different states, which represent three different conformations of the Na^+ channel protein: resting, activated, or inactivated. Upon depolarization the channel goes from the resting (closed) state to the activated (open) state (see Figure 6-6C). If the depolarization is brief, the channels go directly back to the resting state upon repolarization. If the depolarization is maintained, the channels go from the open to the inactivated (closed) state. Once the channel is inactivated it cannot be opened by further depolarization. The inactivation can be reversed only by repolarizing the membrane to its negative resting potential, which allows the channel to switch from the inactivated to the resting state. This switch takes some time because channels leave the inactivated state relatively slowly (Figure 9-8).

These variable, time-dependent effects of depolarization on g_{Na} are determined by the kinetics of the gating reactions that control the Na^+ channels. Each Na^+ channel has two kinds of gates that must be opened simultaneously for the channel to conduct Na^+ ions. An *activation gate* is closed when the membrane is at its negative resting potential and is rapidly opened by de-

Box 9-2 Calculation of Membrane Conductances From Voltage-Clamp Data

Membrane conductance can be calculated from voltage-clamp currents using equations derived from an equivalent circuit of the membrane that includes the membrane capacitance (C_m) and leakage conductance (g_l), as well as g_{Na} and g_K (Figure 9-5). In this context g_l represents the conductance of all of the resting K^+, Na^+, and Cl^- channels (see Chapter 7); g_{Na} and g_K represent the conductances of the voltage-gated Na^+ and K^+ channels. The ionic battery of the resting (leakage) channels, E_l, is equal to the resting potential. The voltage-sensitive Na^+ and K^+ conductances are in series with their appropriate ionic batteries.

The current through each class of voltage-gated channel may be calculated from Ohm's law:

$$I_K = g_K(V_m - E_K)$$

and

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

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

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

and

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

To solve these equations, one must know V_m, E_K, E_{Na}, I_K, and I_{Na}. 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 9-3C). The remaining variables, E_K and E_{Na}, are constants; they 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*. For example, as V_m is stepped to very positive values, the inward I_{Na} becomes smaller because of the smaller inward electrochemical driving force on Na^+. When V_m equals E_{Na}, I_{Na} is zero owing to the lack of a net driving force. At potentials that are positive to E_{Na}, I_{Na} becomes outward (corresponding to a net efflux of Na^+ ions from the axon) because of a net outward driving force on Na^+.

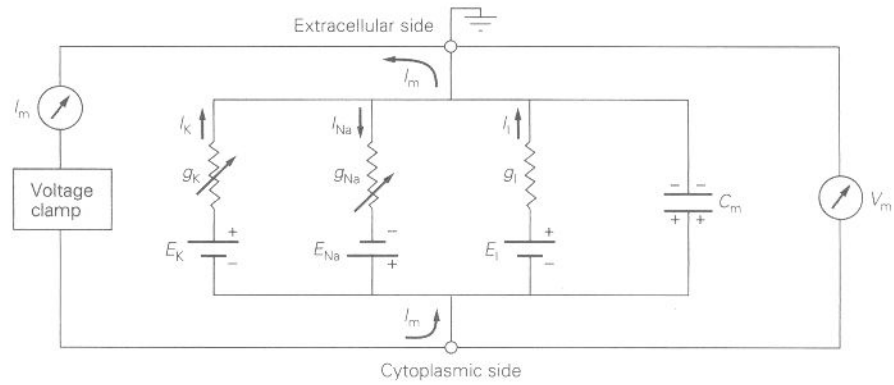


Figure 9-5 Electrical equivalent circuit of a nerve cell being held at a depolarized potential under voltage-clamp conditions. The voltage-gated conductance pathways (g_K and g_{Na}) are represented by the symbol for variable conductance—a conductor (resistor) with an arrow through it.

polarization; an *inactivation gate* is open at the resting potential and closes slowly in response to depolarization. The channel conducts only for the brief period during a depolarization when both gates are open. Repolarization reverses the two processes, closing the activation gate rapidly and opening the inactivation gate more slowly. After the channel has returned to the resting state, it can again be activated by depolarization (Figure 9-9).

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 changes to a set of empirical equations that completely describe variations in membrane Na^+ and K^+ conductances as functions of membrane potential and time. Using these equations

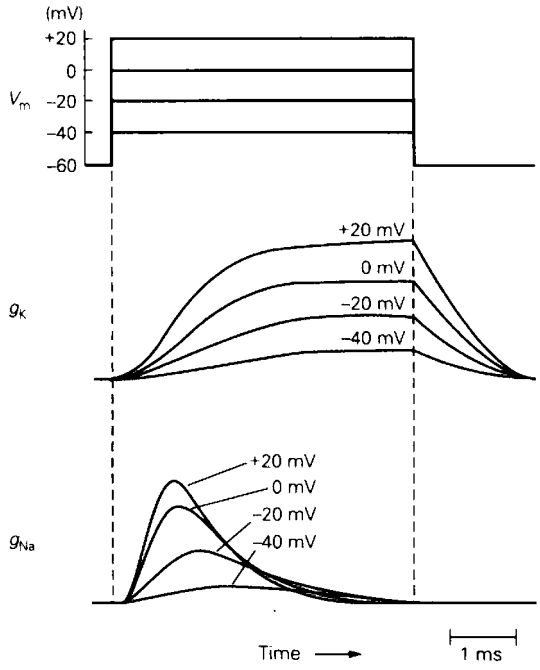


Figure 9-6 Voltage-clamp experiments show that Na^+ channels turn on and off more rapidly than K^+ channels over a wide range of membrane potentials. The increases and decreases in the Na^+ and K^+ conductances (g_{Na} and g_K) shown here reflect the shifting of thousands of voltage-gated channels between the open and closed states.

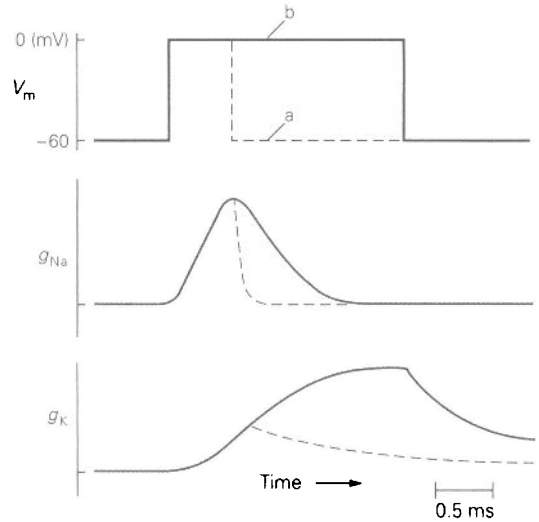


Figure 9-7 Sodium and potassium channels respond differently to long-term depolarization. If the membrane is repolarized after a brief depolarization **(line a)**, both g_{Na} and g_K return to their initial values. If depolarization is maintained **(line b)**, the Na^+ channels close (or inactivate) before the depolarization is terminated, whereas the K^+ channels remain open and g_K increases throughout the depolarization.

and measured values for the passive properties of the axon, they computed the expected shape and the conduction velocity of the propagated action potential. The calculated waveform of the action potential matched the waveform recorded in the unclamped axon almost perfectly! This close agreement indicates that the voltage and time dependence of the active Na^+ and K^+ channels, calculated from the voltage-clamp data, accurately describe the properties of the channels that are essential for generating and propagating the action potential. A half century later, the Hodgkin-Huxley model stands as the most successful quantitative computational model in neural science if not in all of biology.

According to the Hodgkin-Huxley model, an action potential involves the following sequence of events. A 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, there-

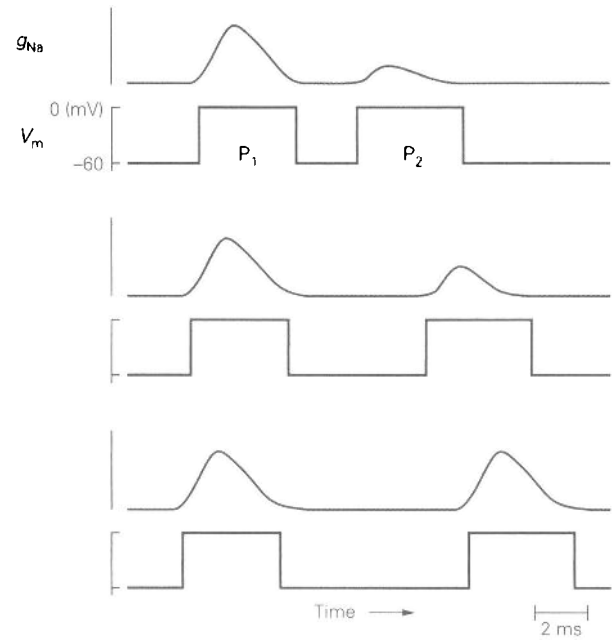


Figure 9-8 Sodium channels remain inactivated for a few milliseconds after the end of a depolarization. Therefore if the interval between two depolarizing pulses (P_1 and P_2) is brief, the second pulse produces a smaller increase in g_{Na} because many of the Na^+ channels are inactivated. The longer the interval between pulses, the greater the increase in g_{Na}, because a greater fraction of channels will have recovered from inactivation and returned to the resting state when the second pulse begins. The time course of recovery from inactivation contributes to the time course of the refractory period.

