NEUROSCIENCE

Exploring the Brain

Enhanced Fourth Edition

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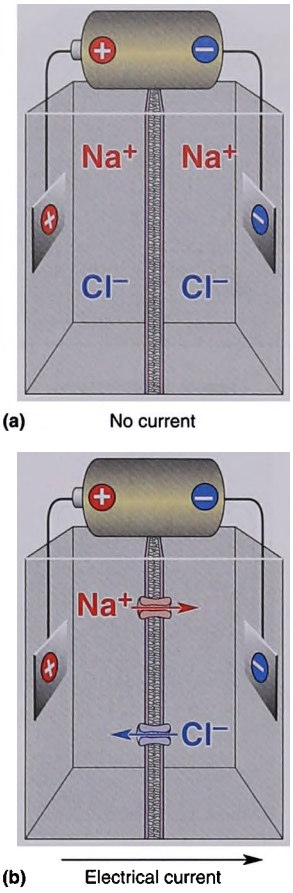


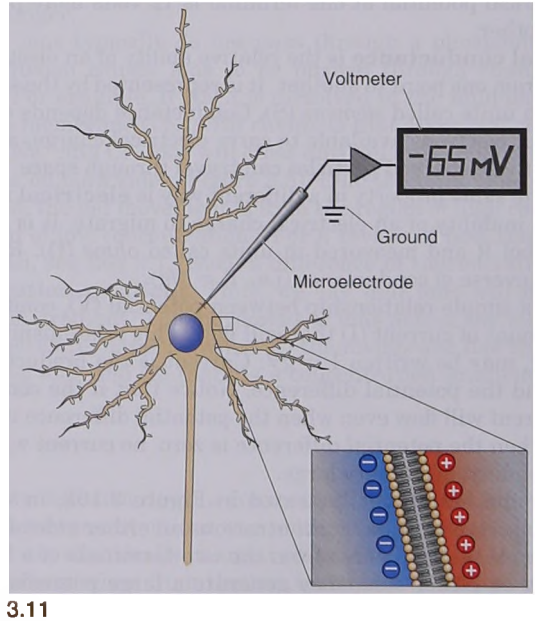
◀ FIGURE 3.10
Electrical current flow across a membrane. (a) A voltage applied across a phospholipid bilayer causes no electrical current because there are no channels to allow the passage of electrically charged ions from one side to the other; the conductance of the membrane is zero. (b) Inserting channels in the membrane allows ions to cross. Electrical current flows in the direction of cation movement (from left to right, in this example).

and (2) there is an electrical potential difference across the membrane (Figure 3.10b).

The stage is now set. We have electrically charged ions in solution on both sides of the neuronal membrane. Ions can cross the membrane only by way of protein channels. The protein channels can be highly selective for specific ions. The movement of any ion through its channel depends on the concentration gradient and the difference in electrical potential across the membrane. Now let's use this knowledge to explore the resting membrane potential.

THE IONIC BASIS OF THE RESTING MEMBRANE POTENTIAL

The **membrane potential** is the voltage across the neuronal membrane at any moment, represented by the symbol V_m. Sometimes V_m is "at rest"; at other times it is not (such as during an action potential). V_m can be measured by inserting a microelectrode into the cytosol. A typical **microelectrode** is a thin glass tube with an extremely fine tip (diameter 0.5 μm) that can penetrate the membrane of a neuron with minimal damage. It is filled with an electrically conductive salt solution and is connected to a device called a *voltmeter*. The voltmeter measures the electrical potential difference between the tip of this microelectrode and a wire placed outside the cell (Figure 3.11). This method reveals that electrical charge is unevenly distributed across the neuronal membrane. The inside of the neuronal membrane is electrically negative relative to



▲ FIGURE 3.11
Measuring the resting membrane potential. A voltmeter measures the difference in electrical potential between the tip of a microelectrode inside the cell and a wire placed in the extracellular fluid, conventionally called "ground" because it is electrically continuous with the earth. Typically, the inside of the neuron is about −65 mV with respect to the outside. This potential is caused by the uneven distribution of electrical charge across the membrane (enlargement).

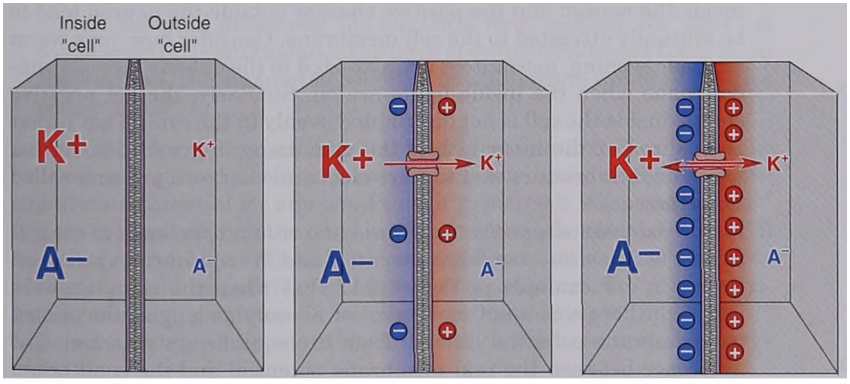
the outside. This steady difference, the resting potential, is maintained whenever a neuron is not generating impulses.

The resting potential of a typical neuron is about −65 millivolts (1 mV = 0.001 volts). Stated another way, for a neuron at rest, V_m = −65 mV. This negative resting membrane potential of the neuron is an absolute requirement for a functioning nervous system. To understand the negative membrane potential, we look to the ions that are present and how they are distributed inside and outside the neuron.

