CHAPTER 3

PROPERTIES OF EXCITABLE MEMBRANES: THE MEMBRANE POTENTIAL

In attempting to account for how the nervous system produces behavior, we should first turn our attention to what a single neuron does. Then, having the properties of single neurons in mind, we can proceed to put neurons together, studying their properties in networks. Actually, the general principles described here apply to all kinds of excitable cells, neurons included, but the details vary from kind to kind.

Membrane structure.

Neurons, like most living cells, have the full complement of intracellular organelles—nucleus, endoplasmic reticulum, Golgi apparatus—but it is the membrane of neurons that has received the most attention in scientific investigations. Other parts of nerve cells are beginning to occupy more prominent positions in ideas about nerve function (e.g., see Chapter 18 on trophic functions), yet the rapid events typical of nerve cells are membrane events.

Like all cells, neurons are bounded by a membrane, the plasma membrane or plasmalemma. It is in many ways like the membranes of all animal cells, being composed of lipids (40%; phospholipids, cholesterol, and glycolipids) and proteins (60%). The lipids of membranes are usually amphipathic, i.e., their molecules have both polar and nonpolar regions. The polar regions are hydrophilic, meaning that in water the lipids orient themselves on the surface so that the polar ends are in water and the nonpolar ends project into the air as shown in Figure 3-1. Because the cell membrane separates two aqueous fluids, the extracellular fluid and the intracellular fluid or cytoplasm, the lipids of the membrane align themselves in two parallel rows, with polar ends toward the aqueous fluids and nonpolar ends facing the nonpolar ends of molecules in the other row, as shown in Figure 3-2. This arrangement, called a lipid bilayer or bimolecular leaflet, forms the skeleton of the membrane, and is approximately 5-10 nm (1 nm = 10^-9 meters) thick. Electron microscopic views of membranes showed them to be uniformly trilaminar in appearance with two dark stripes.
Figure 3-3. Fluid mosaic model of membrane.
Lipid bilayer forms a fluid sea in which protein molecules “float” either on the surface, as extrinsic or structural proteins, or with the fluid, as intrinsic proteins. Channels are thought to be intrinsic proteins or aggregates of such proteins, in contact with both intra- and extracellular fluids, surrounding a water-filled pore through which ions pass. Voltage-gated channels open when the transmembrane potential changes, and chemically gated channels open when they are contacted by a transmitter molecule, indicated here as acetylcholine. (de Robertis E: Science 171:963-971, 1971)

Proteins that are more hydrophobic may extend across much of the bilayer; these are the intrinsic proteins. When the proteins have hydrophilic ends and large hydrophobic regions, they may extend completely through the lipid bilayer and jut out into the adjacent fluids (Fig. 3-3).

The lipid bilayer is considered to be a fluid in or on which the proteins float. Many of the proteins appear to have relatively free movement; others appear to be firmly attached to a substructure and therefore do not move. Certainly, the polarization of neurons, with axons and dendrites having distinct, relatively fixed functions and properties, suggests that some of the proteins must be fixed. On the other hand, the apparent migration of acetylcholine receptors (binding sites for the transmitter substance) away from a previous endplate region (point of synaptic contact with motoneurons) in denervated muscle and their reaggregation upon reinnervation suggests that the mobility of some proteins within the membrane may be limited by forces external to the cell.

What distinguishes one membrane from another is the nature of the proteins in the membrane. In nerve, there appear to be five functional types of membrane proteins. These are: structural elements, enzymes, receptors, channels or pores, and pumps. The structural proteins may be involved in holding cells together at junctions, stabilizing other proteins within the membrane, or maintaining some aspects of subcellular structure. Enzyme proteins facilitate chemical reactions, and presumably those located in or on the membrane are involved in chemical reactions involved in membrane function. The membrane of the cell must be able to recognize particular molecules (e.g., transmitter substances) and bind selectively with those molecules. Receptor proteins provide binding sites, some of high
affinity and selectivity. Charged molecules do not pass readily through lipid media; thus, the membrane must contain specific channels through which ions can move to enter or leave the cell. These channels are provided by certain protein molecules or groups of molecules. As we shall see shortly, the concentrations of different ions are not the same inside and outside the cell; yet the membrane is leaky to these ions. In order to maintain these differing ion concentrations that are fundamental to neuronal function, pumps expend energy to expel certain ions from the cell and bring other ions into the cell. The pump proteins are intimately involved in this process.

It is not necessary that a given protein molecule serves only one of the functions above. In fact, at the neuromuscular junction the same protein is thought to act as a receptor for acetylcholine and a channel for both Na$^+$ and K$^+$ ions. We shall have more to say about channels and pumps later.

Membrane permeability.

The nerve membrane is permeable to a large number of compounds and ions. In general, lipid-soluble substances move through the membrane more rapidly than lipid-insoluble substances, and smaller molecules move through more rapidly than larger ones. Medium and large molecules move through the membrane only if they are lipid-soluble, but small molecules move through whether they are lipid-soluble or not. The existence of pores or channels through the membrane was postulated to explain how lipid-insoluble substances could diffuse through a lipid membrane in which they cannot dissolve. No one has yet seen a pore under the microscope, but it seems likely that the pores are tortuous pathways through protein molecules or between them. It appears that there is a spectrum of pore diameters and permeabilities, some admitting most ions less than a certain maximum size and others selectively permitting only particular ions to pass through. Divalent ions usually pass through less readily than monovalent ions. For some cells, the permeability of the membrane to monovalent ions is

Cs$^+$ > Rb$^+$ > K$^+$ > Na$^+$ > Li$^+$ and F$^-$ > Br$^-$ > Cl$^-$ > F$^-$

for other cells, the sequence is different. These sequences are not determined strictly by molecular weight or diameter; Na$^+$ ions are 30% smaller in diameter than K$^+$, yet the membrane of most cells is 20-30 times more permeable to K$^+$. This surprising result is explained in part by the tendency of ions to be hydrated. There is an attractive force between the positive charge of the ion and the negative end of the water dipole, so that water molecules accumulate around the ion, forming a shell. The number of water molecules attracted to an ion is a function of the ion's charge density. Both sodium and potassium have the same charge, but because sodium is smaller it has a larger charge density. That means sodium attracts more water molecules. Therefore, the radius of the hydration shell for sodium is larger than that for potassium; it has an effectively larger diameter. As we shall see, the two ions share some channels and also have separate channels in the membrane, and the ease with which they pass through the channels is a function of interactions with parts of the channel structure and the positions of water molecules within the channel.
Electrical properties of membranes.