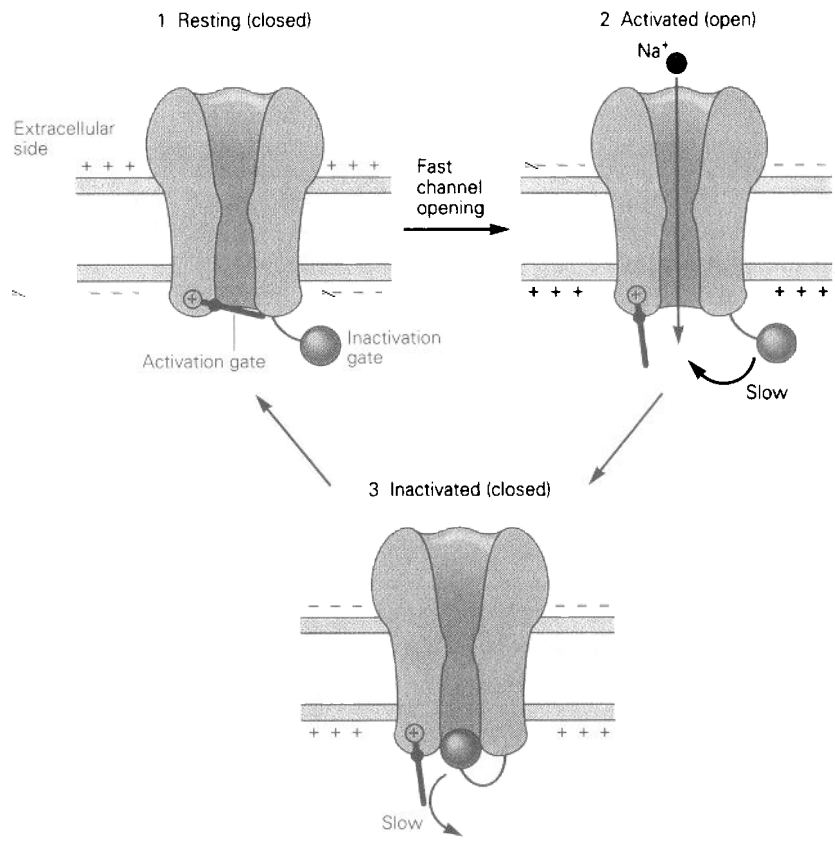


Figure 9-9 Voltage-gated Na^+ channels have two gates, which respond in opposite ways to depolarization. In the resting (closed) state the activation gate is closed and the inactivation gate is open (1). Upon depolarization a rapid opening of the activation gate allows Na^+ to flow through the channel (2). As the inactivation gates close, the Na^+ channels enter the inactivated (closed) state (3). Upon repolarization, first the activation gate closes, then the inactivation gate opens as the channel returns to the resting state (1).

by 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.[1] The depolarizing state of the action potential then limits the duration of the action potential in two ways: (1) It gradually inactivates the 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 9-10).

In most nerve cells the action potential is followed by a transient hyperpolarization, the *after-potential*. This brief increase in membrane potential occurs because the K^+ channels that open during the later phase of the action potential close some time after V_m has returned to its resting value. It takes a few milliseconds for all of the voltage-gated K^+ channels to return to the closed state. During this time, when the permeability of the membrane to K^+ is greater than during the resting state, V_m is hyperpolarized slightly with respect to its normal resting value, resulting in a V_m closer to E_K (Figure 9-10).

The action potential is also followed by a brief period of diminished excitability, or refractoriness, which can be divided into two phases. The *absolute refractory period* comes immediately after the action potential; during this period it is impossible to excite the cell no matter how great a stimulating current is applied. This phase is followed directly by the *relative refractory period*, during which it is possible to trigger an action potential but only by applying stimuli that are stronger than those normally required to reach threshold. These periods of refractoriness, which together last just a few milliseconds, are caused by the residual inactivation of Na^+ channels and increased opening of K^+ channels.

1. It may at first seem paradoxical that to depolarize the cell experimentally one passes *outward* current across the membrane (see Figure 7-2C), while at the same time attributing the depolarization during the upstroke of the action potential to an *inward* Na^+ current. However, in both cases the current flow across the passive components, the nongated leakage channels (g_l) and the capacitance of the membrane (C_m), is outward because positive charge is injected into the cell in one case through an intracellular electrode (see Figure 7-2) and in the other case by the opening of voltage-gated Na^+ channels. It is a matter of convention that when we refer to current injected through a microelectrode we refer to the direction in which the current crosses the membrane capacitance and leakage channels, whereas when we refer to current that flows through channels we refer to the direction of movement of charge through the channels.

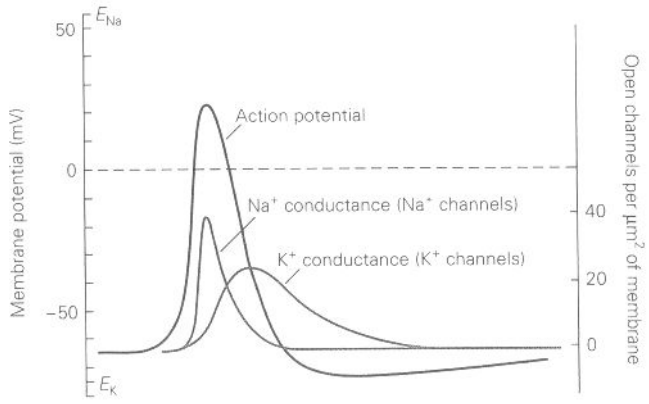


Figure 9-10 The sequential opening of voltage-gated Na^+ and K^+ channels generates the action potential. One of Hodgkin and Huxley's great achievements was to separate the total conductance change during an action potential, first detected by Cole and Curtis (see Figure 9-1) into separate components 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.

Another feature of the action potential predicted by the Hodgkin-Huxley model is its all-or-none behavior. A fraction of a millivolt can be the difference between a subthreshold depolarizing stimulus and a stimulus that generates an action potential. This all-or-none phenomenon may seem surprising when one considers that the Na^+ conductance increases in a strictly *graded* manner as depolarization is increased (see Figure 9-6). Each increment of depolarization increases the number of voltage-gated Na^+ channels that switch from the closed to the open state, thereby causing a gradual increase in Na^+ influx. Why then is there an abrupt 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 force on K^+ and Cl^-. In addition, the depolarization augments the K^+ conductance, g_K, by gradually opening more voltage-gated K^+ channels (see Figure 9-6). As I_K and I_l increase with depolarization, they tend to resist the depolarizing action of the Na^+ influx. However, because of the great voltage sensitivity and rapid kinetics of activation of the Na^+ channels, the depolarization eventually reaches a point where the increase in inward I_{Na} exceeds the increase in outward I_K and I_l. At this point there is a net inward current producing a further depolarization so that the depolarization becomes regenerative. The specific value of V_m at which the *net* ionic current ($I_{Na} + I_K + I_l$) just changes from outward to inward, depositing a net positive charge on the inside of the membrane capacitance, is the threshold.

Variations in the Properties of Voltage-Gated Ion Channels Increase the Signaling Capabilities of Neurons

The basic mechanism of electrical excitability identified by Hodgkin and Huxley in the squid giant axon—whereby voltage-gated ion channels conduct an inward ionic current followed by an outward ionic current—appears to be universal in all excitable cells. However, dozens of different types of voltage-gated ion channels have been identified in other nerve and muscle cells, and the distribution of specific types varies not only from cell to cell but also from region to region within a cell. These differences in the pattern of ion channel expression have important consequences for the details of membrane excitability, as we shall now explore.

