PHYSIOEX 3.0 EXERCISE 18B: NEUROPHYSIOLOGY OF NERVE IMPULSES

Objectives
1. To define the following: irritability, conductivity, resting membrane potential, polarized, sodium-potassium pump, threshold stimulus, depolarization, action potential, repolarization, hyperpolarization, absolute refractory period, relative refractory period, nerve impulse, synaptic cleft, compound action potential, conduction velocity.
2. To list at least four different stimuli capable of generating an action potential.
3. To list at least two agents capable of inhibiting an action potential.
4. To describe the relationship between nerve size and conduction velocity.
5. To describe the relationship between nerve myelination and conduction velocity.

Neurons have two major physiologic properties: irritability, or the ability to respond to stimuli and convert them into nerve impulses, and conductivity, the ability to transmit an impulse (in this case, to take the neural impulse and pass it along the cell membrane.) In the resting neuron (i.e. a neuron that does not have any neural impulses), the exterior of the cell membrane is positively charged and the interior of the neuron is negatively charged. This difference in electrical charge across the plasma membrane is referred to as the resting membrane potential and the membrane is said to be polarized. The sodium-potassium pump in the membrane maintains the difference in electrical charge established by diffusion of ions. This active transport mechanism moves 3 sodium ions out of the cell while moving in 2 potassium ions. Therefore, the major cation outside of the cell in the extracellular fluid is sodium, while the major cation inside of the cell is potassium. The inner surface of the cell membrane is more negative than the outer surface, mainly due to intracellular proteins, which, at body pH, tend to be negatively charged.

The resting membrane potential can be measured with a voltmeter by putting a recording electrode just inside of the cell membrane with a reference, or ground, electrode outside of the membrane. In the giant squid axon, (where most early neural research was conducted), or in the frog axon that will be used in this exercise, the resting membrane potential is measured at –70 mv. (In humans, the resting membrane potential typically measures between -40 mv to -90mv.)

When a neuron is activated by a stimulus of adequate intensity, known as a threshold stimulus, the membrane at its trigger zone, typically the axon hillock, briefly becomes more permeable to sodium ions (sodium gates in the cell membrane open.) Sodium ions rush into the cell, increasing the number of positive ions inside of the cell and changing the membrane polarity. The interior surface of the membrane becomes less negative and the exterior surface becomes less positive, a phenomenon called depolarization. When
depolarization reaches a certain called **threshold**, an **action potential** is initiated and the polarity of the membrane reverses.

When the membrane depolarizes, the resting membrane potential of -70 mv becomes less negative. When the membrane potential reaches 0 mv, indicating there is no charge difference across the membrane, the sodium ion channels close and potassium ion channels open. By the time the sodium ions channels finally close, the membrane potential has reached +35 mv. The opening of the potassium ion channels allows potassium ions to flow out of the cell down their electrochemical gradient-- remember that like ions are repelled from each other. The flow of potassium ions out of the cell causes the membrane potential to move in a negative direction. This is referred to as **repolarization**. This repolarization occurs within a millisecond of the initial sodium influx and reestablishes the resting membrane potential. Actually, by the time the potassium gates close, the cell membrane has undergone a **hyperpolarization**, slipping to perhaps –75 mv. With the gates closed, the resting membrane potential is quickly returned to the normal resting membrane potential.

When the sodium gates are open, the membrane is totally insensitive to additional stimuli, regardless of the force of stimulus. The cell is in what is called the **absolute refractory period**. During repolarization, the membrane may be stimulated if a very strong stimulus is used. This period is called the **relative refractory period**.

The action potential, once started, is a self-propagating phenomenon, spreading rapidly along the neuron membrane. The action potential follows the **all-or-none** law, in which the neuron membrane either depolarizes 100% or not at all. In neurons, the action potential is also called a **nerve impulse**. When it reaches the axon terminal, it triggers the release of neurotransmitters into a gap, known as the **synaptic cleft**. Depending on the situation, the neurotransmitter will either excite or inhibit the postsynaptic neuron.