The nerve cell membrane behaves like a simple electrical circuit containing resistance and capacitance. To understand its behavior it is important to review the behavior of electrical circuits. First, let's recall a few definitions. A voltage or potential difference is a separation of unlike charge in space; the greater the amount of charge separated, the larger the voltage, \( V \), and the greater the tendency for the charges to flow toward each other. Voltage is always measured at one point with respect to another point. There cannot be a voltage at one point in space. The unit of voltage is a volt, \( V \). A flow of electrical charges is a current, \( i \), measured in coulombs/sec or amperes, \( A \). Resistance is a measure of the difficulty with which current flows in a circuit; the greater the difficulty, the greater the resistance, \( R \). The unit of resistance is the ohm, \( \Omega \). The reciprocal of the resistance is called the conductance, \( g \), a measure of the ease with which a current flows in a circuit. The unit of conductance is the siemen (the older term was mho).

Current, voltage, and resistance are related to each other by Ohm's law: \( i = V/R \). This relationship for simple resistive circuits says that for a given resistance, the current increases linearly with the voltage or, alternatively, that the current driven by a particular voltage depends upon the resistance. For those not familiar with electrical concepts, a useful analogy might be carrying rocks up a hill. Current would then be like the number of rocks you could carry per trip, resistance like the steepness of the hill, and voltage like the amount of energy you have available to use in carrying the rocks. Clearly, the less steep the hill (the smaller the resistance) and the higher your energy level (the greater the voltage), the greater will be the number of rocks you can carry up the hill per trip. Of course, Ohm's law can be rewritten \( i = gV \), where \( g = 1/R \). In this form of the equation, current is a direct function of both the voltage and the conductance. We will have occasion to use this form later.

Consider the simple circuit in Figure 3-4. The circuit contains an active element, a voltage source, \( V \), indicated by the battery, and a passive element, the resistance, \( R \). Think of the voltage source as a supplier of current or energy. Likewise, think of the resistance as a load or consumer of energy. Kirchhoff's law states that current flows only in complete or closed circuits. In this case, it may help to think of water flowing through a pipe. If there are no holes, all the water that flows in one end must flow out the other end. The circuit shown is complete. Because we have a potential difference and a resistance, we know there will be current \( (i) \) in the circuit that can be measured by an ammeter, \( I \).

![Figure 3-4. A simple resistance circuit. A battery, \( V \), is used to apply a voltage of 10 V across a resistance, \( R \), of 10 ohms. The voltage and current in the circuit are measured using a voltmeter, \( Mv \), in parallel with \( R \) and an ammeter, \( Mi \), in series with \( R \). In this case, the voltmeter reads 10 V, with polarity as indicated by +,- signs, and the ammeter reads 1 A.](image-url)
That current can be calculated from Ohm’s law as follows:

Let $V = 10 \text{ V}$
And let $R = 10 \Omega$
Then, $i = \frac{V}{R} = 1 \text{ A}$.

The current flowing through the resistor produces a voltage drop (a difference in electrical potential) across the resistor that can be measured by the meter, M, a voltmeter. We can assume that the meter is perfect (real meters seldom are), i.e., no current flows through it, and it does not influence the operation of the circuit; thus, the entire voltage, $V$, is imposed across $R$. The measured voltage drop across the resistor, $R$, will be $10 \text{ V}$ with the polarity of the voltage as shown. The wires of the circuit have a very low resistance, so there will be essentially no voltage drop due to the wires (from Ohm’s law again, $V = iR$; if $R = 0$, then $V = 0$), and the potential will be the same at the upper ends of the resistor and the battery. The same argument applies to the lower ends.

The direction of current flow in the external circuit, that part of the circuit outside the battery, is taken, by convention, to be the direction a positive particle would move (single, semi-circular, counter-clockwise arrow). Actually, current in this type of circuit is carried by electrons, but the direction of current flow is opposite to their movement, i.e., from + to -. In order to complete the circuit, current flows from - to + within the battery (the internal circuit; wide, downward arrow). We will see that most charge carriers for membrane events are positively charged ions, or cations; currents, by convention, will flow the way they move. Bear in mind though, that when an anion, or negatively charged ion, moves, the current is said to be in the direction opposite its motion. This fact frequently gives students problems when considering chloride ions, $\text{Cl}^{-}$.

Also note that when the current enters a resistor (the bottom in Figure 3–4), that end of the resistor becomes positive with respect to the other end. As we will see later, current passing outward through a membrane at rest will make the membrane more positive on the inside with respect to the outside. The reverse is true for inwardly directed currents.

This observation holds for current flow through all passive elements like resistors and “resting” cell membranes. Refer back to the figure to see that current in the source or active element (the battery in this case) flows from negative to positive (again, the wide downward arrow). Therefore, an inward current through active membrane will make the membrane more positive inside with respect to outside, i.e., it will hypopolarize it. We will soon see that an inward sodium current hypopolarizes the active nerve or muscle membrane, whereas an outward current in resting membrane (passive) hypopolarizes it.

A capacitor is a device that is capable of separating and storing charge. Usually a capacitor is made of two parallel conducting plates separated by a nonconductor or dielectric material. The capacitance of a capacitor is symbolized by $C$ and related to voltage by $C = \frac{Q}{V}$, where $Q$ is the charge. The unit of capacitance is the farad. The larger the capacitance, the larger is the amount of charge that must be added to the plates to

\[ \text{It might be good to commit to memory the phrase: “Outward currents hypopolarize passive membrane; inward currents hypopolarize active membrane.”} \]
Figure 3-5. A simple resistance-capacitance circuit. The same circuit as in Figure 3-4, but with a capacitor, \( C \), placed in parallel with the resistance, \( R \). The voltages across the resistance and the capacitance are the same as the battery voltage, \( V \), and the same as read by the voltmeter, \( M_v \). The polarity of the voltages is as indicated by +,- signs.

bring the voltage between them to a given voltage. Looked at in another way, the larger the capacitance, the larger will be the amount of charge stored on the plates for a given voltage between them. We speak of increasing the charge stored on a capacitor as "charging the capacitor" and decreasing the charge stored as "discharging the capacitor."