The Nervous System Expresses a Rich Variety of Voltage-Gated Ion Channels

Although the voltage-gated Na^+ and K^+ channels in the squid axon described by Hodgkin and Huxley have been found in almost every type of neuron examined, several other kinds of channels have also been identified. For example, most neurons contain voltage-gated Ca^{2+} channels that open in response to membrane depolarization. A strong electrochemical gradient drives Ca^{2+} into the cell, so these channels give rise to an inward I_{Ca}. Some neurons and muscle cells also have voltage-gated Cl^- channels. Finally, many neurons have monovalent cation-permeable channels that are slowly activated by hyperpolarization and are permeable to both K^+ and Na^+. The net effect of the mixed permeability of these rather nonselective channels, called *h-type*, is the generation of an inward, depolarizing current in the voltage range around the resting potential.

Each basic type of ion channel has many variants. For example, there are four major types of voltage-activated K^+ channels that differ in their kinetics of activation, voltage activation range, and sensitivity to various ligands. These variants are particularly common in the nervous system. (1) The slowly activating channel described by Hodgkin and Huxley is called the *delayed rectifier*. (2) A *calcium-activated K^+ channel* is activated by intracellular Ca^{2+}, but its sensitivity to intracellular Ca^{2+} is enhanced by depolarization. It requires both a rise in internal Ca^{2+} (mediated by voltage-gated Ca^{2+} channels) and depolarization to achieve a maximum

probability of opening. (3) The *A-type K^+ channel* is activated rapidly by depolarization, almost as rapidly as the Na^+ channel; like the Na^+ channel, it also inactivates rapidly if the depolarization is maintained. (4) The *M-type K^+ channel* is very slowly activated by small depolarizations from the resting potential. One distinctive feature of the M-type channels is that they can be closed by a neurotransmitter, acetylcholine (ACh).

Similarly, there are at least five subtypes of voltage-gated Ca^{2+} channels and two or more types of voltage-gated Na^+ channels. Moreover, each of these subtypes has several structurally and functionally different isoforms.

The squid axon can generate an action potential with just two types of voltage-gated channels. Why then are so many different types of voltage-gated ion channels found in the nervous system? The answer is that neurons with an expanded set of voltage-gated channels have much more complex information-processing abilities than those with only two types of channels. Some ways in which this large variety of voltage-gated channels influences neuronal function are described below.

Gating of Voltage-Sensitive Ion Channels Can Be Influenced by Various Cytoplasmic Factors

In a typical neuron the opening and closing of certain voltage-gated ion channels can be modulated by various cytoplasmic factors, resulting in increased flexibility of the neuron's excitability properties. Changes in such cytoplasmic modulator substances may result from the normal intrinsic activity of the neuron itself or from the influences of other neurons.

The flow of ionic current through membrane channels during an action potential generally does not result in appreciable changes in the intracellular concentrations of most ion species. Calcium is a notable exception to this rule. Changes in the intracellular concentration of Ca^{2+} can have important modulatory influences on the gating of various channels. The concentration of free Ca^{2+} in the cytoplasm of a resting cell is extremely low, about 10^{-7} M, several orders of magnitude below the external Ca^{2+} concentration. For this reason the intracellular Ca^{2+} concentration may increase significantly as a result of inward current flow through voltage-gated Ca^{2+} channels.

The transient increase in Ca^{2+} concentration near the inside of the membrane has several effects. It enhances the probability that Ca^{2+}-activated K^+ channels will open. Some Ca^{2-} channels are themselves sensitive to levels of intracellular Ca^{2+} and are inactivated when incoming Ca^{2+} binds to their intracellular surface. In other channels the influx of Ca^{2+} activates a Ca^{2+}-sensitive protein phosphatase, calcineurin, which dephosphorylates the channel, thereby inactivating it (see Figure 6-7C).

Thus, in some cells the Ca^{2+} influx during an action potential can have two opposing effects: (1) The positive charge that it carries into the cell contributes to the regenerative depolarization, while (2) the increase in cytoplasmic Ca^{2+} concentration results in the opening of more K^+ channels and the closing of Ca^{2+} channels. Because of the opening of K^+ channels and the closing of Ca^{2+} channels, outward ionic current increases while inward ionic current decreases; the resulting net efflux of positive charge causes the cell to repolarize. In this way the depolarizing influx of Ca^{2+} through voltage-gated Ca^{2+} channels is self-limited by two processes that aid repolarization: an increase in K^+ efflux and a decrease in Ca^{2+} influx.

Calcium's role in modulating the gating of ion channels is the simplest example of a variety of second-messenger systems that control channel activity. Gating of ion channels can also be modulated by changes in the cytoplasmic level of small organic second-messenger compounds as a result of synaptic input from other neurons. The gating properties of several voltage-gated channels that are directly involved in generating action potentials are modified when their phosphorylation state is changed by a protein kinase (eg, the cAMP-dependent protein kinase) whose activity is controlled by changes in the concentration of synaptically activated second messengers (eg, cAMP). The importance of Ca^{2+} and other second messengers in the control of neuronal activity will become evident in many contexts throughout this book.

Excitability Properties Vary Between Regions of the Neuron

Different regions of the cell perform specific signaling tasks. The axon, for example, usually specializes in carrying signals faithfully over long distances. As such, it functions as a relatively simple relay line. In contrast, the input, integrative, and output regions of a neuron (see Figure 2-8) typically perform more complex processing of the information they receive before passing it along. The signaling function of a discrete region of the neuron depends on the particular set of ion channels that it expresses.

In many types of neurons the dendrites have voltage-gated ion channels, including Ca^{2+}, K^+, and in some cases Na^+ channels. When activated, these channels modify the passive, electrotonic conduction of synaptic potentials. In some neurons action potentials may be propagated from their site of initiation at the

trigger zone back into the dendrites, thereby influencing synaptic integration in the dendrites. In other neurons the density of dendritic voltage-gated channels may even support the orthograde propagation of a dendritic impulse to the cell soma and axon hillock.

The trigger zone of the neuron has the lowest threshold for action potential generation, in part because it has an exceptionally high density of voltage-gated Na^+ channels. In addition, it typically has voltage-gated ion channels that are sensitive to relatively small deviations from resting potential. These channels are important in determining whether synaptic input will drive the membrane potential to spike threshold. They thus play a critical role in the transformation of graded, analog changes in synaptic or receptor potentials into a temporally patterned, digital train of all-or-none action potentials. Examples include the M-type and certain A-type K^+ channels, the hyperpolarization-activated h-type channels, and a class of low voltage-activated Ca^{2+} channels (see below).

As the action potential is carried down the axon it is mediated primarily by voltage-gated Na^+ and K^+ channels that function much like those in the squid axon. At the nodes of Ranvier of myelinated axons the mechanism of action potential repolarization is particularly simple—the spike is terminated by fast inactivation of Na^+ channels combined with a large outward leakage current. Voltage-gated K^+ channels do not play a significant role in action potential repolarization at the nodal membrane.

Presynaptic nerve terminals at chemical synapses commonly have a high density of voltage-gated Ca^{2+} channels. Arrival of an action potential in the terminal opens these channels, causing Ca^{2+} influx, which in turn triggers transmitter release.

Excitability Properties Vary Among Neurons

The computing power of an entire neural circuit is enhanced when cells in the circuit represent a wide range of functional properties, because specific functions within the circuit can be assigned to cells with the most appropriate dynamic properties. Thus, while the function of a neuron is determined to a great extent by its anatomical relationships to other neurons (its inputs and its outputs), the biophysical properties of the cell also play a critical role.

How a neuron responds to synaptic input is determined by the proportions of different types of voltage-gated channels in the cell's integrative and trigger zones. Cells with different combinations of channels respond to a constant excitatory input differently. Some cells respond with only a single action potential, others with a constant-frequency train of action potentials, and still others with either an accelerating or decelerating train of action potentials. Some neurons even fire spontaneously in the absence of any external input because of the presence of h-type channels that generate endogenous pacemaker currents (Figure 9-11).

In certain neurons small changes in the strength of synaptic inputs produce a large increase in firing rate, whereas in other cells large changes in synaptic input are required to modulate the firing rate. In many neurons a steady hyperpolarizing input makes the cell less responsive to excitatory input by reducing the resting inactivation of the A-type K^+ channels. In other neurons such a steady hyperpolarization makes the cell *more* excitable, because it removes the inactivation of a particular class of voltage-gated Ca^{2+} channels. In many cases the firing properties of a neuron can be modulated by second messenger–mediated changes in the function of voltage-gated ion channels (Figure 9-11).

The Signaling Functions of Voltage-Gated Channels Can Be Related to Their Molecular Structures

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 primarily in terms of changes in membrane conductance and current flow. They tell little about the molecular structure of the voltage-gated channels and the molecular mechanisms by which they are activated. Fortunately, technical advances such as those described in Chapter 6 have made it possible to examine the structure and function of the voltage-gated Na^+, K^+, and Ca^{2+} channels in detail at the molecular level.