Equilibrium Potentials

Consider a hypothetical cell in which the inside is separated from the outside by a pure phospholipid membrane with no proteins. Inside this cell, a concentrated potassium salt solution is dissolved, yielding K^+ and A^- anions (any molecules with a negative charge). Outside the cell is a solution with the same salt but diluted twentyfold with water. Although a large concentration gradient exists between the inside of the cell and the outside, there is no net movement of ions because the phospholipid bilayer, having no channel proteins, is impermeable to charged, hydrophilic atoms. Under these conditions, a microelectrode would record no potential difference between the inside and the outside of the cell. In other words, V_m would be equal to 0 mV because the ratio of K^+ to A^- on each side of the membrane equals 1; both solutions are electrically neutral (Figure 3.12a).

Consider how this situation would change if potassium channels were inserted into the phospholipid bilayer. Because of the selective permeability of these channels, K^+ would be free to pass across the membrane, but A^- would not. Initially, diffusion rules: K^+ ions pass through the channels out of the cell, down the steep concentration gradient. Because A^- is left behind, however, the inside of the cell membrane immediately begins to acquire a net negative charge, and an electrical potential difference is established across the membrane (Figure 3.12b). As the inside



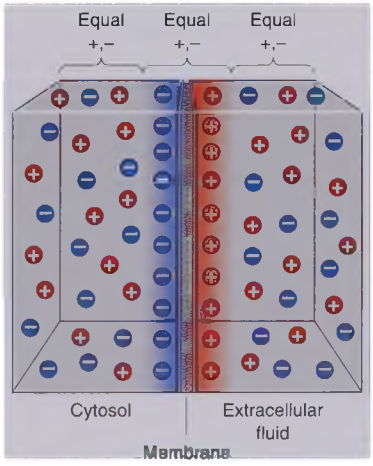
▲ FIGURE 3.12

Establishing equilibrium in a selectively permeable membrane. (a) An impermeable membrane separates two regions: one of high salt concentration (inside) and the other of low salt concentration (outside). The relative concentrations of potassium (K^+) and an impermeable anion (A^-) are represented by the sizes of the letters. (b) Inserting a channel that is selectively permeable to K^+ into the membrane initially results in a net movement of K^+ down their concentration gradient, from left to right. (c) A net accumulation of positive charge on the outside and negative charge on the inside retards the movement of positively charged K^+ from the inside to the outside. Equilibrium is established such that there is no net movement of ions across the membrane, leaving a charge difference between the two sides.

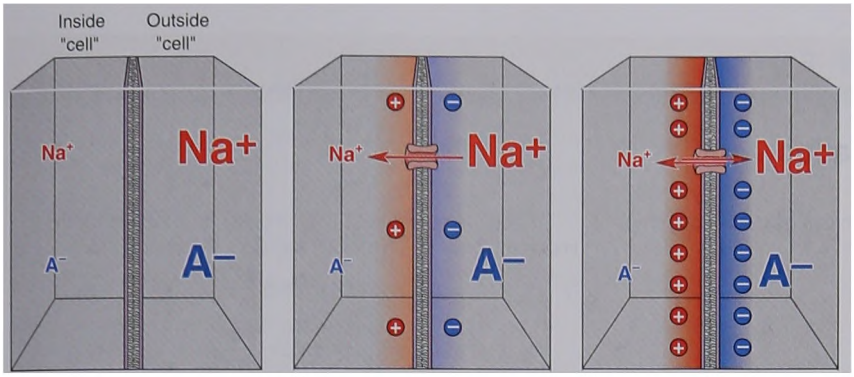
acquires more and more net negative charge, the electrical force starts to pull positively charged K^+ ions back through the channels into the cell. When a certain potential difference is reached, the electrical force pulling K^+ ions inside exactly counterbalances the force of diffusion pushing them out. Thus, an *equilibrium* state is reached in which the diffusional and electrical forces are equal and opposite, and the net movement of K^+ across the membrane ceases (Figure 3.12c). The electrical potential difference that exactly balances an ionic concentration gradient is called an **ionic equilibrium potential**, or simply **equilibrium potential**, and it is represented by the symbol E_{ion}. In this example, the equilibrium potential will be about −80 mV.

The example in Figure 3.12 demonstrates that generating a steady electrical potential difference across a membrane is a relatively simple matter. All that is required is an ionic concentration gradient and selective ionic permeability. Before moving on to the situation in real neurons, however, we can use this example to make four important points.

1. *Large changes in membrane potential are caused by minuscule changes in ionic concentrations.* In Figure 3.12, channels were inserted, and K^+ ions flowed out of the cell until the membrane potential went from 0 mV to the equilibrium potential of −80 mV. How much does this ionic redistribution affect the K^+ concentration on either side of the membrane? Not very much. For a cell with a 50 μm diameter, containing 100 mM K^+, it can be calculated that the concentration change required to take the membrane from 0 to −80 mV is about 0.00001 mM. That is, when the channels were inserted and the K^+ flowed out until equilibrium was reached, the internal K^+ concentration went from 100 to 99.99999 mM—a negligible drop in concentration.
2. *The net difference in electrical charge occurs at the inside and outside surfaces of the membrane.* Because the phospholipid bilayer is so thin (less than 5 nm thick), it is possible for ions on one side to interact electrostatically with ions on the other side. Thus, the negative charges inside the neuron and the positive charges outside the neuron tend to be mutually attracted to the cell membrane. Consider how, on a warm summer evening, mosquitoes are attracted to the outside face of a window pane when the inside lights are on. Similarly, the net negative charge inside the cell is not distributed evenly in the cytosol but rather is localized at the inner face of the membrane (Figure 3.13). In this way, the membrane is said to store electrical charge, a property called *capacitance*.
3. *Ions are driven across the membrane at a rate proportional to the difference between the membrane potential and the equilibrium potential.* Notice in our example in Figure 3.12 that when the channels were inserted, there was a net movement of K^+ only as long as the electrical membrane potential differed from the equilibrium potential. The difference between the real membrane potential and the equilibrium potential ($V_m - E_{ion}$) for a particular ion is called the **ionic driving force**. We'll talk more about this in Chapters 4 and 5 when we discuss the movement of ions across the membrane during the action potential and synaptic transmission.
4. *If the concentration difference across the membrane is known for an ion, the equilibrium potential can be calculated for that ion.* In our example in Figure 3.12, we assumed that K^+ was more concentrated inside the cell. Based on this knowledge, we were able to deduce that the equilibrium potential would be negative if the membrane were selectively permeable to K^+. Let's consider another example, in which Na^+ is more