Introduction of a capacitor, as in Figure 3-5, changes the behavior of the circuit. No electrons actually flow between the plates of a capacitor because of the dielectric material between them, but when the battery is connected in the circuit, positive charges build up on the upper plate and are drawn off of the lower plate, producing a voltage between the plates. The charge flowing onto or off of a plate is a current, the capacitor current, \( i_c \). This current is related to capacitance and voltage by the equation:

\[
   i_c = C \frac{dV}{dt},
\]

where \( dV/dt \) is the rate of change of voltage between the plates. Before the battery is hooked up, the voltage is zero and unchanging; therefore, \( dV/dt = 0 \) and \( i_c = 0 \). When the battery is hooked up, the plates start to charge and continue until the voltage between the plates equals \( V \), the battery voltage. At this time, \( dV/dt \) again becomes zero and \( i_c = 0 \). Thus, there will only be a capacitative current when the voltage is actually changing.

If there were no resistance in the circuit of Figure 3-5, the battery could be disconnected and the voltage between the plates of the capacitor would remain at \( V \) (at least for a perfect capacitor). The resistor provides a current pathway through which the capacitor can discharge, with current flowing from the positive plate onto the negative plate. The larger \( R \) is the less current will flow per unit of time and the less rapidly the capacitor voltage will fall. In fact, the time for discharge varies directly with both \( R \) and \( C \).

The time required for the voltage to attain \( (1-1/e) \Delta V \) or about \( 0.63 \Delta V \) (\( \Delta V \) is the total voltage change) is called the time constant, \( \tau \), and is given by the equation \( \tau = R \times C \). If \( R \) is expressed in ohms and \( C \) in farads, then \( \tau \) will be expressed in seconds. In circuits, such as
that in Figure 3-5, without rectification\(^2\), the charging and discharging time constants are equal. As we shall see, cell membranes have rectifying properties which make them unequal.

In electrical circuits, resistances in series simply add. Thus, \( R \) in Figure 3-4 can be replaced by two resistors of value \( R/2 \) in series (i.e., the output of one is the input of the other) without changing the properties of the circuit. However, parallel resistances, those that share the same input and combine their outputs, are another matter. Consider the parallel resistive circuit in Figure 3-6. The potential difference across both \( R_1 \) and \( R_2 \) will be the same, namely \( V \). Thus, the current through \( R_1 \), will be

\[
i_{R_1} = \frac{V}{R_1}
\]

and that through \( R_2 \) will be

\[
i_{R_2} = \frac{V}{R_2}
\]

The total current will be as follows:

\[
i = \frac{V}{R_{\text{total}}} = i_{R_1} + i_{R_2} = \frac{V}{R_1} + \frac{V}{R_2}
\]

\(^2\) Here rectification is used in the engineer’s sense of a lower resistance to current flow in one direction than the other through a circuit element.
Dividing by \( V \) gives:

\[
\frac{1}{R_{\text{total}}} = \frac{1}{R_1} + \frac{1}{R_2}.
\]

Thus, in a parallel resistive circuit, resistances add reciprocally. Capacitors in parallel each store charge; therefore, more charges are stored for the same voltage. In parallel, \( C_{\text{total}} = C_1 + C_2 \). When capacitors are in series, each sees a voltage that is smaller and thus stores fewer charges. In series:

\[
\frac{1}{C_{\text{total}}} = \frac{1}{C_1} + \frac{1}{C_2}.
\]

Two additional circuits are worth considering before beginning our discussion of membrane events. These are shown in Figure 3-7. Note that the batteries in A are both driving current in the same direction; thus the voltage between points \( a \) and \( c \) will simply be the sum of the two battery voltages, 150 V, with point \( a \) being positive with respect to point \( c \). The total resistance of the circuit (the resistors are in series) is 100 \( \Omega \). Therefore, the current in the circuit is 150 V/100 \( \Omega \) or 1.5 A. When 1.5 A flows through 10 \( \Omega \), a potential drop of 1.5 A x 10 \( \Omega \) or 15 V is produced, point \( a \) being positive with respect to point \( b \). The potential difference between points \( b \) and \( d \), the value shown on the meter, will be 100 V - 15 V or 85 V, with point \( b \) positive with respect to point \( d \). Calculation of the voltage using the 90 \( \Omega \) resistor portion of the circuit must yield the same value and can be done as an exercise.

Figure 3-7B is the same as 3-7A except that the values of the resistors are interchanged. The current in the circuit is still 1.5 A, and the potential drop across 10 \( \Omega \) is still 15 V, but this time the meter will read 50 V - 15 V or 35 V, with point \( b \) negative with respect to point \( d \). Again, by symmetry, the calculation for the 90 \( \Omega \) resistor will give the same value. Note that the potential difference between points \( b \) and \( d \), is changed in magnitude and polarity simply by changing the resistance (or conductance), without altering the battery voltages. This observation will become important later when we consider the origin of the action potential.

Electrically, the nerve axon behaves like a very poor conductor of electricity. The axoplasm, the cytoplasm in the axon, has a very high resistance. For the squid axon, the longitudinal resistivity is 30 to 60 \( \Omega \) cm. This is extremely high compared with a copper wire of the same diameter which has a resistivity of only 1.8 x 10\(^{-6}\) \( \Omega \) cm. This difference in resistance comes about because the density of charge carriers is smaller (ions in cytoplasm are less dense than electrons in copper wire) and their mobility is less (ions in solution move much less readily than electrons in copper wire). The voltage drop (loss) along 1 cm of cytoplasm is about 10\(^7\) times greater than that occurring.
along 1 cm of wire. In other words, you wouldn't want to replace the cord on your toaster with the same length of nerve axon.