One of the first clues that Na^+ channels are distinct physical entities came from studies that measured the binding of radiolabeled tetrodotoxin to nerve membranes. The density of voltage-gated Na^+ channels in different nerves was estimated by measuring the total amount of tritium-labeled tetrodotoxin bound when specific axonal binding sites are saturated. In nonmyelinated axons the density of channels is quite low, ranging from 35 to 500 Na^+ channels per square micrometer of axon membrane in different cell types. In myelinated axons, where the Na^+ channels are concentrated at the nodes of Ranvier, the density is much higher—between 1000 and 2000 channels per square micrometer of nodal membrane. The greater the density of Na^+ channels in the membrane of an axon, the greater the velocity at

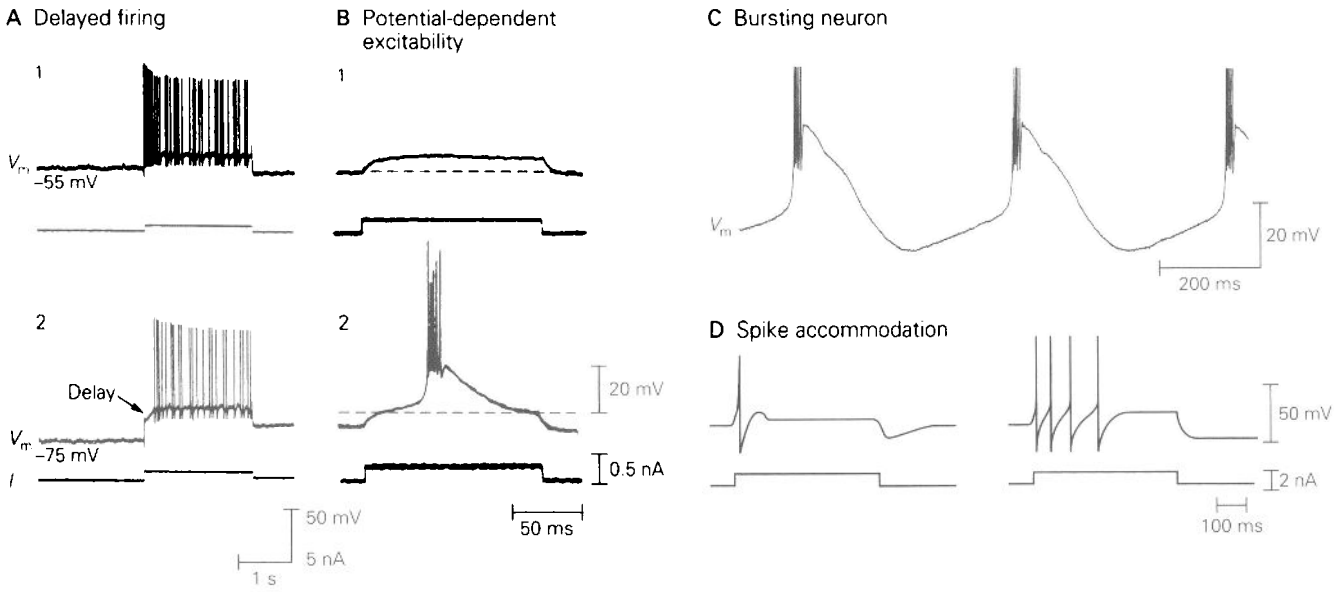


Figure 9-11 Repetitive firing properties vary widely among different types of neurons because the neurons differ in the types of voltage-gated ion channels they express.

A. Injection of a depolarizing current pulse into a neuron from the nucleus tractus solitarius normally triggers an immediate train of action potentials **(1)**. If the cell is first held at a hyperpolarized membrane potential, the depolarizing pulse triggers a spike train after a delay **(2)**. The delay is caused by the A-type K^+ channels, which are activated by depolarizing synaptic input. The opening of these channels generates a transient outward K^+ current that briefly drives V_m away from threshold. These channels typically are inactivated at the resting potential (−55 mV), but steady hyperpolarization removes the inactivation, allowing the channels to be activated by depolarization. (From Dekin and Getting 1987.)

B. When a small depolarizing current pulse is injected into a thalamic neuron at rest, only an electrotonic, subthreshold depolarization is generated **(1)**. If the cell is held at a hyperpolarized level, the same current pulse triggers a burst of action potentials **(2)**. The effectiveness of the current pulse is enhanced because the hyperpolarization causes a type of voltage-gated Ca^{2+} channel to recover from inactivation. The dashed line indicates the level of the resting potential. (From Llinás and Jahnsen 1982.)

The data in **A** and **B** demonstrate that steady hyperpolarization, such as might be produced by inhibitory synaptic input to a neuron, can profoundly affect the spike train pattern that a neuron generates. This effect varies greatly among cell types.

C. In the absence of synaptic input, thalamocortical relay neurons can fire spontaneously in brief bursts of action potentials. These endogenously generated bursts are produced by current flow through two types of voltage-gated ion channels. The gradual depolarization that leads to a burst is driven by inward current flowing through the h-type channels, whose activation gates have the unusual property of opening in response to hyperpolarizing voltage steps. The burst is triggered by inward Ca^{2+} current through voltage-gated Ca^{2+} channels that are activated at relatively low levels of depolarization. This Ca^{2+} influx generates sufficient depolarization to reach threshold and drive a train of Na^+-dependent action potentials. The strong depolarization during the burst causes the h-type channels to close and inactivates the Ca^{2+} channels, allowing the interburst hyperpolarization to develop. This hyperpolarization then opens the h-type channels, initiating the next cycle in the rhythm. (From McCormick and Huguenard 1992.)

D. The firing properties of sympathetic neurons in autonomic ganglia are regulated by a neurotransmitter. A prolonged depolarizing current normally results in only a single action potential. This is because depolarization turns on a slowly activated K^+ current, the M current. The slow activation kinetics of the M-type channels allow the cell to fire one action potential before the efflux of K^+ through the M-type channels becomes sufficient to shift the membrane to more negative voltages and prevent the cell from firing more action potentials (a process termed *accommodation*). The neurotransmitter acetylcholine (ACh) closes the M-type channels, allowing the cell to fire many action potentials in response to the same stimulus. (From Jones and Adams 1987.)

which the axon conducts action potentials. A higher density of voltage-gated Na^+ channels allows more current to flow through the excited membrane and along the axon core, thus rapidly discharging the capacitance of the unexcited membrane downstream (see Figure 8-6).

Opening of Voltage-Gated Channels Is All-or-None

The current flow through a single channel cannot be measured in ordinary voltage-clamp experiments for two reasons. First, the voltage clamp acts on a large area of membrane in which thousands of channels are open-

ing and closing randomly. Second, the background noise caused by the flow of current through passive membrane channels is much larger than the flow of current through any one channel. Both these problems can be circumvented by electrically isolating a tiny piece of membrane in a patch-clamp electrode (see Box 6-1).

Patch-clamp experiments demonstrate that voltage-gated channels generally have only two conductance states, open and closed. Each channel opens in an all-or-none fashion and, when open, permits a pulse of current to flow with a variable duration but constant amplitude (Figure 9-12). The conductances of single voltage-gated Na^+, K^+, and Ca^{2+} channels in the open state typically range from 1 to 20 pS, depending on channel type. One class of Ca^{2+}-activated K^+ channels has an unusually large conductance of about 200 pS.

Redistribution of Charges Within Voltage-Gated Sodium Channels Controls Channel Gating

In their original study of the squid axon, Hodgkin and Huxley suggested that a voltage-gated channel has a net charge, the *gating charge*, somewhere within its wall. They postulated that a change in membrane potential causes this charged structure to move within the plane of the membrane, resulting in a conformational change that causes the channel to open or close. They further predicted that such a charge movement would be measurable. For example, when the membrane is depolarized a positive gating charge would move from near the inner surface toward the outer surface of the membrane, owing to its interaction with the membrane electric field. Such a displacement of positive charge would reduce the net separation of charge across the membrane and hence tend to hyperpolarize the membrane. To keep the membrane potential constant in a voltage-clamp experiment, a small extra component of outward capacitive current, called *gating current*, would have to be generated by the voltage clamp. When the membrane current was examined by means of very sensitive techniques, the predicted gating current was found to flow at the beginning and end of a depolarizing voltage-clamp step prior to the opening or closing of the Na^+ channels (Figure 9-13).