▲ FIGURE 3.13
The distribution of electrical charge across the membrane. The uneven charges inside and outside the neuron line up along the membrane because of electrostatic attraction across this very thin barrier. Notice that the bulk of the cytosol and extracellular fluid is electrically neutral.



▲ FIGURE 3.14

Another example of establishing equilibrium in a selectively permeable membrane. **(a)** An impermeable membrane separates two regions: one of high salt concentration (outside) and the other of low salt concentration (inside). **(b)** Inserting a channel that is selectively permeable to Na^+ into the membrane initially results in a net movement of Na^+ down its concentration gradient, from right to left. **(c)** A net accumulation of positive charge on the inside and negative charge on the outside retards the movement of positively charged Na^+ from the outside to the inside. Equilibrium is established such that there is no net movement of ions across the membrane, leaving a charge difference between the two sides; in this case, the inside of the cell is positively charged with respect to the outside.

concentrated *outside* the cell (Figure 3.14). If the membrane contains sodium channels, Na^+ would flow down the concentration gradient *into* the cell. The entry of positively charged ions would cause the cytosol on the inner surface of the membrane to acquire a net positive charge. The positively charged interior of the cell membrane would now repel Na^+ ions, tending to push them back out through their channels. At a certain potential difference, the electrical force pushing Na^+ ions out would exactly counterbalance the force of diffusion pushing them in. In this example, the membrane potential at equilibrium would be positive on the inside.

The examples in Figures 3.12 and 3.14 illustrate that if we know the ionic concentration difference across the membrane, we can figure out the equilibrium potential for any ion. Prove it to yourself. Assume that Ca^{2+} is more concentrated on the outside of the cell and that the membrane is selectively permeable to Ca^{2+}. See if you can figure out whether the inside of the cell would be positive or negative at equilibrium. Try it again, assuming that the membrane is selectively permeable to Cl^-, and that Cl^- is more concentrated outside the cell. (Pay attention here; note the charge of the ion.)

The preceding examples show that each ion has its own equilibrium potential—the steady electrical potential that would occur if the membrane were permeable only to that ion. Thus, we can speak of the potassium equilibrium potential, E_K; the sodium equilibrium potential, E_{Na}; the calcium equilibrium potential, E_{Ca}; and so on. And knowing the electrical charge of the ion and the concentration difference across the membrane, we can easily deduce whether the inside of the cell would be positive or negative at equilibrium. In fact, the *exact* value of an equilibrium potential in mV can be calculated using an equation derived from the principles of physical chemistry, the **Nernst equation**, which takes into consider-

BOX 3.2 BRAIN FOOD

The Nernst Equation

The equilibrium potential for an ion can be calculated using the Nernst equation:

$$E_{ion} = 2.303 \frac{RT}{zF} \log \frac{[ion]_o}{[ion]_i}$$

where

E_{ion} = ionic equilibrium potential
R = gas constant
T = absolute temperature
z = charge of the ion
F = Faraday's constant
log = base 10 logarithm
$[ion]_o$ = ionic concentration outside the cell
$[ion]_i$ = ionic concentration inside the cell

The Nernst equation can be derived from the basic principles of physical chemistry. Let's see if we can make some sense of it.

Remember that equilibrium is the balance of two influences: diffusion, which pushes an ion down its concentration gradient, and electricity, which causes an ion to be attracted to opposite charges and repelled by like charges. Increasing the thermal energy of each particle increases diffusion and therefore increases the potential difference achieved at equilibrium. Thus, E_{ion} is proportional to T. On the other hand, increasing the electrical charge of each particle decreases the potential difference needed to balance diffusion. Therefore, E_{ion} is inversely proportional to the charge of the ion (z). We need not worry about R and F in the Nernst equation because they are constants.

At body temperature (37°C), the Nernst equation for the important ions—K^+, Na^+, Cl^-, and Ca^{2+} — simplifies to:

$$E_K = 61.54 \text{ mV} \log \frac{[K^+]_o}{[K^+]_i}$$

$$E_{Na} = 61.54 \text{ mV} \log \frac{[Na^+]_o}{[Na^+]_i}$$

$$E_{Cl} = -61.54 \text{ mV} \log \frac{[Cl^-]_o}{[Cl^-]_i}$$

$$E_{Ca} = 30.77 \text{ mV} \log \frac{[Ca^{2+}]_o}{[Ca^{2+}]_i}$$

Therefore, to calculate the equilibrium potential for a certain type of ion at body temperature, all we need to know is the ionic concentrations on either side of the membrane. For instance, in the example we used in Figure 3.12, we stipulated that K^+ was twentyfold more concentrated inside the cell:

If $\frac{[K^+]_o}{[K^+]} = \frac{1}{20}$

and $\log \frac{1}{20} = -1.3$

then $E_K = 61.54 \text{ mV} \times -1.3$
$= -80 \text{ mV}.$

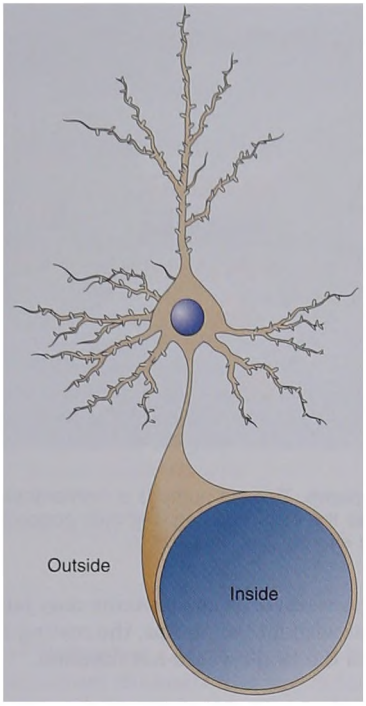
Notice that there is no term in the Nernst equation for permeability or ionic conductance. Thus, calculating the value of E_{ion} does not require knowledge of the selectivity or the permeability of the membrane for the ion. There is an equilibrium potential for each ion in the intracellular and extracellular fluid. E_{ion} is the membrane potential that would just balance the ion's concentration gradient, so that no net ionic current would flow if the membrane were permeable to that ion.

ation the charge of the ion, the temperature, and the ratio of the external and internal ion concentrations. Using the Nernst equation, we can calculate the value of the equilibrium potential for any ion. For example, if K^+ is concentrated twentyfold on the inside of a cell, the Nernst equation tells us that $E_K = -80$ mV (Box 3.2).

The Distribution of Ions Across the Membrane

It should now be clear that the neuronal membrane potential depends on the ionic concentrations on both sides of the membrane. Approximate values for these concentrations appear in Figure 3.15. The important point is that *K^+ is more concentrated on the inside, and Na^+ and Ca^{2+} are more concentrated on the outside.*

How do these concentration gradients arise? Ionic concentration gradients are established by the actions of ion pumps in the neuronal membrane. Two ion pumps are especially important in cellular neurophysiology: the sodium-potassium pump and the calcium pump. The



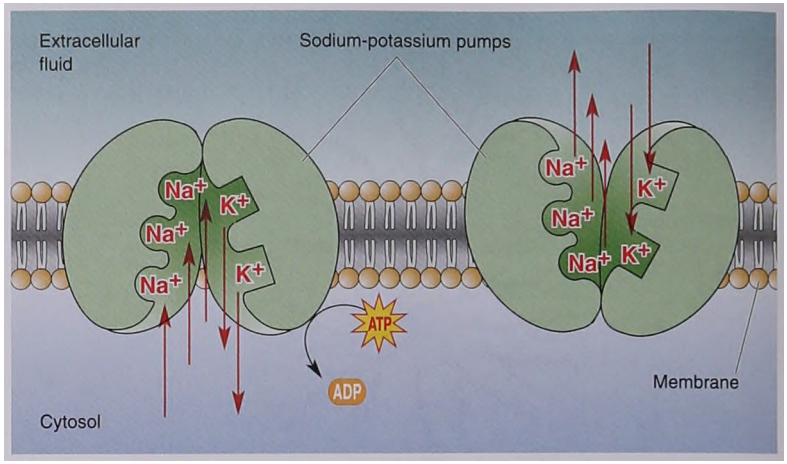
Ion	Concentration outside (in mM)	Concentration inside (in mM)	Ratio Out : In	E_{ion} (at 37∞C)
K^+	5	100	1 : 20	−80 mV
Na^+	150	15	10 : 1	62 mV
Ca^{2+}	2	0.0002	10,000 : 1	123 mV
Cl^-	150	13	11.5 : 1	−65 mV

▲ FIGURE 3.15
Approximate ion concentrations on either side of a neuronal membrane. E_{ion} is the membrane potential that would be achieved (at body temperature) if the membrane were selectively permeable to that ion.

sodium-potassium pump is an enzyme that breaks down ATP in the presence of internal Na^+. The chemical energy released by this reaction drives the pump, which exchanges internal Na^+ for external K^+. The actions of this pump ensure that K^+ is concentrated inside the neuron and that Na^+ is concentrated outside. Notice that the pump pushes these ions across the membrane against their concentration gradients (Figure 3.16). This work requires the expenditure of metabolic energy. Indeed, it has been estimated that the sodium-potassium pump expends as much as 70% of the total amount of ATP utilized by the brain.

The **calcium pump** is also an enzyme that actively transports Ca^{2+} out of the cytosol across the cell membrane. Additional mechanisms decrease intracellular $[Ca^{2+}]$ to a very low level (0.0002 mM); these include intracellular calcium-binding proteins and organelles, such as mitochondria and types of endoplasmic reticulum, which sequester cytosolic calcium ions.

Ion pumps are the unsung heroes of cellular neurophysiology. They work in the background to ensure that the ionic concentration gradients



▲ FIGURE 3.16
The sodium-potassium pump. This ion pump is a membrane-associated protein that transports ions across the membrane against their concentration gradients at the expense of metabolic energy.

are established and maintained. These proteins may lack the glamour of a gated ion channel, but without ion pumps, the resting membrane potential would not exist and the brain would not function.

