INTRODUCTION

In the last self-instructional package we dealt with the ionic mechanisms of the resting potential. We learned that ion pumps create ionic electrochemical potential gradients and that the flow of ions down their electrochemical potential gradients creates the resting membrane potential. In this package we deal with the ionic mechanisms of the action potentials of excitable cells. We will learn that the action potential is also due to the flow of ions down their electrochemical potential gradients. The ionic currents of the action potential occur in a particular time sequence that is caused by dramatic increases in the membrane conductances for Na\(^+\), K\(^+\), and sometimes other ions as well. These conductance increases are time-dependent and also depend on the membrane potential. Understanding the ionic mechanisms of action potentials will enhance our appreciation of muscle physiology, cardiac physiology, and the cellular functions of neurons.

OBJECTIVES

1. Draw a generalized nerve action potential and label the resting potential, threshold, spike, overshoot, and hyperpolarizing afterpotential. Put in approximate membrane potential values for the resting potential, threshold, and peak of the overshoot. Indicate the approximate duration of the action potential.

2. Draw the time courses of the conductance changes to Na\(^+\) and K\(^+\) that have been shown to occur during a nerve action potential. Show the relationships in time between the conductance changes and the resulting action potential.

3. Predict the effects of increased membrane conductance of Na\(^+\), K\(^+\), Cl\(^-\) on the resting membrane potential and on the shape of a hypothetical action potential.

4. State the cellular ionic concentration changes in a single action potential are negligible, so that the Na, K-pump does not play a direct role in generating the action potential.

5. Explain the following in terms of membrane ionic conductances.
   A. Why the overshoot falls short of \(E_{Na}\)
   B. Threshold.
   C. Absolute refractory period.
   D. Relative refractory period.
PRACTICE CYCLE 1

INPUT 1

The action potential is a transient change of the membrane potential. In a particular cell type, the form of the action potential is almost invariant under normal conditions. The action potential in a typical vertebrate nerve cell looks something like this:

Cover the schematic depiction of the action potential and practice reproducing it until you can draw it and label all the features labeled above.

FEEDBACK 1

Now that you can draw and describe an action potential, we should pause to note some of its important characteristics that you will learn about in more detail later in this package. Note that once the threshold is reached, the spike proper begins and that the membrane depolarizes very quickly and then repolarizes almost as quickly. Note that the overshoot (peak + 50 mV) never reaches the Na⁺ equilibrium potential (+65 mV).
We should point out that the form of the action potential differs considerably among different types of excitable tissues (figure just above). This Package deals primarily with the action potentials of a "typical" mammalian nerve cell, because in nerve cells the action potential is relatively simple, as is also the case in skeletal muscle. In cardiac muscle and in smooth muscle electrical activity has somewhat different forms. relatively simple, as is also the case in skeletal muscle. In cardiac muscle and in smooth muscle electrical activity has somewhat different forms.

**PRACTICE CYCLE 2**

**INPUT2**

For quite a long time it was suspected that changes in the permeability of the plasma membrane to ions were involved in generating the action potential. Bernstein in the early 1900’s proposed that the action potential was caused by a transient increase in the permeability of the membrane to all ions. A later version of this theory postulated that increased in the permeability of only Na⁺ and K⁺ were involved.

The conductance changes that do occur during the action potential of the giant axion of the squid were elucidated in the early 1950’s by A.L. Hodgkin and A.F. Huxley. Both the peculiarities of the squid and the availability of a new technique contributed to the success of their endeavors. Squid giant axons range in diameter from 100 µm to 500 µm. This is large enough that a fine wire may be placed inside the axon along its long axis with no damage to the plasma membrane. The new method they employed is called the voltage clamp technique. This method allowed them to estimate the changes in conductances to Na⁺ and K⁺ that occur during an action potential.