Analysis of the gating current reveals that activation and inactivation of Na^+ channels are coupled processes. During a short depolarizing pulse net outward movement of gating charge within the membrane at the beginning of the pulse is balanced by an opposite inward movement of gating charge at the end of the pulse. However, if the pulse lasts long enough for Na^+ inactivation to take place, the movement of gating charge back across the membrane at the end of the pulse is delayed. The gat-

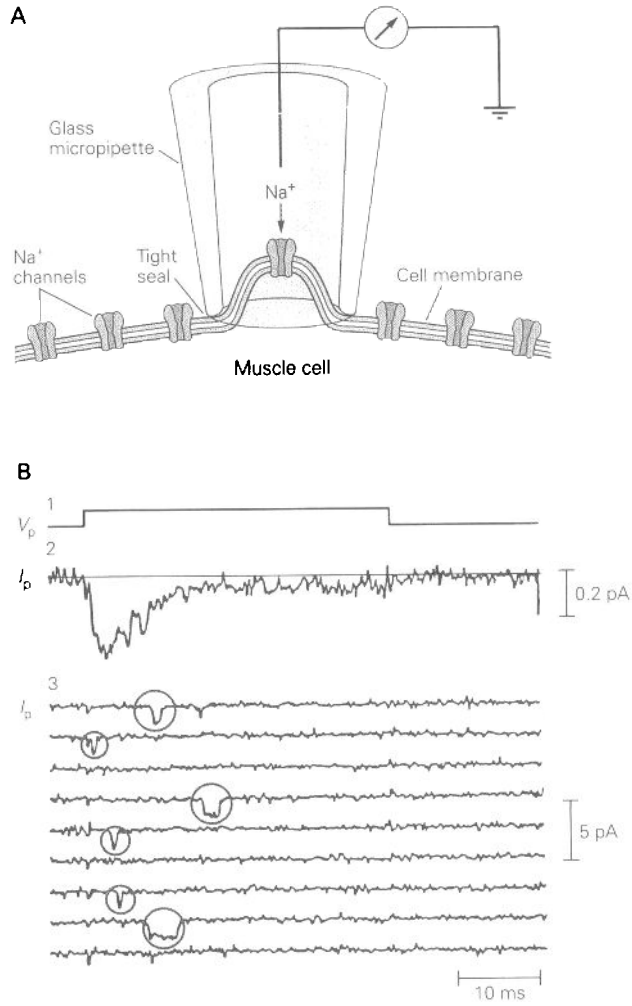


Figure 9-12 Individual voltage-gated channels open in an all-or-none fashion.

A. A small patch of membrane containing only a single voltage-gated Na^+ channel is electrically isolated from the rest of the cell by the patch electrode. The Na^+ current that enters the cell through these channels is recorded by a current monitor connected to the patch electrode.

B. Recordings of single Na^+ channels in cultured muscle cells of rats. **1.** Time course of a 10 mV depolarizing voltage step applied across the patch of membrane (V_p = potential difference across the patch). **2.** The sum of the inward current through the Na^+ channels in the patch during 300 trials (I_p = current through the patch of membrane). The trace was obtained by blocking the K^+ channels with tetraethylammonium and subtracting the capacitive current electronically. **3.** Nine individual trials from the set of 300, showing six individual Na^+ channel openings **(circles)**. These data demonstrate that the total Na^+ current recorded in a conventional voltage-clamp record (see Figure 9-3C) can be accounted for by the all-or-none opening and closing of individual Na^+ channels. (From Sigworth and Neher 1980.)

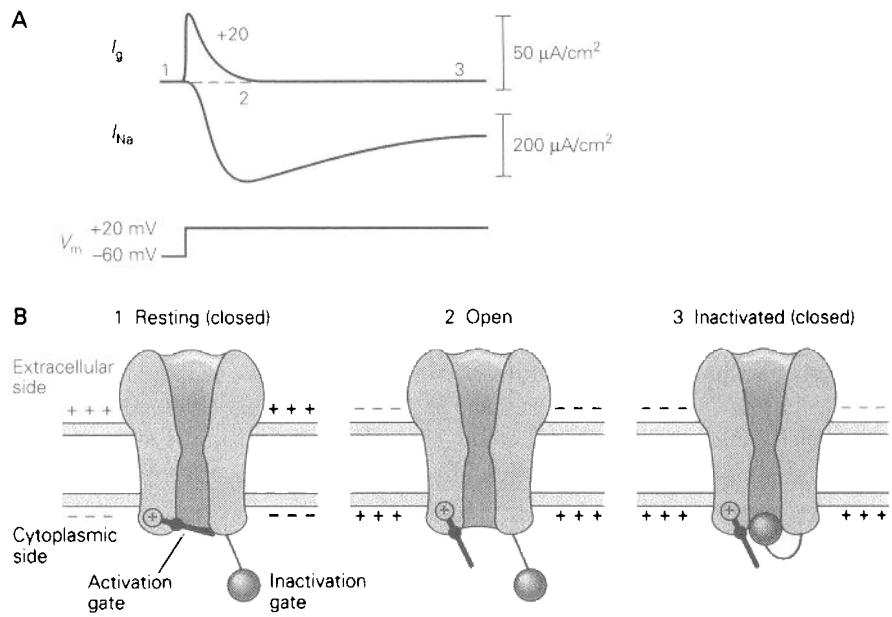


Figure 9-13 Gating currents directly measure the changes in charge distribution associated with Na^+ channel activation.

A. When the membrane is depolarized the Na^+ current (I_{Na}) first activates and then inactivates. The activation of the Na^+ current is preceded by a brief outward gating current (I_g), reflecting the outward movement of positive charge within the Na^+ channel protein associated with the opening of the activation gate. To detect the small gating current it is necessary to block the flow of ionic current through the Na^+ and K^+ channels and mathematically subtract the capacitive current due to charging of the lipid bilayer.

B. Illustration of the position of the activation and inactivation gates when the channel is at rest **(1)**, when the Na^+ channels have been opened **(2)**, and when the channels have been inactivated **(3)**. It is the movement of the positive charge on the activation gate through the membrane electric field that generates the gating current.

ing charge is thus temporarily immobilized; only as the Na^+ channels recover from inactivation is the charge free to move back across the membrane. This charge immobilization indicates that the gating charge cannot move while the channel is in the inactivated state, ie, while the inactivation gate is closed (see Figure 9-9).

To explain this phenomenon, Clay Armstrong and Francisco Bezanilla proposed that Na^+ channel inactivation occurs when the open (activated) channel is blocked by a tethered plug (the ball and chain mechanism), thereby preventing the closure of the activation gate. In support of this idea, exposing the inside of the axon to proteolytic enzymes selectively removes inactivation, causing the Na^+ channels to remain open during a depolarization, presumably because the enzymes clip off the inactivation "ball."

The Voltage-Gated Sodium Channel Selects for Sodium on the Basis of Size, Charge, and Energy of Hydration of the Ion

After the gates of the Na^+ channel have opened, how does the channel discriminate between Na^+ and other ions? The channel's selectivity mechanism can be probed by measuring the channel's relative permeability to several types of organic and inorganic cations that differ in size and hydrogen-bonding characteristics. As we learned in Chapter 6, the channel behaves as if it contains a filter or recognition site that selects partly on the basis of size, thus acting as a molecular sieve (see Figure 6-3). The ease with which ions with good hydrogen-bonding characteristics pass through the channel suggests that part of the inner wall of the channel is made up of negatively polarized or charged amino acid residues that can substitute for water. When the pH of the fluid surrounding the cell is lowered, the conductance of the open channel is gradually reduced, consistent with the titration of important negatively charged carboxylic acid residues.

The selectivity filter of the Na^+ channel is made up of four loops within the molecule (the P region) that are similar in structure (see below). A glutamic acid residue is situated at equivalent points in two of these loops. A lysine and an alanine residue are situated at the equivalent site in the other two loops. The channel is thought to select for Na^+ ions by the following mechanism. The negatively charged carboxylic acid groups of the glutamic acid residues, which are located at the outer mouth of the pore, perform the first step in the selection process by attracting cations and repelling anions. The cations then encounter a constricted portion of the pore, the selectivity filter, with rectangular dimensions of 0.3×0.5 nm. This cross section is just large enough to accommodate one Na^+ ion contacting one water molecule. Cations that are larger in diameter cannot pass through

the pore. Cations smaller than this critical size pass through the pore, but only after losing most of the waters of hydration they normally carry in free solution.