The membrane of a neuron behaves like a resistor, that is, when current is passed through the membrane, there is a voltage drop that is predictable from Ohm's law. Values of membrane resistance are usually expressed as area specific resistance in $\Omega \text{cm}^2$. (Membrane resistance decreases with increases in membrane area; therefore it is measured in ohms x cm$^2$, whereas membrane capacitance increases with increases in area and is expressed as $\mu\text{F per cm}^2$.) Motoneurons of the lobster (a large neuron in which such measurements are easily made) have a membrane resistance of 2300 $\Omega \text{cm}^2$.

The neural membrane also behaves like a capacitor, that is, it is capable of separating and storing charge. The capacitance of most neural membranes is of the order of 1 $\mu\text{F/cm}^2$, meaning that the membrane can separate and store a charge of $1 \times 10^6$ coulomb/volt of potential across the membrane per cm$^2$. Thus, the membrane can separate charges due to $10^{-12}$ mole of univalent ions/volt (Faraday's constant: 96,516 coulombs/mole of univalent ions). We will see shortly that the membrane does in fact separate considerable charge.

The effect of membrane resistance and capacitance are shown in Figure 3-8. The drawing shows the set-up of the experiments. A pair of micropipette electrodes are inserted through the membrane of the axon. The pipettes are made of 1 to 2 mm glass capillary tubing drawn out into a fine tip (less than 1 $\mu\text{m}$ in diameter) and filled with a conducting solution such as KCl. A fine wire is inserted into each of the pipettes, and one is connected to a stimulator that produces a square current pulse, while the other is connected to the input of an amplifier, A. The other amplifier input (remember: we must measure the voltage between $two$ points in space) contacts the extracellular fluid, and thus the transmembrane voltage is amplified and can be displayed on an oscilloscope, M. When a square current pulse, such as that shown in the upper trace (labeled ‘Injected current’), is passed outward through the membrane from the pipette on the right, the recording system sees a voltage such as that shown in the second trace. The recorded voltage rises more slowly to a maximum than the current does, and then, after the current is turned off, the voltage falls slowly back to its initial value. The greater the distance between the stimulating and recording electrodes (as indicated to the right of each trace), the more slowly the voltage will rise and fall and the smaller will be the maximum value (lower traces in Fig. 3-8). This experiment illustrates why the time constant of the membrane should be measured at the site of current injection; values at other sites will be inaccurate because of the influences of membrane length. Values for $\tau$ for neurons range from 0.5 to 5.0 msec.

It is useful in attempting to understand the properties of the nerve membrane to construct a circuit model as done in Figure 3-9. We know that the membrane has both resistance and capacitance and that the intracellular and extracellular fluids both have a resistance. We can symbolize these as $R_m$, $C_m$, $R_i$, and $R_o$, respectively. The longitudinal resistance, whether inside the axon or out, is inversely related to the area of the pathway over which current can flow. Although the intracellular area is small and bounded by the axis cylinder, the interstitial (extracellular) medium is large. The extracellular resistance is therefore much smaller than the intracellular.

Erratum: Since this was posted online, David Miller has pointed out an error. "The minimum charge separation, assuming a typical capacitance of $1\mu\text{F per cm}^2$, is $10\text{pmole/V}$, not $1\text{pmole as you have there. As I'm sure you know, 1pmole/cm}^2$ will account for a membrane potential of $100\text{mV which is approximately the physiological scale. (With nearly }10^6\text{ coulombs per F (monovalent) we need }10^6/10^6\text{ moles per V=10^{-11} mole per V (monovalent). An annoying power-of-ten slip! I hope this little detail helps." Thanks David.
resistance, and it can be neglected. The figure on the left shows the membrane resistance, $R_m$, in parallel with the membrane capacitance. This arrangement is shown repeated in each adjacent patch of membrane on the right. These patches are linked by the intracellular fluid resistance and the extracellular fluid resistance, the latter treated here as zero. The changes in the membrane voltage are the result of current flow through the membrane only, but to reach each patch of membrane the current must flow through the intracellular fluid resistance, $R_i$. The intracellular resistances are in series so the farther a given patch is from the source of the current the larger will be the effective intracellular resistance and the greater the attenuation of the voltage ($V = iR$).

The distance along the membrane at which the imposed voltage, $V$, has been attenuated to $1/e \times V$ is called the space constant, $\lambda$. Because $e = 2.72$ or about 3, $\lambda$ is the distance along the membrane at which $V$ has fallen to about a of its initial value. The space constant is related to axon resistances as:

$$\lambda = \sqrt{R_m/(R_o + R_i)},$$

where $R_m$ is the transmembrane resistance, and $R_o$ and $R_i$ are external and internal longitudinal resistances, respectively. For most neurons, $\lambda$ is about 2 mm. Thus, if information to be transmitted is expressed as a membrane voltage and this voltage is conducted passively or electrotonically along the neuron, distances over 5 mm would be well outside the conduction ability of the membrane; the voltage would be essentially zero (less than 1/10 V) 5 mm away from its source.

What happens in transatlantic telephone cables is similar, but the space constant there is longer. Still, the telephone signal must be amplified at intervals across the Atlantic Ocean in order for any telephone message sent from New York to be heard in London.

The membrane capacitances shown in Figure 3-9 are in parallel so the farther away from the current source the greater will be the capacitance. The greater the capacitance and resistance of a circuit the greater the time constant and the more slowly voltage changes occur. It is not surprising, in view of these drastic attenuations and distortions over short distances, that neural transmission occurs by a specialized process, the self-regenerative action potential.

**Membrane ionic environment.**

The nerve membrane is capable of storing, transmitting, and releasing electrical energy and yet it contains no metallic conductors. Neither are such metallic conductors found in the intra- or extracellular fluids. It is, therefore, unlikely that membrane currents represent flows of free electrons, but it is to the electrolytes, the ions, that we must look for carriers of membrane currents.

The intracellular fluid consists of an aqueous solution containing relatively large amounts of potassium, but small amounts of chloride, sodium, calcium, and magnesium. In addition, it contains some organic anions (negatively charged molecules) to which the membrane is impermeable, i.e., they cannot leave the cell. Table 3-1 shows both intracellular and extracellular concentrations of ions for the giant axon of the squid mantle (a cylinder of membrane of about 0.5-mm diameter), frog muscle fibers, and motoneurons in the cat spinal cord. Relative to intracellular fluid, extracellular fluid is rich in sodium, chloride, calcium, and magnesium, but poor in
potassium.