The voltage clamp method allows the investigator to set the membrane potential of a cell to whatever value he chooses and then to "clamp" it at that value. For those students that want to learn about the voltage clamp technique and how it allows us to determine the ionic currents that flow across the membrane, an Appendix to this package describes this method and how it was applied to the squid axon. Hodgkin and Huxley showed that the action potential is due to an influx of Na⁺ into the axon followed by an efflux of K⁺. Having measured the ionic currents and the membrane potential, they computed the membrane conductances for Na⁺ and K⁺ (i.e. 

\[ g_{Na} = \frac{I_{Na}}{E_m - E_{Na}} \quad \text{and} \quad g_{K} = \frac{I_{K}}{E_m - E_{K}} \]

Hodgkin and Huxley showed that changes in the membrane conductances to Na⁺ and K⁺ (shown below) occur during an action potential in a squid axon.
PRACTICE 2

Cover the diagram above and practice reproducing it until you do so accurately and label the three curves.

FEEDBACK 2

Note that the sodium conductance \((g_{Na})\) turns on rapidly and also turns off rapidly. The rapid inactivation of the sodium conductance is an important feature that will be discussed later. Note that the increase in potassium conductance \((g_{K})\) is delayed with respect to the increase in \(g_{Na}\). We now will learn how these conductance changes cause the action potential.

PRACTICE CYCLE 3

INPUT 3

In the Package on the resting potential you learned that each permeable ion tends to bring the resting membrane potential toward its own equilibrium potential. The greater the conductance of the membrane to a particular ion, the more power that ion has to influence the membrane potential. The table below shows the ion distributions across the membrane of a typical mammalian nerve fiber and the equilibrium potentials of the ions.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Intracellular Conc. (mM)</th>
<th>Extracellular Conc. (mM)</th>
<th>Equilibrium Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K^+)</td>
<td>140</td>
<td>4</td>
<td>-92.6</td>
</tr>
<tr>
<td>(Na^+)</td>
<td>10</td>
<td>120</td>
<td>+64.8</td>
</tr>
<tr>
<td>(Cl^-)</td>
<td>8.2</td>
<td>120</td>
<td>-70</td>
</tr>
</tbody>
</table>

Resting Membrane Potential = -70 mV

As we previously learned, the resting potential is closer to \(E_K\) than \(E_{Na}\) because the resting \(g_K\) is about 6 times larger than \(g_{Na}\) for the typical vertebrate neuron. Chloride ions are distributed in an
equilibrium fashion and resting $g_{Cl}$ is a good deal smaller than the other conductances in a typical neuron.

**PRACTICE 3**

Qualitatively explain the form of the action potential in terms of the changes in $g_{Na}$ and $g_{K}$ that occur.

**FEEDBACK 3**

As you said, the initial increase in $g_{Na}$ causes the membrane potential to move toward the Na$^+$ equilibrium potential. This causes the rising phase of the action potential spike. The inactivation of $g_{Na}$ , plus the delayed increase in $g_{K}$ causes the membrane potential to rapidly return toward the K$^+$ equilibrium potential.

In most neurons resting $g_{Cl}$ is much smaller than the resting $g_{K}$ and $g_{Na}$. For this reason chloride currents do not play an important role in the action potential in these cells. In skeletal muscle cells, however, the resting Cl$^-$ conductance is quite high. At the resting potential (-90 mV) chloride is close to equilibrium. Thus at rest there is little Cl$^-$ current. When the membrane potential is changed however, Cl$^-$ is no longer in equilibrium and a net Cl$^-$ current will flow that will tend to bring $E_m$ back toward $E_{Cl}$. In this way Cl$^-$ currents in skeletal muscle cells oppose the rising phase of the action potential and hasten the repolarization phase.

**PRACTICE CYCLE 4**

**INPUT 4**

Before leaving the subject of the way in which changing ionic conductances cause the action potential, let's pause to be sure these ideas are fully understood. In order to test our understanding let's do some "thought experiments".

**Example: Question 1.** What would happen to the action potential should the $g_{Na}$ change as we have described, but the delayed increase in $g_{K}$ fail to occur?

**Answer 1:** The rising phase of the action potential would not change much, but the overshoot might be a bit higher since the delayed increase in $g_{K}$ opposes the overshoot. The membrane would still return to the resting potential when the Na$^+$ conductance inactivated, but would repolarizes much more slowly since the increase in $g_{K}$ hastens the repolarization. Thus the action potential would be prolonged.
**PRACTICE 4**

**Question 2.** What would happen to the action potential if the K⁺ conductance failed to turn off and g_k remained at a high level?