The negative carboxylic acid group, as well as other oxygen atoms that line the pore, can substitute for these waters of hydration, but the degree of effectiveness of this substitution varies among ion species. The more effective the substitution, the more readily ions can traverse the Na^+ channel. The Na^+ channel excludes K^+ ions, in part because the larger-diameter K^+ ion cannot interact as effectively with the negative carboxylic group. The lysine and alanine residues also contribute to the selectivity of the channel. When these residues are changed to glutamic acid residues by site-directed mutagenesis, the Na^+ channels can act as Ca^{2+}-selective channels! (The mechanism whereby K^+ selectivity is achieved was discussed in Chapter 6.)

Genes Encoding the Potassium, Sodium, and Calcium Channels Stem From a Common Ancestor

Since a change in two amino acid residues can cause a Na^+ channel to behave as a Ca^{2+} channel, it is reasonable to believe that the Na^+ and Ca^{2+} channels may be closely related. Detailed molecular studies have revealed that all voltage-gated ion channels—those for K^+, Na^+, and Ca^{2+}—share several functionally important domains and are indeed quite similar. In fact, there is now strong evidence from studies of bacteria, plants, invertebrates, and vertebrates that most voltage-sensitive cation channels stem from a common ancestral channel—perhaps a K^+ channel—that can be traced to a single-cell organism living over 1.4 billion years ago, before the evolution of separate plant and animal kingdoms. The amino acid sequences conserved through evolution help identify the domains within contemporary cation channels that are critical for function.

Molecular studies of the voltage-sensitive cation channels began with the identification of Na^+ channel molecules. Three subunits have been isolated: one large glycoprotein (α) and two smaller polypeptides ($\beta 1$ and $\beta 2$). The α-subunit is ubiquitous, and insertion of this subunit into an artificial lipid bilayer reconstitutes the basic features of Na^+ channel function. Therefore the α-subunit is presumed to form the aqueous pore of the channel. The smaller subunits, whose presence varies in different regions of the nervous system, regulate various aspects of α-subunit function.

Examination of the amino acid sequence encoded by the cloned gene for the α-subunit of the Na^+ channel reveals two fundamental features of the structure of the Na^+ channel. First, the α-subunit is composed of four internal repeats (domains I–IV), with only slight variations, of a sequence that is approximately 150 amino acids in length. Each of the four repeats of this sequence domain is believed to have six membrane-spanning hydrophobic regions (S1–S6) that are primarily α-helical in form. A seventh hydrophobic region the P region that connects the S5 and S6 segments, appears to form a loop that dips into and out of the membrane (Figure 9-14). The four repeated domains are thought to be arranged roughly symmetrically, with the P region and some of the membrane-spanning regions forming the walls of the water-filled pore (Figure 9-15).

The second structural feature of the Na^+ channel revealed by amino acid sequence analysis is that one of the six putative membrane-spanning regions, the S4 region, is structurally quite similar in the Na^+ channels of many different species. This strict conservation suggests that the S4 region is critical to Na^+ channel function. Moreover, the S4 region of the Na^+ channel is similar to corresponding regions of the voltage-gated Ca^{2+} and K^+ channels (Figure 9-14) but is lacking in K^+ channels that are not activated by voltage (see below). For this reason the S4 region may be the voltage sensor—that part of the protein that transduces depolarization of the cell membrane into a gating transition within the channel, thereby opening the channel. This idea is supported by the observation that the S4 region contains a distinctive pattern of amino acids. Every third amino acid along the S4 helix is positively charged (lysine or arginine) while the intervening two amino acids are hydrophobic. This highly charged structure is therefore likely to be quite sensitive to changes in the electric field across the membrane. Experiments using site-directed mutagenesis show that reducing the net positive charge in one of the S4 regions of the channel lowers the voltage sensitivity of Na^+ channel activation.

Structure-function studies based on genetic engineering of the α-subunit have led to a hypothesis about how the charges in the S4 region move across the membrane during channel gating. According to the scheme, at rest one of the charged residues on the S4 α-helix is completely buried in the wall of the channel, where its positive charge is stabilized by interaction with a negatively charged amino acid residue in one of the other membrane-spanning segments of the channel (Figure 9-16). The other positive charges are located on parts of the S4 helix that are within a water-filled lacuna in the wall of the channel that is continuous with the cytoplasm. When the membrane is depolarized the change in electrostatic force causes movement of the S4 helix relative to the surrounding channel wall, translocating some of the positively charged residues to the outside of

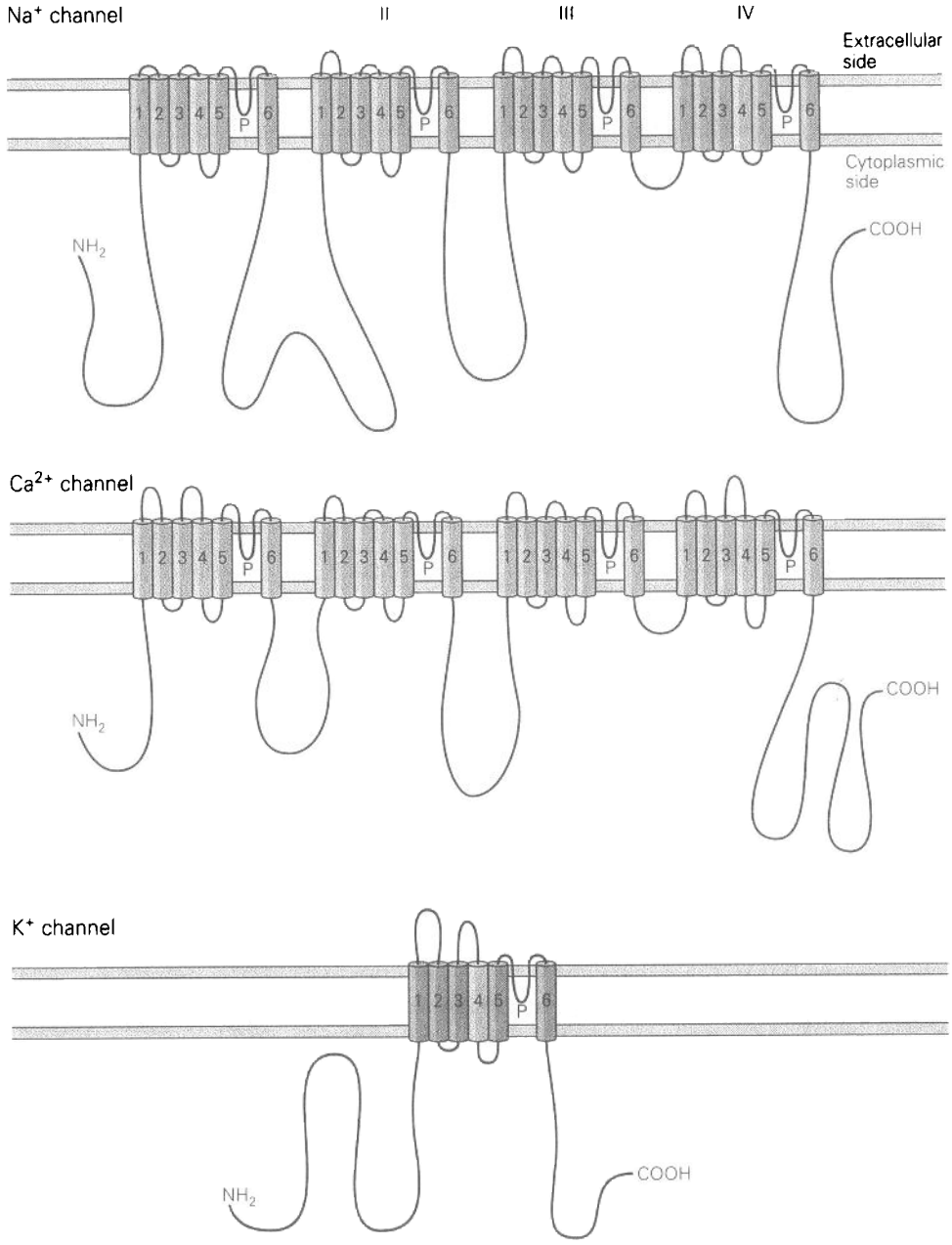


Figure 9-14 The pore-forming subunits of the voltage-gated Na^+, Ca^{2+}, and K^+ channels are composed of a common repeated domain. The α-subunit of the Na^+ and Ca^{2+} channels consists of a single polypeptide chain with four repeats (I-IV) of a domain that contains six membrane-spanning α-helical regions (S1-S6). A stretch of amino acids, the P region between α-helices 5 and 6, forms a loop that dips into and out of the membrane. The S4 segment is shown in red, representing its net positive charge. The fourfold repetition of the P region is believed to form a major part of the pore lining (see Figure 9–15). The K^+ channel, in contrast, has only a single repeat of the six α-helices and the P region. Four K^+ channel subunits are assembled to form a complete channel (see Figure 6–12). (Adapted from Catterall 1988, Stevens 1991.)

the membrane. This movement is somehow transduced into opening of the activation gate.