Relative Ion Permeabilities of the Membrane at Rest

The pumps establish ionic concentration gradients across the neuronal membrane. With knowledge of these ionic concentrations, we can use the Nernst equation to calculate equilibrium potentials for the different ions (see Figure 3.15). Remember, though, that an equilibrium potential for an ion is the membrane potential that would result if a membrane were *selectively permeable* to that ion alone. In reality, however, neurons are not permeable to only a single type of ion. How does that affect our understanding?

Let's consider a few scenarios involving K^+ and Na^+. If the membrane of a neuron were permeable only to K^+, the membrane potential would equal E_K, which, according to Figure 3.15, is −80 mV. On the other hand, if the membrane of a neuron were permeable only to Na^+, the membrane potential would equal E_{Na}, 62 mV. If the membrane were equally permeable to K^+ and Na^+, however, the resulting membrane potential would be some average of E_{Na} and E_K. What if the membrane were 40 times more permeable to K^+ than it is to Na^+? The membrane potential again would be between E_{Na} and E_K but much closer to E_K than to E_{Na}. This approximates the situation in real neurons. The actual resting membrane potential of −65 mV approaches, but does not reach, the potassium equilibrium potential of −80 mV. This difference arises because, although the membrane at rest is highly permeable to K^+, there is also a steady leak of Na^+ into the cell.

The resting membrane potential can be calculated using the **Goldman equation**, a mathematical formula that takes into consideration the relative permeability of the membrane to different ions. If we concern ourselves only with K^+ and Na^+, use the ionic concentrations in Figure 3.15, and assume that the resting membrane permeability to K^+ is fortyfold greater than it is to Na^+, then the Goldman equation predicts a resting membrane potential of −65 mV, the observed value (Box 3.3).

BOX 3.3 BRAIN FOOD

The Goldman Equation

If the membrane of a real neuron were permeable only to K^+, the resting membrane potential would equal E_K, about −80 mV. But it does not; the measured resting membrane potential of a typical neuron is about −65 mV. This discrepancy is explained because real neurons at rest are not exclusively permeable to K^+; there is also some Na^+ permeability. Stated another way, the *relative permeability* of the resting neuronal membrane is quite high to K^+ and low to Na^+. If the relative permeabilities are known, it is possible to calculate the membrane potential at equilibrium by using the Goldman equation. Thus, for a membrane permeable only to Na^+ and K^+ at 37° C:

$$V_m = 61.54 \text{ mV} \log \frac{P_K[K^+]_o + P_{Na}[Na^+]_o}{P_K[K^+]_i + P_{Na}[Na^+]_i}$$

where V_m is the membrane potential, P_K and P_{Na} are the relative permeabilities to K^+ and Na^+, respectively, and the other terms are the same as for the Nernst equation.

If the resting membrane ion permeability to K^+ is 40 times greater than it is to Na^+, then solving the Goldman equation using the concentrations in Figure 3.15 yields:

$$V_m = 61.54 \text{ mV} \log \frac{40\,(5) + 1\,(150)}{40\,(100) + 1\,(15)}$$

$$= 61.54 \text{ mV} \log \frac{350}{4015}$$

$$= -65 \text{ mV}$$

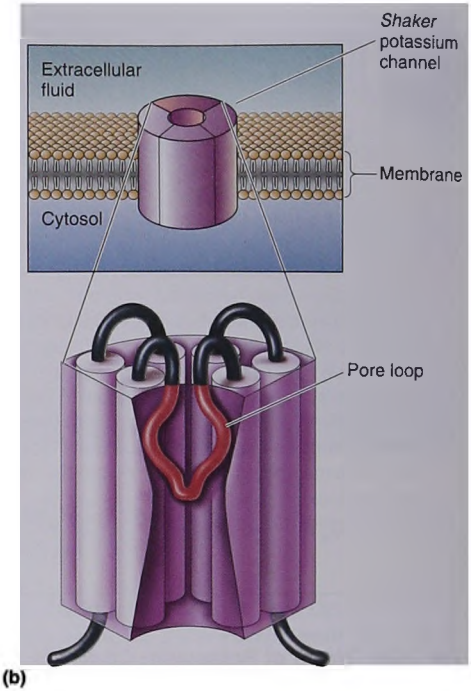
The Wide World of Potassium Channels. As we have seen, the selective permeability of potassium channels is a key determinant of the resting membrane potential and therefore of neuronal function. What is the molecular basis for this ionic selectivity? Selectivity for K^+ ions derives from the arrangement of amino acid residues that line the pore regions of the channels. It was a major breakthrough in 1987 when researchers succeeded in determining the amino acid sequences of a family of potassium channels in the fruit fly *Drosophila melanogaster*. While these insects may be annoying in the kitchen, they are extremely valuable in the lab because their genes can be studied and manipulated in ways that are not possible in mammals.

Normal flies, like humans, can be put to sleep with ether vapors. While conducting research on anesthetized insects, investigators discovered that flies of one mutant strain responded to the ether by shaking their legs, wings, and abdomen. This strain of fly was designated *Shaker*. Detailed studies soon explained the odd behavior by a defect in a particular type of potassium channel (Figure 3.17a). Using molecular biological techniques, it was possible to map the gene that was mutated in *Shaker*. Knowledge of the DNA sequence of what is now called the *Shaker* potassium channel enabled researchers to find the genes for other potassium channels based on sequence similarity. This analysis has revealed the existence of a very large number of different potassium channels, including those responsible for maintaining the resting membrane potential in neurons.

Most potassium channels have four subunits that are arranged like the staves of a barrel to form a pore (Figure 3.17b). Despite their diversity, the subunits of different potassium channels have common structural features that bestow selectivity for K^+. Of particular interest is a region called the *pore loop*, which contributes to the *selectivity filter* that makes the channel permeable mostly to K^+ (Figure 3.18).