**Table 3-1**

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<th>Ionic Environments of the Membrane</th>
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<tbody>
<tr>
<td>Preparation</td>
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<td>Frog muscle(^4) ((-100 &lt; V_r &lt; -70) mV)</td>
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<tr>
<td>Cat motoneuron(^5) ((-80 &lt; V_r &lt; -60) mV)</td>
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\(^3\)Kuffler SW, Nichols JG: *From Neuron to Brain*, Sunderland, MA: Sinauer Assoc., 1976

\(^4\)Conway EJ: *Physiol. Rev.* 37:84-132, 1957

\(^5\)Coombs JS, Curtis, DR, Eccles, JC: *J. Physiol. (Lond.)* 130:326-373, 1955
**Diffusion.** The behavior of ions in solution is such that these differences in concentration across the membrane could not long be maintained unless the membrane formed an effective barrier between the two aqueous phases or some energy-requiring process existed to maintain them. We have already seen that ions in this situation should seek equilibrium by diffusion.

**Equilibrium potentials.** Thus far, we have not considered the effect of ionic charge on movement of the ions in solution. In Figure 3-10A, the compartments of the vessel are again separated by a membrane, but this time let us suppose that the membrane is permeable only to sodium. If we now add salt to one compartment as before, there will be a diffusion of sodium ions (recall that NaCl ionizes almost completely in water), from side 1 to side 2 (as before) because of the concentration gradient. Chloride would move with sodium to preserve electrical neutrality, but it cannot penetrate this membrane. As sodium crosses the membrane, an excess of positive charge (sodium is a positively charged ion or cation) builds up on side 2, leaving an excess of negative charge on side 1 due to unpaired chloride ions. This separation of charge is by definition a voltage. We speak of this voltage as being an **electrical gradient.** The excess of positive charge acts as a repellent to further movement of sodium across the membrane (like charges repel) or, alternatively, the excess negative charge on side 1 attracts sodium ions back across the membrane (unlike charges attract). The greater the accumulation of sodium ions and excess positive charge on side 2, the greater will be the repulsive force against further diffusion. This is the principle of operation of a concentration cell or battery; voltage is generated by differences in concentration across a semi-permeable membrane.

The value of the membrane potential at electrochemical equilibrium for a particular ion is called the **equilibrium potential** for that ion.

At some point, the concentration force promoting diffusion of sodium ions will be exactly balanced by the electrical force preventing diffusion (Fig. 3-10B), and a condition of equilibrium will result, in which
This is called **electrochemical equilibrium**. The value of the membrane potential at electrochemical equilibrium for a particular ion is called the **equilibrium potential** for that ion and is abbreviated, $\varepsilon$. A subscript is added to show the ion at equilibrium, in the case of sodium, $\text{Na}^+$. At equilibrium, $J = 0$ because there is no net ion movement. Thus,

$$W_c = -W_e . \tag{1}$$

From thermodynamics,

$$W_c = RT \ln( [\text{Na}^+]_1 / [\text{Na}^+]_2 ),$$

where $R$ is the gas constant; $T$, the absolute temperature; $[\text{Na}^+]_1$ is the sodium concentration on side 1; and $[\text{Na}^+]_2$ is the sodium concentration on side 2. This is the work required to maintain the concentration gradient. At chemical equilibrium $[\text{Na}^+]_1 = [\text{Na}^+]_2$ and $W_e = 0$; no work need be done to maintain equal concentrations.

The work necessary to move ions against the electrical gradient is

$$W_e = zF\varepsilon,$$

where $z$ is the valence of the ion, $F$ is Faraday's constant and $\varepsilon$ is the potential difference between the two compartments. Substitution for $W_c$ and $W_e$ in equation (1) yields

$$W_c = -W_e = -RT \ln( [\text{Na}^+]_1 / [\text{Na}^+]_2 ) = zF\varepsilon.$$

Rearranging terms:

$$\varepsilon = (-RT/zF) \ln( [\text{Na}^+]_1 / [\text{Na}^+]_2 ) . \tag{5}$$

This equation is known as the **Nernst equation**. Substitution of values for the gas constant, Faraday's constant and physiological temperature allows the Nernst equation to be simplified, using logarithms to base 10, to the

**Figure 3-11.** The equilibrium condition of the experiment in Figure 3-10B and C. Electrochemical equilibrium develops in which the $\text{Na}^+$ concentration gradient, indicated by the upper arrow, is just balanced by the electrical gradient, indicated by the lower arrow. At this point, there is no net flux of $\text{Na}^+$ to either side.
The Nernst equation specifies the transmembrane voltage necessary to maintain a given concentration difference or the voltage that will result from maintenance of a given concentration difference. In its most general form, the Nernst equation is written:

$$\epsilon_X = (-58/z) \log_{10} \left( \frac{[X]_1}{[X]_2} \right), \quad (2)$$

where \(X\) is any ion.

The Nernst equation specifies the transmembrane voltage necessary to maintain a given concentration difference or the voltage that will result from maintenance of a given concentration difference.

The membrane potential.

Looking back at Table 3-1, we can see that essentially all ions experience a non-zero concentration gradient across the cell membrane. This is true for the squid axon, the frog muscle, and the cat motoneuron, in fact, for most if not all living cells. Thus, in all cells sodium, chloride, calcium and magnesium ions experience a chemical driving force toward the inside of the cell, that is, if the concentrations were the only factors involved in determining ion distributions, all of these ions would diffuse into the cell. The only diffusible ion that experiences a reverse gradient, from inside to outside of the cell, is \(K^+\).