The genes encoding the major α-subunits of several voltage-gated Ca^{2+} channels have also been cloned. Their sequences reveal that the Ca^{2+} channels are also composed of four repeating domains, each with six hydrophobic transmembrane regions and one P loop, which have amino acid sequences homologous to those of the voltage-gated Na^+ channel (see Figure 9-14).

The K^+ channel genes contain only one copy of the domain that is repeated four times in the genes for Na^+ and Ca^{2+} channels. Nevertheless, the basic channel structure is similar for the three channel types, as four α-subunits must aggregate symmetrically around a central pore to form a K^+ channel. It is this striking homology among the voltage-gated Na^+, Ca^{2+}, and K^+ channels that suggests that all three channels belong to the same gene family and have evolved by gene duplication and modification from a common ancestral structure, presumably a K^+ channel.

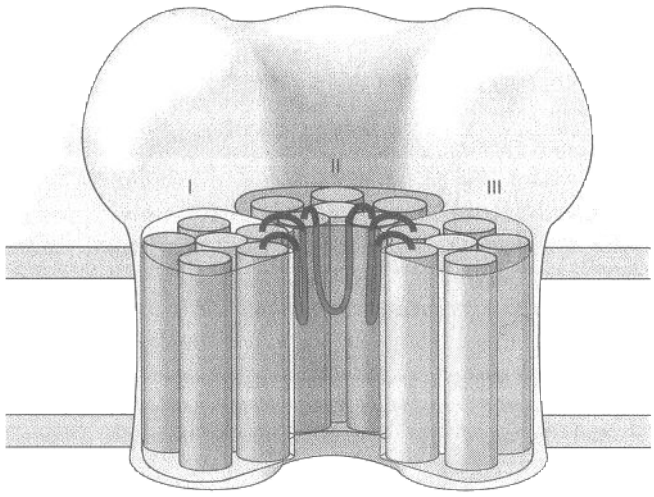


Figure 9-15 The four membrane-spanning domains of the α-subunit in voltage-gated Na^+ and Ca^{2+} channels form the channel pore. The tertiary structure of the channels proposed here is based on the secondary structures shown in Figure 9-14. The central pore is surrounded by the four internally repeated domains (M-I to M-IV). (Only three of the domains are shown here for clarity.) Each quadrant of the channel includes six cylinders, which represent six putative membrane-spanning α-helices. The S4 segment (in red) is thought to be involved in gating because it contains a significant net charge. The protruding loop in each quadrant represents the P region segment that dips into the membrane to form the most narrow region of the wall of the pore.

The conservative mechanism by which evolution proceeds—creating new structural or functional entities by modifying, shuffling, and recombining existing gene sequences—is illustrated by the modular design of various members of the extended gene family that includes the voltage-gated Na^+, K^+, and Ca^{2+} channels. For example, the basic structures of both a Ca^{2+}-activated K^+ channel, an h-type cation channel activated by hyperpolarization and intracellular cycle neucleotides, and a voltage-independent cation channel activated by intracellular cyclic nucleotides are the same as the structures of other members of the gene family (six membrane-spanning α-helices and a P region), with some modifications. The functional differences between these two channels are due primarily to the addition of regulatory domains that bind Ca^{2+} or cyclic nucleotides, respectively, to the C-terminal ends of the proteins. As we saw in Chapter 6, the subunits that comprise the inward-rectifying K^+ channels are truncated versions of the fundamental domain, consisting of the P region and its two flanking membrane-spanning regions. Four such subunits combine to form a functional channel (Figure 9-17).

The modular design of this extended gene family is also illustrated by a comparison of activation and inactivation mechanisms of various channels within the family. The S4 membrane-spanning region, which is thought to be the voltage sensor in channels of this family, has a relatively large net charge in the Na^+, K^+, and Ca^{2+} channels that open in response to depolarization. In contrast, the S4 regions in cyclic nucleotide-gated channels, which are only weakly sensitive to voltage, have significantly less net charge, and h-type channels lack certain conserved S4 residues. Moreover, inward-rectifying K^+ channels, which have essentially no intrinsic voltage sensitivity, completely lack the S4 region. These inward-rectifying channels are activated by the effect of hyperpolarization on freely diffusible, positively charged blocking particles in the cytoplasm. Depending on the subspecies of channel, this blocker may be either Mg^{2+} or various organic polyamines. These channels open when the cation-blocking particle is electrostatically drawn out of the channel at negative potentials around the resting potential.

Inactivation of voltage-gated ion channels is also mediated by different molecular modules. For example, the rapid inactivation of both the A-type K^+ channel and the voltage-gated Na^+ channel can be attributed to a tethered plug that binds to the inner mouth of the channel when the activation gate opens. In the A-type K^+ channel the plug is formed by the cytoplasmic N terminus of the channel, whereas in voltage-gated Na^+ channels the cytoplasmic loop connecting domains III and IV of the α-subunit forms the plug.

Various Smaller Subunits Contribute to the Functional Properties of Voltage-Gated Sodium, Calcium, and Potassium Channels

Most, perhaps all, voltage-gated Na^+, K^+, and Ca^{2+} channels have β- and, in some cases, γ- and δ-subunits that modulate the functional properties of the channel-forming α-subunits. The modulatory function of these subunits, which may be either cytoplasmic or membrane-spanning, depends on the type of channel. For example, the subunits may enhance the efficiency of coupling of depolarization to activation or inactivation gating. They may also shift the gating functions to different voltage ranges. In some K^+ channels in which the α-subunit lacks a tethered inactivation plug, addition of a set of β-subunits with their own N-terminal tethered plugs can endow the channel with the ability to rapidly inactivate. In contrast to the α-subunits, there is no known homology among the β-, γ-, and δ-subunits from the three-major subfamilies of voltage-gated channels.

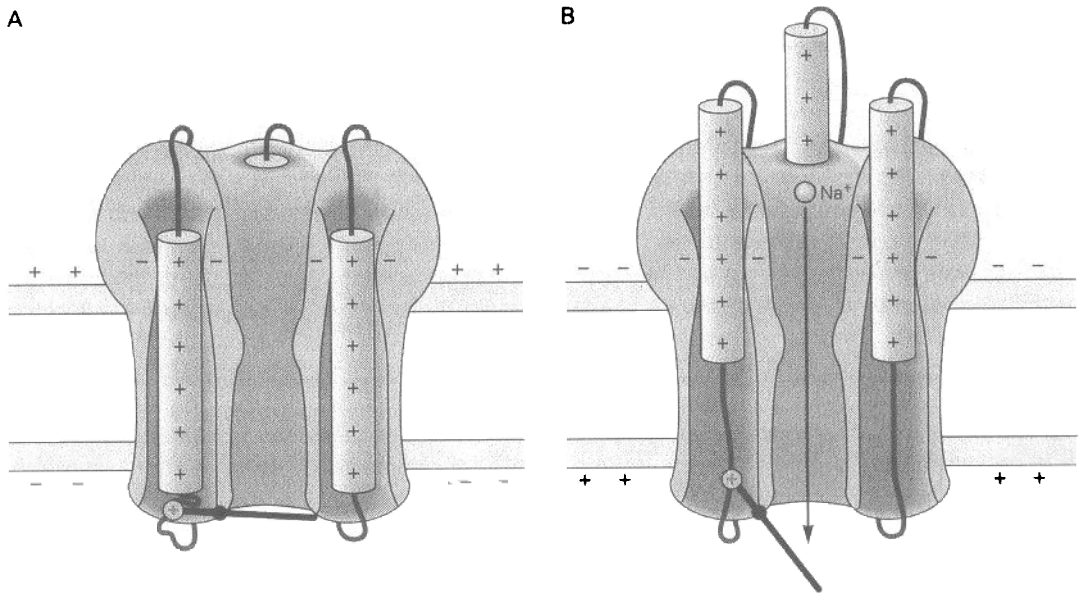


Figure 9-16 Gating of the Na^+ channel is thought to rely on redistribution of net charge in the S4 region.

A. At rest, the inside-negative electric field across the membrane biases the positively charged S4 helix toward the inside of the membrane. One of the positive charges is stabilized by interaction with a negative charge in another part of the channel. The remainder of the charged region lies in a water-filled cavity in the channel wall that is continuous with the cytoplasm.

B. When the cell is depolarized the change in electrical field across the membrane drives the S4 region toward the extracellular face of the membrane. This change in configuration opens the activation gate by a mechanism that is not well understood. (Adapted from Yang et al. 1996.)