In addition to flies, the deadly scorpion also made an important contribution to the discovery of the pore loop as the selectivity filter. In 1988, Brandeis University biologist Chris Miller and his student Roderick MacKinnon observed that scorpion toxin blocks potassium channels

(a)

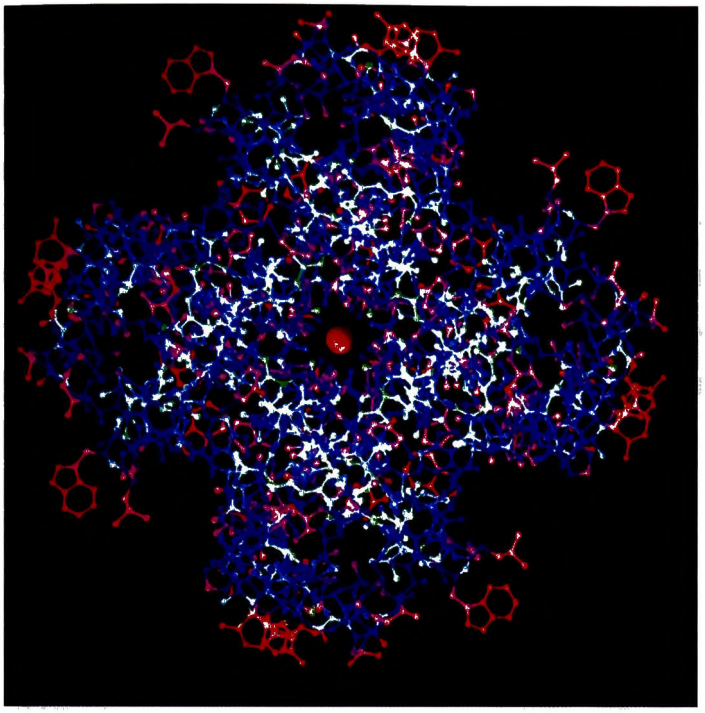


(b)

▲ FIGURE 3.17
The structure of a potassium channel. **(a)** *Shaker* potassium channels in the cell membrane of the fruit fly *Drosophila*, viewed from above with an electron microscope. (Source: Li et al., 1994; Fig. 2.) **(b)** The *Shaker* potassium channel has four subunits arranged like staves of a barrel to form a pore. Enlargement: The tertiary structure of the protein subunit contains a pore loop, a part of the polypeptide chain that makes a hairpin turn within the plane of the membrane. The pore loop is a critical part of the filter that makes the channel selectively permeable to K^+.

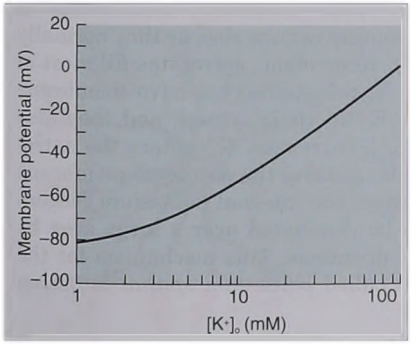
(and poisons its victims) by binding tightly to a site within the channel pore. They used the toxin to identify the precise stretch of amino acids that forms the inside walls and selectivity filter of the channel (Box 3.4). MacKinnon went on to solve the three-dimensional atomic structure of a potassium channel. This accomplishment revealed, at long last, the physical basis of ion selectivity and earned MacKinnon the 2003 Nobel Prize in Chemistry. It is now understood that mutations involving only a single amino acid in this region can severely disrupt neuronal function.

An example of this is seen in a strain of mice called *Weaver*. These animals have difficulty maintaining posture and moving normally. The defect has been traced to the mutation of a single amino acid in the pore loop of a potassium channel found in specific neurons of the cerebellum, a region of the brain important for motor coordination. As a consequence of the mutation, Na^+ as well as K^+ can pass through the channel. Increased sodium permeability causes the membrane potential of the neurons to become less negative, thus disrupting neuronal function. (Indeed, the absence of the normal negative membrane potential in these cells is believed to be the cause of their untimely death.) In recent years, it has become increasingly clear that many inherited neurological disorders in humans, such as certain forms of epilepsy, are explained by mutations of specific potassium channels.



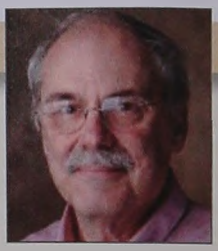
▲ FIGURE 3.18
A view of the potassium channel pore. The atomic structure of potassium-selective ion channels has recently been solved. Here we are looking into the pore from the outside in a three-dimensional model of the atomic structure. The red ball in the middle is a K^+. (Source: Doyle et al., 1998.)

The Importance of Regulating the External Potassium Concentration. Because the neuronal membrane at rest is mostly permeable to K^+, the membrane potential is close to E_K. Another consequence of high K^+ permeability is that the membrane potential is particularly sensitive to changes in the concentration of extracellular potassium. This relationship is shown in Figure 3.19. A tenfold change in the K^+ concentration outside the cell, $[K^+]_o$, from 5 to 50 mM, would change the membrane potential from −65 to −17 mV. A change in membrane potential from the normal resting value (−65 mV) to a less negative value is called a **depolarization** of the membrane. Therefore, *increasing extracellular potassium depolarizes neurons.*



◀ FIGURE 3.19
The dependence of membrane potential on external potassium concentration. Because the neuronal membrane at rest is mostly permeable to potassium, a tenfold change in $[K^+]_o$, from 5 to 50 mM, causes a 48 mV depolarization of the membrane. This function was calculated using the Goldman equation (see Box 3.3).