In order to study nerve physiology, we will use a frog nerve and several electronic instruments. The first instrument we will use is the **electronic stimulator**. Nerves can be stimulated by chemicals, touch or electric shock. The electronic stimulator administers an electric shock that is pure DC, and allows duration, frequency, and voltage of the shock to be precisely controlled. The stimulator has two output terminals; the positive terminal is red and the negative terminal is black. Voltage leaves the stimulator via the red terminal, passes through the item to be stimulated (in this case, the nerve), and returns to the stimulator at the black terminal to complete the circuit.

The second instrument is the **oscilloscope**, an instrument that measures voltage changes over a period of time. The face of the oscilloscope is similar to a black-and-white TV screen. The screen of the oscilloscope is the front of a tube with a filament at the other end. The filament is heated and gives off a beam of
electrons. The beam passes to the front of the tube. Electronic circuitry allows for the electron beam to be brought across the screen in pre-set time intervals. When the electrons hit the phosphorescent material on the inside of the screen, a spot on the screen will glow. When we apply a stimulus to a nerve, the oscilloscope screen will display one of the following three results: no response, a flat line, or a graph with a peak. A graph with a peak indicates that an action potential has been generated.

While performing the following experiments, keep in mind that you are working with a nerve, which consists of many neurons— you are not just working with a single neuron. The action potential you will see on the oscilloscope screen reflects the cumulative action potentials of all the neurons in the nerve, called a compound nerve action potential. Although an action potential follows the all-or-none law within a single neuron, it does not necessarily follow this law within an entire nerve. When you electrically stimulate a nerve at a given voltage, the stimulus may result in the depolarization of most of the neurons, but not necessarily all of them. To achieve depolarization of all of the neurons, a higher stimulus voltage may be needed.

Eliciting a Nerve Impulse

In the following experiments, you will be investigating what kinds of stimuli trigger an action potential. To begin, select Neurophysiology of Nerve Impulses from the main menu. The opening screen will appear in a few seconds. Note that a sciatic nerve from a frog has been placed into the nerve chamber. Leads go from the stimulator output to the nerve chamber. Leads also go from the nerve chamber to the oscilloscope. Notice that these leads are red and black. The stimulus travels along the red lead to the nerve. When the nerve depolarizes, it will generate an electrical impulse that will travel along the red wire to the oscilloscope, and back to the nerve along the black wire.

Activity: Electrical Stimulation

1. Set the voltage at 1.0 V by clicking the (+) button next to the Voltage display.

2. Click Single Stimulus.

Do you see any kind of response on the oscilloscope screen?

If you saw no response, or a flat line indicating no action potential, click the Clear button on the oscilloscope, increase the voltage, and click Single Stimulus again until you see a trace (deflection of the line) that indicates an action potential.
What was the threshold voltage, or the voltage at which you first saw an action potential? _____________ V

Click **Record Data** on the data collection box to record your results.

3. If you wish to print your graph, click **Tools** and then **Print Graph**. You may do this each time after you have generated a graph on the oscilloscope screen.

4. Increase the voltage by 0.5 V and click **Single Stimulus**.

How does this tracing compare to the one trace that was generated at the threshold voltage? (Hint: look very carefully at the tracings.)

What reason can you give for your answer?

Click **Record Data** on the data collection box to record your results.

5. Continue to increase the voltage by 0.5 V and clicking **Single Stimulus** until you find the point beyond which no further increase occurs in the peak of the action potential trace. Record this maximal voltage here: ______________ V

Click **Record Data** to record your results.

Now that we have seen that an electrical impulse can cause an action potential, let's try some other methods of stimulating a nerve.

**Activity: Mechanical Stimulation**

1. Click the **Clear** button on the oscilloscope.

2. Using the mouse, click and drag the glass bar over to the nerve and place it over the nerve. When the glass rod is over the nerve, release the mouse button. This will indicate that the rod is now touching the nerve. What do you see on the oscilloscope screen?

How does this tracing compare with the other tracings that you have generated?
Click **Record Data** to record your results. Leave the graph on the screen so that you can compare it to the graph you will generate in the next activity.

**Activity: Thermal Stimulation**

1. Click on the glass rod and drag it to the heater, releasing the mouse button. Click on the **Heat** button. When the rod turns red, indicating that it has been heated, click and drag the rod over the nerve and release the mouse button. What happens?