These concentration gradients should form concentration cells and one would expect to find a potential difference (or voltage) between the inside and outside of a living cell. But, what should be the value and polarity of the potential? These can be calculated from Ohm's law and the Nernst equation. Any current flowing across the membrane will result in a voltage drop, predictable from Ohm's law:

$$i = gV.$$

That current will have to be carried by ions because the membrane contains no metallic conductors, so the total current will be equal to the sum of the currents carried by each ion:

$$i = (i_{K^+}) + (i_{Na^+}) + (i_{Cl^-}) + (i_{HCO_3^-}) + (i_{Mg^{++}}) + (i_{Ca^{++}}) \ldots$$

The current carried by any ion will be determined by its driving force and the conductance of the membrane to it. The driving force is the electrical force experienced by the ion, and it is determined by the deviation of the membrane voltage from the equilibrium potential for the ion. The conductance, as you remember, is a measure of how easily the ion passes through the membrane. Thus:

6 You should always keep in mind that conductance and driving force are independent quantities. Driving force depends upon the membrane potential and the equilibrium potential. The equilibrium potential depends, in turn, upon the concentration difference for the ion. Conductance is a measure of the qualities of the membrane itself, how easily it admits the ion. Driving force does not depend in any way upon the qualities of the membrane.
\[ i_{K^+} = g_{K^+}(V_m - \varepsilon_{K^+}) \]
\[ i_{Na^+} = g_{Na^+}(V_m - \varepsilon_{Na^+}) \]
\[ i_{Cl^-} = g_{Cl^-}(V_m - \varepsilon_{Cl^-}), \]

and so on, where \( V_m \) is the membrane potential. When \( V_m = \varepsilon_{K^+} \), there is no driving force on \( K^+ \) and no \( K^+ \) current. Any potassium current will be influenced by both the driving force and the membrane conductance for potassium. This is true for any ion.

**The driving force** is the electrical force experienced by the ion, and it is determined by the deviation of the membrane voltage from the equilibrium potential for the ion.

\[ i = g_{K^+}(V_m - \varepsilon_{K^+}) + g_{Na^+}(V_m - \varepsilon_{Na^+}) + g_{Cl^-}(V_m - \varepsilon_{Cl^-}) \ldots \hspace{0.5cm} (3) \]

Because of their very low concentrations, \( Ca^{++} \) and \( Mg^{++} \) ions contribute only slightly to the membrane potential, though they play a significant role in the functioning of both nerve and muscle. We shall ignore these ions for the present.

We want to determine the value of \( V_m \) in equation 3 above. To do this, we have to know the values of the equilibrium potentials for the various ions. Let's choose the squid giant axon for our example and calculate the equilibrium potentials from values in Table 3-1.

It is customary in neurophysiology to express membrane potentials as potential inside with respect to outside. Therefore, the Nernst equation takes the form:

\[ \varepsilon_X = (-58/z) \log_{10}(\frac{[X]_{\text{inside}}}{[X]_{\text{outside}}}), \hspace{0.5cm} (4) \]

Substituting values for \( K^+ \), we get:

\[ \varepsilon_{K^+} = (-58/1) \log_{10}(400/20) = -58 \log_{10}(20) = -75.5 \text{ mV}. \]

Thus, \( K^+ \) will be in electrochemical equilibrium at a 20/1 concentration ratio (inside/outside) when the inside is 75.5 mV negative with respect to the outside. At any potential less negative than this value, \( K^+ \) will leave the cell; at any potential more negative than this value, \( K^+ \) will enter the cell. We can calculate the equilibrium potentials for both \( Na^+ \) and \( Cl^- \) in a similar way:

\[ \varepsilon_{Na^+} = (-58/1) \log_{10}(50/440) = +58 \log_{10}(440/50) = +58 \log_{10}(8.8) = +54.8 \text{ mV} \]
\[ \varepsilon_{Cl^-} = (-58/-1) \log_{10}(40/560) = -58 \log_{10}(560/40) = -58 \log_{10}(14) = -66.5 \text{ mV}. \]

It is customary to express membrane potentials as potential inside with respect to outside.

In cell membranes, the conductance to chloride ions is high. Nerve membrane conductance to chloride is slightly lower than for potassium, but muscle membrane has a higher conductance to chloride than to potassium. Because of the high conductance, chloride is able to redistribute across the membrane by simple diffusion in response to changes in the membrane voltage. Thus, the chloride ion will always be at equilibrium in the vicinity of the resting membrane potential. (This might not be true for every cell, but it's true for...
the one we are considering here.) The contribution of chloride to the membrane potential can be ignored because of its tendency to redistribute itself.

In order to maintain electrical neutrality (the net charge in any solution must be zero), some positive ions must be present inside to balance the charge of the large anions that cannot leave the cell. The low sodium conductance of the resting membrane and the presence of the sodium pump prevent sodium ions from redistributing to provide the positive charge to balance the large intracellular anions; so potassium ions must remain inside the cell in high concentration.

That leaves us with only K\textsuperscript{+} and Na\textsuperscript{+} to contribute to the membrane potential. At rest, the membrane is at equilibrium, i.e., there is no net transmembrane current flow, so i=0, therefore

\[ 0 = i = g_{\text{K}^+} (V_m - \varepsilon_{\text{K}^+}) + g_{\text{Na}^+} (V_m - \varepsilon_{\text{Na}^+}) \]

or

\[ g_{\text{K}^+}(V_m - \varepsilon_{\text{K}^+}) = - g_{\text{Na}^+}(V_m - \varepsilon_{\text{Na}^+}), \]

where \( V_r \) is the value of \( V_m \) at equilibrium and is called the **resting membrane potential**. Rearranging terms:

\[ \frac{g_{\text{K}^+}}{g_{\text{Na}^+}} = \frac{(V_r - \varepsilon_{\text{Na}^+})}{(V_r - \varepsilon_{\text{K}^+})} \]

The nerve membrane is permeable to both Na\textsuperscript{+} and K\textsuperscript{+}, but the permeability to K\textsuperscript{+} is much higher. The ratio of the conductance of the membrane to K\textsuperscript{+} to the conductance to Na\textsuperscript{+} varies from 10 to 30 in different cells. If the ratio is taken to be 20, then

\[ 20/1 = -(V_r - \varepsilon_{\text{Na}^+}) / (V_r - \varepsilon_{\text{K}^+}) . \]

Solving for \( V_r \), we see that

\[ V_r = 20/21 \varepsilon_{\text{K}^+} + 1/21 \varepsilon_{\text{Na}^+} . \]