The Diversity of Voltage-Gated Channel Types Is Due to Several Genetic Mechanisms

A single ion species can cross the membrane through several distinct types of ion channels, each with its own characteristic kinetics, voltage sensitivity, and sensitivity to different modulators. In voltage-gated channels this diversity may be due to any of five genetic mechanisms: (1) More than one gene may encode related α-subunits within each class of channel. (2) A single gene product may be alternatively spliced in different classes of neurons, resulting in different variants of the mRNA that encodes the α-subunit. (3) The four α-subunits that coalesce to form a K^+ channel may be encoded by different genes. After translation the gene products are mixed and matched in various combinations, thus forming different subclasses of heteromultimeric channels. (4) A given α-subunit may be combined with different β-, γ- or δ-subunits to form functionally different channel types. (5) The diversity of some β-subunits is increased either by alternative splicing of the pre-mRNA molecule or by the encoding of different variants of a basic β-subunit type on different genes. These various sources of diversity endow the nervous system with tremendous opportunities for regional diversity of functional properties.

Mutations in Voltage-Gated Channels Cause Specific Neurological Diseases

Several inherited neurological disorders are now known to be caused by mutations in voltage-gated ion channels. Patients with hyperkalemic periodic paralysis have episodes of muscle stiffness (myotonia) and muscle weakness (paralysis) in response to the elevation of K^+ levels in serum after vigorous exercise. Genetic studies have shown that the disease is caused by a point mutation in the α-subunit of the gene for the voltage-gated Na^+ channel found in skeletal muscle. Voltage-clamp

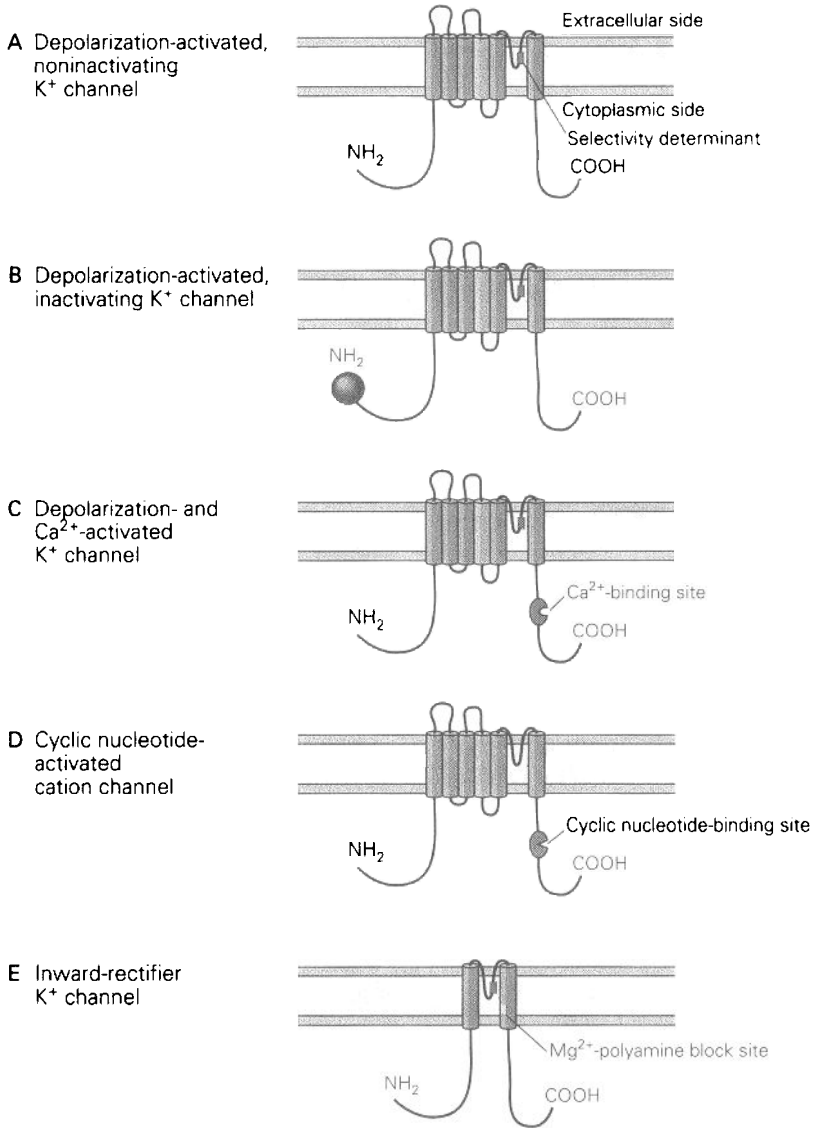


Figure 9-17 Ion channels belonging to the extended gene family of voltage-gated channels are variants of a common molecular design.

A. Depolarization-activated, noninactivating K^+ channels are formed from four copies of an α-subunit, the basic building block of voltage-gated channels. The α-subunit is believed to have six membrane-spanning regions and one membrane-embedded region (the P region). The P region contains a K^+-selective sequence (denoted by the rectangle).

B. Many K^+ channels that are first activated and then inactivated by depolarization have a ball-and-chain segment on their N-terminal ends that inactivates the channel by plugging its inner mouth.

C. Potassium channels that are activated by both depolarization and intracellular Ca^{2+} have a Ca^{2+}-binding sequence attached to the C-terminal end of the channel.

D. Cation channels gated by cyclic nucleotides have a cyclic nucleotide-binding domain attached to the C-terminal end. One class of such channels is the voltage-independent, cyclic nucleotide-gated channels important in the transduction of olfactory and visual sensory signals. Another class of channels is the hyperpolarization-activated h-type channels important for pacemaker activity (see Figure 9-11C).

E. Inward-rectifying K^+ channels, which are gated by blocking particles available in the cytoplasm, are formed from truncated versions of the basic building block, with only two membrane-spanning regions and the P region.

studies of cultured skeletal muscle cells obtained from biopsies of patients with this disorder demonstrate that the voltage-gated Na^+ channels fail to completely inactivate. This defect is exacerbated by elevation of external K^+. The prolonged opening of the Na^+ channels is thought to cause muscles to fire repetitive trains of action potentials, thus producing the muscle stiffness. As the fraction of channels with altered inactivation increases (as a result of continued K^+ elevation), the muscle resting potential eventually reaches a new stable depolarized level (around −40 mV), at which point most Na^+ channels become inactivated so that the membrane fails to generate further action potentials (paralysis).

Patients with episodic ataxia exhibit normal neurological function except during periods of emotional or physical stress, which can trigger a generalized ataxia due to involuntary muscle movements. The disease has been shown to result from one of several point mutations in a delayed-rectifier, voltage-gated K^+ channel. These mutations decrease current through the channel, in part by enhancing the rate of inactivation. As a result, because less outward K^+ current is available for repolarization, the tendency of nerves and muscle cells to fire repetitively is enhanced. (Remarkably, the first K^+ channel gene to be cloned was identified based on a genetic strategy involving a similar mutation in a *Drosophila* K^+ channel gene, which gives rise to the so-called Shaker phenotype.) Muscle diseases involving mutations in Cl^- channels (myotonia congenita) and Ca^{2+} channels (hypokalemic periodic paralysis) have also been identified.

An Overall View

An action potential is produced by the movement of ions across the membrane through voltage-gated channels. This ion movement, which occurs only when the channels are open, changes the distribution of charges on either side of the membrane. An influx of Na^+, and in some cases Ca^{2+}, depolarizes the membrane, initiating an action potential. An outflow of K^+ then repolarizes the membrane by restoring the initial charge distribution. A particularly important subset of voltage-gated ion channels opens primarily when the membrane potential nears the threshold for an action potential; these channels have a profound effect on the firing patterns generated by a neuron.

We know something about how channels function from studies using variations on the voltage-clamp technique—these studies let us eavesdrop on a channel at work. And we know something from biochemical and molecular biology studies about the channel's structure—about the primary amino acid sequence of the proteins that form them. Now these two approaches are being combined in a concerted effort to understand the relationship between structure and function in these channels: how they are put together, what their contours and surface map look like, how they interact with other molecules, what the structure of the channel pore is, and how its gate is opened.

Thus, we may soon be able to understand the molecular mechanism for the remarkable ability of voltage-gated channels to generate the action potential. These insights have two important implications: they will allow us to understand better the molecular bases of certain genetic diseases that involve mutations in ion channel genes, and they will enable us to design safer and more effective drugs to treat a variety of diseases that involve disturbances in electrical signaling (such as epilepsy, multiple sclerosis, myotonia, and ataxia).

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