**Question 3.** What would happen to the action potential should a large increase in g_{Cl} occur with the same time course and magnitude as the increase in g_{Na}?

**Question 4.** What would happen should both g_{Na} and g_k fail to inactivate but remain at their maximum values: What would be the resting membrane potential under these conditions?

**FEEDBACK 4**

**Answer 2:** The rising phase of the action potential would be unchanged, as would the early part of the repolarization phase. Because of the elevated g_k the rate of repolarization would be somewhat increased and the membrane potential would return to a new value that is hyperpolarized with respect to the normal resting potential. This is because E_k is -92.6 mV while the normal resting potential is -70 mV. An increase in resting g_k would result in a larger (more negative) resting membrane potential.

**Answer 3:** The increase in the chloride conductance would tend to hold E_m at the resting potential since E_m ≅ E_{Cl}. If the increase in g_{Cl} were sufficiently large, it could block completely the ability of Na⁺ to depolarize the membrane to the threshold. If the increased g_{Cl} were smaller relative to g_{Na} it would at least slow the rising phase of the action potential and lower the overshoot to an extent proportionate to the increase in g_{Cl}. The increase in g_{Cl} would also speed the early part of the repolarization.

**Answer 4:** The rising phase of the action potential would be similar. The overshoot might be higher due to the failure of g_{Na} to inactivate. Since g_{Na} fails to activate the repolarization phase would be markedly different. The membrane would fail to return to the resting membrane potential and would reach a value that is quite depolarized relative to its normal value. The new value of the resting potential would approximate

\[ E_m = \frac{g_{max}^{K}}{g_{K}^{max} + g_{Na}^{max}} E_K + \frac{g_{max}^{Na}}{g_{K}^{max} + g_{Na}^{max}} E_{Na} \]
It is often stated that, while the Na, K-pump sets up the gradients of Na\(^+\) and K\(^+\) that permit electrical excitability, it plays no role in the recovery from a single action potential. The reason for this is that the changes in [Na\(^+\)] and [K\(^+\)] in the cell that result from the inrush of Na\(^+\) and efflux of K\(^+\) during a single action potential are very small.

About 10\(^{-12}\) moles of Na\(^+\) flow across each cm\(^2\) of plasma membrane into the cell during an action potential. An equal amount of K\(^+\) flows out to repolarize the cell. Since the smaller a cell is, the larger its surface area relative to its volume, the smaller the cell, the larger the changes in concentration of Na\(^+\) and K\(^+\). For an example, let us take a small axon with diameter 10 µm. We will assume it to be cylindrical.

Surface area = circumference x length = \(\pi \times 10 \times length\) (μm\(^2\))

We want to consider a segment with a surface area (area) = 1 cm\(^2\) = 10\(^8\) μm\(^2\)

\[
\text{length} = \frac{\text{area}}{10\pi} = \frac{10^8}{10\pi} = \frac{10^7}{\pi}
\]

That is, a segment that is \((10^7 / \pi)\) μm long will have a surface area of 1 cm\(^2\). What will be the volume (V) of such a segment?

\[
V = \pi r^2 \text{length} = \pi \left(\frac{10^7}{\pi}\right)^2 \text{length} = 25\pi \frac{10^7}{\pi} = 2.5 \times 10^8 \mu\text{m}^3
\]

Since 1 cm\(^3\) = 10\(^{12}\) μm\(^3\), this is equal to 2.5 x 10\(^{-4}\) cm\(^3\).

Now during each action potential, about 10\(^{-12}\) moles of Na\(^+\) flow into the axon across 1 cm\(^2\) of membrane area into a volume of 2.5 x 10\(^{-4}\) cm\(^3\) during one action potential. What will be the concentration change that results?

\[
\Delta[Na^+] = \frac{10^{-12} \text{mole}}{2.5 \times 10^{-4} \text{cm}^3} = 4 \times 10^{-9} \text{mole/cm}^3 = 4 \times 10^{-6} \text{mole/liter}
\]

