Ion Concentration Differences Across Membranes Lead to Potential Differences

To understand the emergence of a membrane potential we step back and consider a simplified situation that illustrates the energetic and entropic competition which plays out when there are concentration gradients of charged species. To study this process of electrochemical equilibrium, we consider an isolated system with an internal barrier separating the system into two regions as shown in Figure 17.2. On one side, we have a charge neutral solution of K⁺ and Cl⁻ ions, for example, at concentration $c_1$ and on the other side, we have a charge neutral solution of the same ions, but at concentration $c_2$. If we now remove the constraint of impermeability for the K⁺ ions, these ions will start flowing from the region of high concentration to low concentration. As already highlighted in Chapter 13, the diffusion of these ions down their concentration gradient can be thought of as a response to the driving force tending to maximize the entropy of the system. As these ions flow down their concentration gradient, there will be an ever-increasing charge imbalance and a concomitant increase in the electric energy of the system. Equilibrium is reached when these two effects balance. This occurs at a particular value of the potential difference between the two regions, which depends on the ratio of the final concentrations of K⁺ ions. For numbers typical of cellular processes, the change of concentration that accompanies the transfer of ions across the cell membrane is many orders of magnitude smaller than the concentrations themselves, which we can therefore treat as constant.

To apply these ideas mathematically, we recall from Chapter 9 that the electrical energy of an ion of unit charge $e$, when placed in a region of potential $V_1$, is $eV_1$. For an ion whose valence is $z$ the corresponding charge is $ze$ and the energy is $zeV_1$. Consider two small regions in our salt solution (small so we can consider the electrical potential roughly uniform). In one, the potential is $V_1$ and the concentration of positive ions is $c_1$, while in the other we have $V_2$ and $c_2$ for the potential and the concentration. In equilibrium the probability of finding a positive ion in region 1 or 2 is

$$p_{1,2} = \frac{1}{Z} e^{-eV_1/zkT},$$  (17.1)

resulting from application of the Boltzmann distribution to this problem in which the energies are electrical. The subscript 1, 2 on $p$ and $V$ means the subscript should be either 1 or 2; there is a distinct equation for each box. The ratio of the two probabilities is equal to the ratio of the number of ions in region 1 and the number in region 2, which in turn are proportional to the ion concentrations in the two regions. We can use this proportionality to write the ratio of concentrations as

$$\frac{c_1}{c_2} = \frac{p_1}{p_2} = \frac{e^{-eV_1/kT}}{e^{-eV_2/kT}}.$$  (17.2)

If we take the logarithm of both sides of this equation, we are left with the celebrated Nernst equation which relates the difference of electrical potentials to the ratio of concentrations of ions and is given by

$$V_2 - V_1 = \frac{kT}{e} \ln \frac{c_1}{c_2}.$$  (17.3)

The Nernst equation will be our starting point for trying to understand the way cells manage charge in order to set up membrane potentials.
**Figure 17.10** Mapping of membrane onto an equivalent electrical circuit. The lipid bilayer yields the behavior of a capacitor of capacitance $C$, while the presence of channels can be modeled as resistors whose conductances (inverse resistances) are $g_{Na}$ and $g_K$, for the sodium and potassium channels, respectively. The batteries represent the Nernst potentials for each of the individual ionic species, which account for the ionic currents due to the concentration gradient across the membrane.

<table>
<thead>
<tr>
<th>Ion species</th>
<th>Intracellular concentration (mM)</th>
<th>Extracellular concentration (mM)</th>
<th>Nernst potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K^+$</td>
<td>155</td>
<td>4</td>
<td>-98</td>
</tr>
<tr>
<td>$Na^+$</td>
<td>12</td>
<td>145</td>
<td>67</td>
</tr>
<tr>
<td>$Ca^{2+}$</td>
<td>$10^{-4}$</td>
<td>1.5</td>
<td>130</td>
</tr>
<tr>
<td>$Cl^-$</td>
<td>4</td>
<td>120</td>
<td>-90</td>
</tr>
</tbody>
</table>

**Table 17.1** Ion concentrations and the Nernst potential for small ions within the cell. The numbers are typical of mammalian skeletal muscle cells, which have a resting potential of $V_{mem} = -90$ mV. (Data adapted from B. Hille, Ion Channels of Excitable Membranes, Sunderland, Sinauer Associates, Inc., 2001.)
**THE MOVEMENT OF IONS**

**Diffusion**

Ions and molecules dissolved in water are in constant motion. This temperature-dependent, random movement will tend to distribute the ions evenly throughout the solution. In this way, there will be a net movement of ions from regions of high concentration to regions of low concentration; this movement is called **diffusion**. As an example, consider adding a teaspoon of milk to a cup of hot tea. The milk tends to spread evenly through the tea solution. If the thermal energy of the solution is reduced, as with iced tea, the diffusion of milk molecules will take noticeably longer.