Substituting values for \( \varepsilon_{\text{K}^+} \) and \( \varepsilon_{\text{Na}^+} \) from Table 3-1, we see that \( V_r = -69.3 \text{ mV} \). That is, we expect the membrane potential to be 69.3 mV negative inside with respect to outside. Taking the two extremes of the ratio of conductances, namely 30/1 and 10/1, yields predicted membrane potentials of -71.3 mV and -63.6 mV. The greater the ratio, i.e., the (relatively) more impermeable the membrane is to Na\textsuperscript{+}, the closer \( V_r \) will be to \( \varepsilon_{\text{K}^+} \). Thus, the membrane potential is due mainly to the concentration difference for potassium and is close to \( \varepsilon_{\text{K}^+} \). The greater the relative permeability to Na\textsuperscript{+}, the farther \( V_r \) will deviate from \( \varepsilon_{\text{K}^+} \) or the closer it will deviate toward \( \varepsilon_{\text{Na}^+} \). If the conductances to Na\textsuperscript{+} and K\textsuperscript{+} were equal, \( V_r \) would lie half way between \( \varepsilon_{\text{Na}^+} \) and \( \varepsilon_{\text{K}^+} \).

The accuracy of this theoretical prediction of \( V_r \) can be examined by actually measuring the resting membrane potential. This is done using a micropipette electrode.
as shown in Figure 3-8. When the electrode is in the extracellular fluid, zero potential difference will be recorded because both the microelectrode and the indifferent electrode are in the same fluid and therefore at the same

Figure 3-12. The membrane potential. A graph of the voltage recorded between a movable micropipette electrode and a fixed electrode in the extracellular fluid (ordinate) against time (abscissa). At the origin, both the pipette and the fixed electrode are in the extracellular fluid, and the voltage between them is zero (A). When the micropipette penetrates the membrane, the voltage changes to -70 mV, inside with respect to outside (B). When the electrode is backed out of the cell, the potential returns to zero (C). The positions of \( \varepsilon_{\text{Na}^+} \) and \( \varepsilon_{\text{K}^+} \) are indicated on the ordinate.

potential. This is shown in the first part (A) of the record of Figure 3-12. In this figure, known as a **driving force diagram**, positive membrane voltages are plotted above zero, negative voltages below. The positions of \( \varepsilon_{\text{Na}^+} \) at +54.8 mV and \( \varepsilon_{\text{K}^+} \) at -75.5 mV are shown. As the micropipette penetrates the membrane, the potential it records goes negative by 70 mV (section B of the record). If the membrane seals around the electrode, the recorded voltage will stay at -70 mV, the value of \( V_r \), the resting membrane potential. When the micropipette is withdrawn from the cell, the voltage returns to zero (section C of the record). At times, the membrane is badly damaged by the electrode, and a membrane voltage less negative than the actual \( V \) will be recorded, presumably because the normal ionic concentration gradients are destroyed. Enough cells have been examined to indicate that the membrane potential of the squid axon is about -70 mV in sea water at normal temperatures. Thus, the preceding theoretical analysis is reasonably accurate. Any inaccuracies that exist may be the result of inaccuracies in the measurement of ion concentrations or they may be due to small contributions from \( \text{Ca}^{++} \) or \( \text{Mg}^{++} \) to the membrane potentials. A more accurate prediction is obtained when these ions are also included in the analysis. Their contribution, as noted previously, is small.

Not all cells, not even all neurons, have the same resting membrane potential. In all cells, however, the value of the resting membrane potential depends upon the values of \( \varepsilon_{\text{K}^+} \), \( \varepsilon_{\text{Na}^+} \) and \( g_{\text{K}^+}/g_{\text{Na}^+} \) (for some cells chloride and other ions may also play an important role). The values of \( \varepsilon_{\text{K}^+} \) for the three types of cells in Table 3-1 vary from -75 to -99 mV, and these values are reflected in their membrane potentials. Frog muscle cells have resting potentials of -70 to -100 mV, whereas cat motoneurons vary from -60 to -80 mV. Neurons in the cerebral cortex typically have resting membrane potentials around -50 mV, presumably reflecting either lower values of \( \varepsilon_{\text{K}^+} \) or \( g_{\text{K}^+}/g_{\text{Na}^+} \) or both.

If this analysis of the origin of the resting membrane potential is accurate, then the membrane potential should be highly sensitive to changes the concentration gradient for \( \text{K}^+ \). That this is the case is shown in Figure 3-13 for frog muscle fibers. As the external concentration of \( \text{K}^+ \) is raised from 12 mM (moving to the right on the abscissa), the
The value of the membrane potential depends upon: $g_{K^+}$, $g_{Na^+}$, $\varepsilon_{K^+}$, and $\varepsilon_{Na^+}$.

Membrane potential falls (moving upward on the ordinate) as predicted from the Nernst equation (the solid line). At lower $[K^+]_o$ values, the role of $Na^+$ becomes greater because of the greater driving force (greater deviation of $\varepsilon_{Na^+}$ from $V_r$), and the points deviate from the Nernst relation. Increasing the internal $K^+$ concentration has similar consequences for the concentration gradients suggests an electrical equivalent circuit. The concentration gradients can be viewed as concentration cells or batteries whose voltages are the equilibrium potentials for the ions at these concentrations. We may, therefore, modify the circuit of Figure 3-9 to include the membrane potential. Figure 3-14 includes the $Na^+$ and $K^+$ batteries and separate resistances or conductances for the two ions. The $Na^+$ battery is shown oriented with negative pole toward the outside and the $K^+$ battery with negative pole toward the inside. This matches the orientations of the $Na^+$ and $K^+$ equilibrium potentials. We can see how this circuit accounts for the membrane potential by examining the limiting conditions. Suppose $R_{Na^+}$ were infinitely large. All of the voltage across the membrane would then come from the potassium battery, $\varepsilon_{K^+}$. If $R_{Na^+}$ were zero, then the contribution to the membrane potential from $\varepsilon_{K^+}$ would be negligible compared to that from $\varepsilon_{Na^+}$. Similarly, if $R_{K^+}$ were infinite, $\varepsilon_{Na^+}$

Keep in mind that a concentration gradient is a source of potential energy, energy released if the gradient is allowed to dissipate.
would determine the membrane potential. However, neither resistance is infinite or zero; rather the sodium resistance is 20-30 times larger than the potassium resistance (remember $g_{Na^+} = 1/R_{Na^+}$), so that the membrane potential is dominated by $\epsilon_{K+}$ but not equal to $\epsilon_{K+}$.