Thus the increase in [Na\(^+\)] in this thin axon as a result of one action potential will only be 4 micromolar. The increase in K\(^+\) will be the same. This is a change of about 1 part in 3000 of cellular [Na\(^+\)] and a change of about 1 in 30,000 for [K\(^+\)]. These changes in [Na\(^+\)] and [K\(^+\)] are negligible and the Na, K-pump need not be called into play to help recover from a single action potential. If we completely poisoned the Na, K-pump with ouabain, this axon could fire hundreds of action potentials before it would fail. The squid giant axon, because of its larger volume relative to surface area, can fire close to 100,000 times without assistance from the Na, K-pump in regenerating its ion gradient.
**PRACTICE 5**

**Question 1.** Does the Na, K-pump play a role in a single action potential in a nerve or skeletal muscle cell?

**Question 2.** Why? Or why not?

**FEEDBACK 5**

**Answer 1:** It does not.

**Answer 2:** Because the changes in intracellular concentrations of [Na\(^+\)] and [K\(^+\)] that result from a single action potential are at most at the micromolar level. This is not sufficient to activate the Na, K-pump or to require its activity. Over long periods of time, however, the activity the Na,K-ATPase is required to maintain the transmembrane gradients of Na\(^+\) and K\(^+\).

**PRACTICE CYCLE 6**

**INPUT 6**

You have just learned a good deal about the ionic mechanism of the action potential. You have learned that depolarization of the cell leads to opening of Na\(^+\) channels, which leads to Na\(^+\) entry, which further depolarizes the cell. This positive feedback of Na\(^+\) entry on depolarization causes the explosive rising phase of the action potential. You have also learned that Na\(^+\) channels spontaneously inactivate after they have been opened and that they can not be reopened until the repolarization of the membrane is more or less complete. You have seen that the delayed increased in g\(_K\) is also more leisurely in returning to resting levels.

For some period during an action potential (shown in magenta in the right hand panel), a second action potential cannot be elicited, no matter how strong the stimulus. This is the **absolute refractory period**. For the remainder of the action potential (shown in blue on the same figure), a second action potential may be elicited, but a stronger than normal stimulus is required; this is the **relative refractory period**.

![Diagram](image-url)
**PRACTICE 6**

In terms of the ionic conductance mechanisms involved, explain:

A. Why the overshoot falls short of \( E_{Na} \).
B. The threshold of the action potential
C. The absolute refractory period.
D. The relative refractory period.

**FEEDBACK 6**

A. The overshoot falls short of \( E_{Na} \) for two reasons: First, the inactivation of the \( Na^+ \) channels lowers \( g_{Na} \) and second the delayed increase in \( g_K \) provides an "opposing force" to the depolarization.

B. If the stimulus applied to a nerve or muscle cell is small, the resulting depolarization of the membrane will also be small. As a result only a small number of \( Na^+ \) channels will be opened. Thus the rate of depolarization due to \( Na^+ \) inflow will be slow. The slow rate of depolarization gives time for the two processes that oppose depolarization to come into play. These are the inactivation of the previously opened \( Na^+ \) channels and the delayed opening of \( K^+ \) channels. In order for the action potential to be generated, a critical number of \( Na^+ \) channels must be opened rather quickly. This requires a certain amount of depolarization. Thus the phenomenon of the threshold comes to be.

C. The absolute refractory period occurs when the \( Na^+ \) channels have been inactivated. Until the membrane is almost back to its resting potential most of these channels cannot be opened at all. As a result the cell is absolutely refractory to stimulation. No matter how strong the stimulus, an action potential will not be elicited because the critical number of open \( Na^+ \) channels cannot be recruited.

D. The relative refractory period begins before the repolarization of the membrane is complete. As a result some of the \( Na^+ \) channels are inactivated and cannot be opened by a stimulus. With fewer activatable \( Na^+ \) channels it takes a stronger stimulus to open a sufficient number of \( Na^+ \) channels to produce an action potential. In addition, the potassium conductance (\( g_K \)) is elevated during the relative refractory period. This tends to hold \( E_m \) near the potassium equilibrium potential and opposes any depolarization toward threshold. Thus both the voltage inactivation of \( Na^+ \) channels and the continued elevation of \( g_K \) (because the \( K^+ \) channels remain) open both contribute to make the membrane relatively refractory to stimulation, so that a larger stimulus than normal is required to elicit an action potential.
INPUT 7
Ca\(^{++}\), like Na\(^{+}\), is actively extruded from most cells. Both the concentration gradient for Ca\(^{++}\) and the electrical potential across the membrane favor Ca\(^{++}\) entry into the cell. Thus, if Ca\(^{++}\) channels in a nerve or muscle plasma membrane are opened Ca\(^{++}\) enters and depolarizes the cell.