Although ions typically will not pass through a phospholipid bilayer directly, diffusion will cause ions to be pushed through channels in the membrane. For example, if NaCl is dissolved in the fluid on one side of a permeable membrane (i.e., with channels that allow Na⁺ and Cl⁻ passage), the Na⁺ and Cl⁻ ions will cross until they are evenly distributed in the solutions on both sides (Figure 3.8). As with the previous example, the net movement is from the region of high concentration to the region of low concentration. (For a review of how concentrations are expressed, see Box 3.1.) Such a difference in concentration is called a **concentration gradient**. Thus, we say that ions will flow down a concentration gradient. Driving ions across the membrane by diffusion, therefore, happens when (1) the membrane possesses channels permeable to the ions, and (2) there is a concentration gradient across the membrane.

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 inside the neuron is an absolute requirement for a functioning nervous system. In order to understand the negative membrane potential, we look to the ions that are available 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⁻ anion, any molecule 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 will be 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 immediately begins to acquire a net negative charge, and an electrical potential difference is established across the membrane (Figure 3.12b). As the inside fluid 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 \( E_{\text{ion}} \). 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:

**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⁺ ions 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⁺ ions from the inside to the outside. An equilibrium is established such that there is no net movement of ions across the membrane, leaving a charge difference between the two sides.
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 mM 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 from 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, an 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. From 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 concentrated outside the cell (Figure 3.14). If the membrane contained 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 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 Nernst Equation

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

\[ E_{\text{ion}} = \frac{2.303 \cdot RT}{zF} \log \frac{[\text{ion}]_o}{[\text{ion}]} \]

where

- \( E_{\text{ion}} \) = ionic equilibrium potential
- \( R \) = gas constant
- \( T \) = absolute temperature
- \( z \) = charge of the ion
- \( F \) = Faraday's constant
- \( \log \) = base 10 logarithm
- \([\text{ion}]_o\) = ionic concentration outside the cell
- \([\text{ion}]\) = 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 will therefore increase the potential difference achieved at equilibrium. Thus, \( E_{\text{ion}} \) is proportional to \( T \). On the other hand, increasing the electrical charge of each particle will decrease the potential difference needed to balance diffusion. Therefore, \( E_{\text{ion}} \) 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:

\[ E_{K} = 61.54 \text{ mV} \log \frac{[K^+]_o}{[K^+]} \]
\[ E_{Na} = 61.54 \text{ mV} \log \frac{[Na^+]_o}{[Na^+]} \]
\[ E_{Cl} = -61.54 \text{ mV} \log \frac{[Cl^-]_o}{[Cl^-]} \]
\[ E_{Ca} = 30.77 \text{ mV} \log \frac{[Ca^{2+}]_o}{[Ca^{2+}]} \]

Therefore, in order 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:

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

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 \( E_{\text{ion}} \) 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. \( E_{\text{ion}} \) 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.

The Distribution of Ions Across the Membrane

It should now be clear that the neuronal membrane potential depends on the ionic concentrations on either side of the membrane. Estimates of 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.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Concentration outside (in mM)</th>
<th>Concentration inside (in mM)</th>
<th>Ratio Out : In</th>
<th>( E_{\text{ion}} ) (at 37°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K^+ )</td>
<td>5</td>
<td>100</td>
<td>1 : 20</td>
<td>-80 mV</td>
</tr>
<tr>
<td>( Na^+ )</td>
<td>150</td>
<td>15</td>
<td>10 : 1</td>
<td>62 mV</td>
</tr>
<tr>
<td>( Ca^{2+} )</td>
<td>2</td>
<td>0.0002</td>
<td>10,000 : 1</td>
<td>123 mV</td>
</tr>
<tr>
<td>( Cl^- )</td>
<td>150</td>
<td>13</td>
<td>11.5 : 1</td>
<td>-65 mV</td>
</tr>
</tbody>
</table>
**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 results if a membrane is *selectively permeable* to that ion alone. In reality, however, neurons are not permeable to only a single type of ion. How do we incorporate this detail into our thinking?

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 resting membrane potential of $-65$ mV approaches, but does not achieve, 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).