How does this trace compare to the trace that was generated with the unheated glass bar?

What explanation can you provide for this?

Click **Record Data** to record your results. Then click **Clear** to clear the oscilloscope screen for the next activity.

**Activity: Chemical Stimulation**

1. Click and drag the dropper from the bottle of sodium chloride (salt solution) over to the nerve in the chamber and then release the mouse button to dispense drops. Does this generate an action potential?

2. Using your threshold setting, stimulate the nerve. Does this tracing differ from the original threshold stimulus tracing?

Explain your answer.

Click **Record Data** to record your results.
3. Click the **Clean** button on top of the nerve chamber. This will return the nerve to its original (non-salted) state. Click **Clear** to clear the oscilloscope screen.

4. Click and drag the dropper from the bottle of hydrochloric acid and drag it over the nerve, and release the mouse button to dispense drops. Does this generate an action potential?

5. Does this tracing differ from the original threshold stimulus?

Why?

Click **Record Data** to record your results.

6. Click on the **Clean** button on the nerve chamber to clean the chamber and return the nerve to its untouched state.

To summarize your experimental results, what kinds of stimuli can elicit an action potential?

You have reached the end of this activity. To continue on to the next activity, click the **Experiment** pull-down menu and select **Inhibiting a Nerve Impulse**.

**Inhibiting a Nerve Impulse**

Numerous physical factors and chemical agents can impair the ability of nerve fibers to function. For example, deep pressure and cold temperature both block nerve impulse transmission by preventing local blood supply from reaching the nerve fibers. Local anesthetics, alcohol and numerous other chemicals are also very effective in blocking nerve transmission. In this activity, we will study the effects of various agents on nerve transmission.

The display screen for this activity is very similar to the screen in the first activity. To the left are bottles of several agents that we will test on the nerve. Keep the tracings you printed out from the first activity close at hand for comparison.
Activity: Testing the Effects of Ether

1. Using the mouse, click and drag the dropper from the bottle marked Ether over to the nerve, in between the stimulating electrodes and recording electrodes. Release the mouse button to dispense drops.

2. Click **Stimulate**, using the voltage setting from the threshold stimulus you used in the earlier activities. What sort of trace do you see?

What has happened to the nerve?

Click **Record Data** to record your results.

3. Click on the **Time(min.)** button on the oscilloscope. The screen will now display activity over the course of 10 minutes (the space between each vertical line representing 1 minute each.) Because of the change in time scale, an action potential will look like a sharp vertical spike on the screen.

4. Click the (+) button under **Interval between Stimuli** on the stimulator and set the timer for 2.0 minutes. This will set the stimulus to stimulate the nerve every two minutes. Click on **Stimulate** to start the stimulations. Watch the **Elapsed Time** display.

   How long does it take for the nerve to return to normal?

5. Click on the **Stop** button to stop this action and to return the elapsed timer to 0.0.

6. Click the **Time(msec)** button on the oscilloscope to return it to its normal millisecond display.

7. Click **Clear** to clear the oscilloscope for the next activity.

8. Click the (-) button under **Interval between Stimuli** until it is reset to 0.00.

Activity: Testing the Effects of Curare

Curare is a well-known plant extract that South American Indians used to paralyze their prey. It is an alpha-toxin which binds to acetylcholine binding sites on the post-synaptic cell membrane, which will prevent the acetylcholine from
acting. Curare blocks synaptic transmission by preventing neural impulses to flow from neuron to neuron.

1. Click and drag the dropper from the bottle marked Curare and position the dropper on the nerve, in between the stimulating and recording electrodes. Release the mouse button to dispense drops.

2. Set the stimulator at the threshold voltage and stimulate the nerve. What effect on the action potential is noted?

What explains this effect?

What do you think would be the overall effect of curare on the organism?

Click **Record Data** to record your results.

3. Click on the **Clean** button on the nerve chamber to remove the curare and return the nerve to its original untouched state.