PRACTICE 7
A cell type has ionic conductance properties during an action potential that are shown below. On the figure draw the action potential in the correct time relation to the ionic conductances. \(E_{Na} = +65\) mV, \(E_{Ca} = +100\) mV.

FEEDBACK 7
Did you draw something like this?

Good. Actually this example is not totally imaginary! The conductance changes are a greatly over-simplified version of what occurs in a cardiac ventricular cell. The initial sodium generated spike is not too different from the depolarizing phase of the nerve action potential. The prolonged plateau phase, due to the long-lived increase in \(g_{Ca}\), is characteristic of the cardiac action potential. Repolarization is brought about by the relatively slow inactivation of the Ca\(^{++}\) conductance plus a greatly delayed increase in \(g_{K}\). You will learn more about the electrical properties of cardiac muscle in the Physiology/CTS course next semester.
POST-TEST

1. On the schematic depiction of the nerve action potential, label: resting potential, threshold, spike, overshoot, absolute refractory period, and relative refractory period.

![Schematic of nerve action potential](image)

What are the approximate values for the magnitude of the membrane potential at:
- rest _________________
- threshold_____________
- peak of overshoot______

What is the approximate duration of the action potential in msec?_________

2. Draw the time courses of the conductance changes of Na\(^+\) and K\(^+\) that have been found to occur during the action potential in a squid giant axon. Show their relationship in time to the action potential itself.

3. a. Draw the normal action potential in squid giant axon.
   
b. Draw the action potential that would result if the K\(^+\) conductance increase failed to occur and the Na\(^+\) conductance did not inactivate.
   
c. Draw the action potential that would occur if, in addition to the increase in Na\(^+\) conductance that occurs, a significant increase in the chloride conductance occurred with a similar time course.

4. What is the role of the Na\(^+\), K\(^+\)-pump in generating a single action potential?

5. Why does the overshoot fall short of \(E_{Na}\)?

6. What causes the absolute refractory period?

7. What causes the relative refractory period?

8. Why does the action potential have a threshold?
**ANSWERS TO POST-TEST**

1. 

resting potential = -70 mV  
threshold = -55 mV  
peak of overshoot = +50 mV  
approximate duration = 5 msec

2. 

3a  3b  3c

3a: 

3b: 

3c: normal action potential
4. There is no role of the Na, K-pump in generating a single action potential. On a longer times scale, the pump does create and maintain the ion gradients that make the action potential possible.

5. Because $g_{Na}$ inactivates and there is a delayed increase of $g_{K}$. Both of these tend to return $E_{m}$ toward the resting potential before $E_{m}$ can reach $E_{Na}$.

6. The inactivation of the Na$^{+}$ channels which cannot be opened until the membrane is repolarized almost to the resting potential. The critical number of Na$^{+}$ channels that are needed to begin the action potential are not available to be recruited.

7. The prolonged elevation of $g_{K}$ which makes the membrane harder to depolarize toward threshold and the voltage inactivation of some of the Na$^{+}$ channels makes the membrane require a stronger stimulus to reach threshold.

8. For a small stimulation the rate and extent of depolarization are small. This allows time for voltage inactivation of Na$^{+}$ channels and the delayed increase in $g_{K}$ to squelch the nascent action potential.

**OPTIONAL APPENDIX: THE VOLTAGE CLAMP TECHNIQUE**

Often the introduction of new scientific method leads in a major way to the advance of our understanding of particular phenomena. The voltage clamp technique, developed in the 1940’s, played a vital role in elucidating the ionic mechanisms of the action potential. An understanding of the voltage clamp method is not one of the objectives of this course, but some students will want to learn a bit about it nonetheless.

Kenneth S. Cole and his co-workers developed the voltage clamp technique during the Second World War when electronic feedback control principles were being used in constructing sophisticated weapons. The voltage clamp technique is a relatively straightforward application of feedback control.