Recall that an ion is in equilibrium if its equilibrium potential equals the membrane potential.

The sodium-potassium pump. Few of the ions around the membrane are at electrochemical equilibrium when the membrane is at rest, i.e., when $V_m = V_r$. $V_r$ is far from $\epsilon_{Na^+}$, and it is not equal to $\epsilon_{K+}$. Only Cl- is in equilibrium at $V_r$. There is a weak tendency for $K^+$ to move out of the cell and a strong tendency for $Na^+$ to enter the cell. A rapid equilibration of ionic concentrations is prevented by the low resting sodium conductance of the membrane, but the resting $g_{Na^+}$ is not zero and, therefore, in the absence of any other mechanism, the membrane potential should slowly decline toward zero. As sodium moves into the cell down its concentration gradient, it provides positive charge to balance the internal anions and thus allows $K^+$ to diffuse out of the cell. As $K^+$ leaves, its concentration gradient falls, and $\epsilon_{K+}$ and $V_r$ become less negative.

There exists, within the membrane, a mechanism for expelling $Na^+$ and taking up $K^+$ against their concentration gradients. This mechanism is called the **sodium-potassium pump** or, simply, the **sodium pump**. Obviously, if the ions have a natural tendency to move in one direction, causing them to move in the opposite direction requires the expenditure of energy. The tendency of ions to move down their concentration gradients constitutes a potential energy (as opposed to kinetic energy) that can be counteracted only by expending at least an equal amount of energy. That this process of pumping ions is, indeed, an active process is indicated by its sensitivity to changes in temperature, i.e., cells accumulate more sodium and lose more potassium at low temperature, and by the blockade of the pumping and decline of the membrane potential over a prolonged period when metabolic activity, involving splitting the high-energy phosphate bonds of adenosine triphosphate (ATP), is blocked by the inhibitor ouabain or by cyanide. The extrusion of 3 sodium ions requires the hydrolyzation of 1 molecule of ATP.

The rate of extrusion of $Na^+$ by the sodium pump is proportional to the internal sodium concentration. For example, at an internal concentration of $Na^+$ of 50 mM, the rate of pumping of $Na^+$ ions is about 30 pmol/cm$^2$/sec (pmol = picomoles) in the squid axon, but the rate rises to about 150 pmol/cm$^2$/sec at an internal concentration of 230 mM. The relationship between pumping rate and the internal $Na^+$ concentration appears to be linear for some cells (e.g., squid axon) but not for others (e.g., frog muscle).

In most cells, the pumping of sodium ions is linked to the pumping of potassium. In fact, extrusion of sodium can be reduced by as much as 70% in the absence of potassium in the extracellular fluid. A pump that exchanges one

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8 This is true in the cell we have chosen for our examples, but it may not be true in the majority of cells. In particular, it is not true in cardiac muscle cells.
Na\(^+\) for one K\(^+\) will not produce a net charge transfer, and therefore there will be no change in membrane potential as a result of pump activity. This is called a non-electrogenic pump. However, if the amount of Na\(^+\) transported exceeds the amount of K\(^+\) transported, a potential will develop as a result of the net transfer of charge. This is called an electricgenic pump. An electricgenic pump can add to potentials set up by diffusing ions and cause the ions to redistribute themselves in order to restore equilibrium. The activity of electricgenic pumps can therefore affect ion distributions and membrane potentials either through action on concentration gradients or by altering the membrane potential. In any event, at equilibrium the net fluxes due to pump activity, or any other forces, must still be zero. Active fluxes (pump-related) must exactly balance passive fluxes (diffusional).

The sodium pump of erythrocytes has a Na\(^+\)/K\(^+\) transport ratio that is nearly one, i.e., it is essentially non-electrogenic. In the squid axon, the ratio can be as high as 3 or as low as 1, depending upon whether the intracellular Na\(^+\) concentration is high or low. Thus, the transport ratio need not be fixed, but can vary, providing a mechanism for a relatively constant K\(^+\) transport rate and a variable sodium transport rate.

There is evidence that the transport system involves Na\(^+\)-K\(^+\)-activated ATPases (enzymes that hydrolyze ATP) within the membrane. These have a greater affinity for Na\(^+\) and a lesser affinity for K\(^+\) at the inner surface of the membrane and a lesser affinity for Na\(^+\) and greater affinity for K\(^+\) at the outer surface. They hydrolyze ATP at a rate dependent upon the Na\(^+\) and K\(^+\) concentrations. The actual physical mechanism by which the ions are moved is unknown, but the suggestion has been made that the ATPase is an intrinsic protein that perhaps rotates or otherwise changes shape after picking up intracellular Na\(^+\) and extracellular K\(^+\). In this new orientation or conformation, the affinity for the transported substance is reduced, and Na\(^+\) is released outside and K\(^+\) inside the cell. Finally, the protein rotates back to its original orientation or changes to its original conformation and affinity, and the process repeats.

It has been estimated that each sodium pump can transport up to 200 Na\(^+\) and 130 K\(^+\) per sec, the actual rate being determined by ion concentrations. Other estimates suggest that, on the average, sodium pumps have a density of 100-200 per mm\(^2\) of membrane, but, in some parts of the cell, they can be up to 10 times more dense. Thus, a typical neuron might have a million pumps with a maximum capacity of 200 million Na\(^+\) ions/sec.

The importance of the membrane potential. The importance of the resting membrane potential is seldom stated explicitly, but its importance cannot be over-emphasized. All living cells, not just nerves and muscles, have a resting membrane potential. It is a ubiquitous property of living matter. The work of nerve and muscle cells depends upon the membrane potential. It acts as an energy store, a source of potential energy from which these cells draw to initiate their characteristic activities, the nerve and muscle action potentials and muscle contraction. The secretion of chemical substances by nerve terminals and glands also depends upon the existence of the membrane potential. We will see presently, how the membrane potential results in action potentials, and we shall see later, how the membrane potential is involved in muscle contraction.