BOX 3.4 PATH OF DISCOVERY



Feeling Around Inside Ion Channels in the Dark

by Chris Miller

For me, the practice of scientific discovery has always been tightly linked to play. The self-indulgent pleasure of just fiddling around with a problem is what motivated the early stages of every research project I've ever engaged in. Only later comes the intense itch scratching, scholarship, and sweat needed to attack—and sometimes solve—the puzzles presented by nature. The sandbox I've been playing in for the past 40 years contains what are to me the most fascinating of toys: ion channels, the membrane-spanning proteins that literally *make* the electrical signals of neurons, breathing life into the nervous system. To the extent that the brain is a computer—an inaccurate but evocative analogy—the ion channels are the transistors. In response to biological dictates, these tiny proteinaceous pores form diffusion pathways for ions such as Na^+, K^+, Ca^{2+}, H^+, and Cl^-, which carry electrical charge across membranes, thereby generating, propagating, and regulating cell voltage signals. I fell in love with these proteins long ago when I accidentally stumbled upon an unexpected K^+ channel in experiments initially aimed at capturing a completely different sort of beast, a Ca^{2+}-activated enzyme, and over the years that love has only deepened as I've wandered around in a teeming electrophysiological zoo housing many species of ion channel proteins.

An undergraduate background in physics and subsequent experience as a high school math teacher delivered me in the 1970s to graduate school, post-doctoral training, and my own lab at Brandeis with no formal preparation in (and precious little knowledge of) neurobiology or electrophysiology. Picking up bits and pieces of these subjects from reading the literature and osmosing them from my surroundings, I became increasingly fascinated by how ion channels, at that time only just nailed down as proteins, could do their job of producing bioelectricity; in parallel, I grew increasingly horrified by what struck me as the overwhelming complexity of living cells and the ambiguity in molecular interpretation that would inevitably accompany experiments done exclusively on cellular membranes. This combination of fascination and horror provoked my attraction to simplified "artificial membranes" of defined composition, developed by Paul Mueller in the 1960s, with which to follow the electrical activities of ion channels isolated from their complex cellular homes. I worked out a method for inserting single channel molecules from excitable cells into these chemically controllable membranes and used it to record single K^+ channels at a time when card-carrying neurobiologists were beginning to observe single channels in native excitable membranes with the then-new cellular patch-recording methods. I confess that my early technique-building experiments were just play. To watch and control individual protein molecules dancing electrically before my eyes in real time was—and still is—an indescribable thrill, regardless of the particular tasks the channels carry out for the cell.

The sensitivity of the membrane potential to $[K^+]_o$ has led to the evolution of mechanisms that tightly regulate extracellular potassium concentrations in the brain. One of these is the **blood-brain barrier**, a specialization of the walls of brain capillaries that limits the movement of potassium (and other bloodborne substances) into the extracellular fluid of the brain.

Glia, particularly astrocytes, also possess efficient mechanisms to take up extracellular K^+ whenever concentrations rise, as they normally do during periods of neural activity. Remember, astrocytes fill most of the space between neurons in the brain. Astrocytes have membrane potassium pumps that concentrate K^+ in their cytosol, and they also have potassium channels. When $[K^+]_o$ increases, K^+ enters the astrocyte through the potassium channels, causing the astrocyte membrane to depolarize. The entry of K^+ increases the internal potassium concentration, $[K^+]_i$, which is believed to be dissipated over a large area by the extensive network of astrocytic processes. This mechanism for the regulation of $[K^+]_o$ by astrocytes is called *potassium spatial buffering* (Figure 3.20).

Eventually, this play led me to compelling problems that could be advantageously attacked with this reductionist approach. By the mid-1980s, my lab was home to a collection of supremely talented post-docs—Gary Yellen, Rod MacKinnon, and Jacques Neyton among them—going after the remarkable ion selectivity of various K^+ channels: How do they tell the difference between ions as similar as K^+ and Na^+, as they must do if neurons are to fire action potentials, and if we are to think, feel, and act? Having stumbled, while purposelessly fooling around with natural neurotoxins, on a scorpion venom peptide that blocks K^+ channels, we used the power of single-channel analysis to show that this toxin works by plugging up the protein's K^+-selective pore, just like a cork in a bottle (Figure A). In 1988, Rod took our toxin peptide to a Cold Spring Harbor laboratory course he'd signed up for to learn how to express ion channels by recombinant DNA methods. There he made a key discovery: that the toxin also blocks *Shaker*, the first genetically manipulable K^+ channel, cloned the previous year in the lab of Lily and Yuh-Nung Jan. This chance finding led us, by making specific mutations, to a localized region in the channel's amino acid sequence that forms the outer entryway of the K^+ selective pore, a result immediately applicable to the entire family of voltage-dependent K^+, Na^+, and Ca^{2+} channels. A few years later, Rod and Gary, as newly hatched independent investigators, collaboratively homed in on these pore sequences to find the ion-selectivity hot spots, a result that propelled Rod, 7 years later, to bag the first X-ray crystal structure of a K^+ channel and to begin a whole new "structural era" in ion channel studies.

Looking back at my wrestling matches with ion channels, it is clear that the greatest joy I've derived from this endeavor has arisen from seeing—and being surprised by—new and unexpected elements of beauty and coherence in the natural world. This feeling was described by the great theoretical physicist Richard Feynman who, in a riposte to a W.H. Auden poem that dismisses scientific motivation as merely utilitarian, asserted that research scientists, like poets, are driven mainly by aesthetic forces: "We want knowledge so we can love Nature more."