In the schematic diagram shown below, note that two intracellular and two extracellular electrodes are involved in the voltage clamp circuit. One pair of electrodes is for sensing the transmembrane potential difference (V), the other pair is for passing current (I) across the membrane of the giant axon.

The voltage clamp circuitry allows the experimenter to quickly set the membrane potential to whatever value he chooses. The circuit then holds (or clamps) the membrane potential at that
pre-set value for as long as desired. It does this by sensing the membrane potential \( V \), which is the input to the feedback amplifier. So long as \( V \) is equal to the pre-set value \( V_{\text{set}} \), the amplifier does nothing. If \( V \) should differ from \( V_{\text{set}} \), the amplifier passes the current (amplifier output) across the membrane in such a way to reduce \( V - V_{\text{set}} \) to near zero.

In an actual experiment with squid axon the experimenter might choose to clamp the membrane potential to 0 mV. We know that normally when the squid axon is depolarized past -55 mV it fires an action potential. It tries to do that in this experiment. The Na\(^+\) channels open and Na\(^+\) rushes in, the Na\(^+\) channels close while K\(^+\) channels are opening and K\(^+\) rushes out. The difference is that while these events are occurring the membrane potential remains stable at 0 mV. This happens because, while these ionic currents are flowing, the feedback amplifier is passing currents that are precisely equal in magnitude and opposite in direction to the ionic currents. Thus the currents put out by the voltage clamp feedback amplifier effectively cancel the effects of the net ionic currents on the membrane potential, thus keeping \( E_m \) at the value set by the experimenter.

Since the voltage clamp circuit passes current that at any instant in time is simply equal and opposite to the sum of the membrane ionic currents, the clamp current is a precise measure of the net ionic current flowing at that instant. The clamp current would tell us that the net ionic current that results from clamping the squid giant axon to 0 mV is as shown below:

Note that an early net inward is followed by a net outward current. The current occurs on the same time scale as the normal action potential. This current is the sum of the currents carried by Na\(^+\) and K\(^+\) and whatever other currents flow across the membrane.

Hodgkin and Huxley used various experimental manipulations to separate the Na\(^+\) current from the K\(^+\) current. They replaced most of the NaCl in the external solution by choline chloride,
so that the concentrations of NaCl in the axoplasm and the bathing solution were the same. Then when the axon was clamped to zero mV there was no concentration force on Na\(^+\) and no electrical force on Na\(^+\), hence no movement of Na\(^+\). This left only the outward K\(^+\) current as shown here.

In an axon with normal external medium the inward Na\(^+\) current also vanished when the membrane potential was clamped to +65 mV, the Na\(^+\) equilibrium potential (figure below). If the axon was clamped to greater than +65, there was then a net tendency for Na\(^+\) to leave the axon and the Na\(^+\) current hump reversed to become an outward current. Thus +65 mV is said to be the "reversal potential" for the Na\(^+\) current.

Hodgkin and Huxley clamped the squid giant axon to various membrane potentials. At each potential they used techniques like those just described to separate the Na\(^+\) current from the K\(^+\) current. They were then in a position to reconstruct the ionic currents that flow during an actual action potential when the membrane is not clamped. Knowing the currents for Na\(^+\) and K\(^+\) and the driving forces for each ion, they used the relations

\[
I_{Na} = g_{Na}(E_m - E_{Na}) \quad \text{and} \quad I_{K} = g_{K}(E_m - E_{K})
\]

to calculate the changes in the ionic conductances, \(g_{Na}\) and \(g_{K}\), that occur during the action potential.

Action potentials in mammalian nerve axons and in mammalian skeletal muscle cells are remarkably similar to those in squid giant axon. However, as you will learn later in the course, action potentials in certain other types of cells are somewhat more complex than the action potential of the squid giant axon. For example, action potentials in the cells of the ventricles of mammalian hearts involves Ca\(^{++}\) currents as well as Na\(^+\) and K\(^+\) currents. Action potentials in mammalian smooth muscle cells also involve Ca\(^{++}\) currents. In all these cases the application of the voltage clamp technique has helped to define the ionic mechanisms of the action potentials.