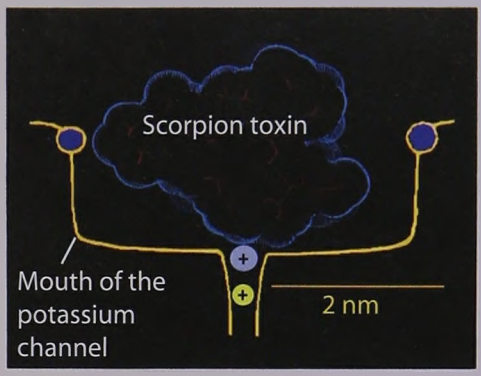
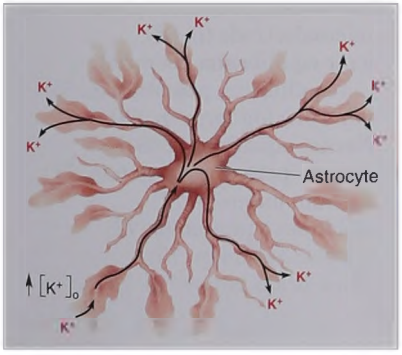


Figure A
The extracellular opening of a K^+ channel with bound scorpion toxin envisioned indirectly in the "pre-structural" days by probing the channel with the toxin of known structure. Points of interaction: site on channel that makes contact with toxin (dark blue circles), key lysine residue on toxin that intrudes into the narrow pore (pale blue circle with +), a K^+ displaced downward into the pore by binding of toxin (yellow circle with +). The yellow scale bar represents 2 nm. (Source: Adapted from Goldstein et al. 1994. *Neuron* 12:1377–1388.)

It is important to recognize that not all excitable cells are protected from increases in potassium. Muscle cells, for example, do not have equivalents to the blood-brain barrier or glial buffering mechanisms. Consequently, although the brain is relatively protected, elevations of $[K^+]$ in the blood can still have serious consequences on body physiology (Box 3.5).



◀ FIGURE 3.20
Potassium spatial buffering by astrocytes. When brain $[K^+]_o$ increases as a result of local neural activity, K^+ enters astrocytes via membrane channels. The extensive network of astrocytic processes helps dissipate the K^+ over a large area.

BOX 3.5 OF SPECIAL INTEREST

Death by Lethal Injection

On June 4, 1990, Dr. Jack Kevorkian shocked the medical profession by assisting in the suicide of Janet Adkins. Adkins, a 54-year-old, happily married mother of three, had been diagnosed with Alzheimer's disease, a progressive brain disorder that always results in senile dementia and death. Mrs. Adkins had been a member of the Hemlock Society, which advocates euthanasia as an alternative to death by terminal illness. Dr. Kevorkian agreed to help Mrs. Adkins take her own life. In the back of a 1968 Volkswagen van at a campsite in Oakland County, Michigan, she was hooked to an intravenous line that infused a harmless saline solution. To choose death, Mrs. Adkins switched the solution to one that contained an anesthetic solution, followed automatically by potassium chloride. The anesthetic caused Mrs. Adkins to become unconscious by suppressing the activity of neurons in part of the brain called the *reticular formation*. Cardiac arrest and death were then caused by the KCl injection. The ionic basis of the resting membrane potential explains why the heart stopped beating.

Recall that the proper functioning of excitable cells (including those of cardiac muscle) requires that their membranes be maintained at the appropriate resting potential whenever they are not generating impulses. The negative resting potential is a result of selective ionic permeability to K^+ and to the metabolic pumps that concentrate potassium inside the cell. However, as Figure 3.19 shows, membrane potential is very sensitive to changes in the extracellular concentration of potassium. A tenfold rise in extracellular K^+ would severely diminish the resting potential. Although neurons in the brain are somewhat protected from large changes in $[K^+]_o$, other excitable cells in the body, such as muscle cells, are not. Without negative resting potentials, cardiac muscle cells can no longer generate the impulses that lead to contraction, and the heart immediately stops beating. Intravenous potassium chloride is, therefore, a lethal injection.

CONCLUDING REMARKS

We have now explored the resting membrane potential. The activity of the sodium-potassium pump produces and maintains a large K^+ concentration gradient across the membrane. The neuronal membrane at rest is highly permeable to K^+, owing to the presence of membrane potassium channels. The movement of K^+ ions across the membrane, down their concentration gradient, leaves the inside of the neuronal membrane negatively charged.

The electrical potential difference across the membrane can be thought of as a battery whose charge is maintained by the work of the ion pumps. In the next chapter, we see how this battery runs our brain.

KEY TERMS

Introduction
action potential (p. 57)
excitable membrane (p. 57)
resting membrane potential (p. 57)

The Cast of Chemicals
ion (p. 58)
cation (p. 59)
anion (p. 59)
phospholipid bilayer (p. 59)
peptide bond (p. 60)
polypeptide (p. 60)
ion channel (p. 63)
ion selectivity (p. 63)
gating (p. 63)
ion pump (p. 63)

The Movement of Ions
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concentration gradient (p. 64)
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electrical potential (p. 65)
voltage (p. 65)
electrical conductance (p. 65)
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Ohm's law (p. 65)

The Ionic Basis of the Resting Membrane Potential
membrane potential (p. 66)
microelectrode (p. 66)
ionic equilibrium potential (equilibrium potential) (p. 68)
ionic driving force (p. 68)
Nernst equation (p. 69)
sodium-potassium pump (p. 71)
calcium pump (p. 71)
Goldman equation (p. 72)
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blood-brain barrier (p. 76)