4. Click **Clear** to clear the oscilloscope screen for the next activity.

**Activity: Testing the Effects of Lidocaine**

Note: Lidocaine is a sodium-channel antagonist.

1. Click and drag the dropper from the bottle marked Lidocaine and position it over the nerve, between the stimulating and recording electrodes. Release the mouse button to dispense drops. Does this generate a trace?

2. Stimulate the nerve at the threshold voltage. What sort of tracing is seen?
Why does lidocaine have this effect on nerve fiber transmission?

Click **Record Data** to record your results.

3. Click on the **Clean** button on the nerve chamber to remove the lidocaine and return the nerve to its original untouched state.

You have reached the end of this activity. To continue on to the next activity, click the **Experiment** pull-down menu and select **Nerve Conduction Velocity**.

**Nerve Conduction Velocity**

As has been pointed out, one of the major physiological properties of neurons is **conductivity**: the ability to transmit the nerve impulse to other neurons, muscles or glands. The nerve impulse, or propagated action potential, occurs when sodium ions flood into the neuron, causing the membrane to depolarize. Although this event is spoken of in electrical terms, and is measured using instruments that measure electrical events, the velocity of the action potential along a neural membrane does not occur at the speed of light. Rather, this event is much slower. In certain nerves in the human, the velocity of an action potential may be as fast as 120 meters per second. In other nerves, conduction speed is much slower, occurring at a speed of less than 3 meters per second.

In this exercise, the oscilloscope and stimulator will be used along with a third instrument, the **bio-amplifier**. The bio-amplifier is used to amplify any membrane depolarization so that the oscilloscope can easily record the event. Normally, when a membrane depolarization sufficient to initiate action potential is looked at, the interior of the cell membrane goes from –70 millivolts to about +40 millivolts. This is easily registered and viewable on an oscilloscope, without the aid of an amplifier. However, in this experiment, it is the change in the membrane potential on the **outside** of the nerve which is being observed. The change which occurs here during depolarization will be so miniscule that it must be amplified in order to be visible on the oscilloscope.

A nerve chamber (similar to the one used in the previous two experiments) will be used. Basically, this is a plastic box with platinum electrodes running across it. The nerve will be laid on these electrodes. Two electrodes will be used to bring the impulse from the stimulator to the nerve and three will be used for recording the membrane depolarization.

In this experiment, we will determine and compare the conduction velocities of different types of nerves. We will examine four nerves: an earthworm nerve,
frog nerve and two rat nerves. The earthworm nerve is the smallest of the four. The frog nerve is a medium-sized myelinated nerve (consult your text's discussion of myelination.) Rat nerve #1 is a medium-sized unmyelinated nerve. Rate nerve #2 is a large, myelinated nerve-- the largest nerve in this group. We will observe the effects of size and myelination on nerve conductivity.

The basic layout of the materials is shown in the opening screen. The two wires (red and black) from the stimulator connect with the top right side of the nerve chamber. Three wires (red, black and a bare wire cable) are attached to connectors on the other end of the nerve chamber and go to the bio-amplifier. The bare cable serves as a “ground reference” for the electrical circuit and provides the reference for comparison of any change in membrane potential. The bio-amplifier is connected to the oscilloscope so that any amplified membrane changes can be observed. The stimulator output, called the “pulse”, has been connected to the oscilloscope so that when the nerve is stimulated, the tracing will start across the oscilloscope screen. Thus, the time from the start of the trace on the left-hand side of the screen (when the nerve was stimulated) to the actual nerve deflection (from the recording electrodes) can be accurately measured. This amount of time, usually in milliseconds, is critical for determining conduction velocity.

Look closely at the screen. The wiring of the circuit may seem complicated, but really is not. First, look at the stimulator, found on top of the oscilloscope. On the left side, red and black wires leave the stimulator to go to the nerve chamber. Remember, the red wire is the “hot” wire that carries the impulse from the stimulator and the black wire is the return to the stimulator that completes the circuit. When the nerve is stimulated, the red recording wire (leaving the left side of the nerve chamber) will pick up the membrane impulse and bring it to the bio-amplifier. The black wire, as before, completes the circuit, and the bare cable wire simply acts as a reference electrode. The membrane potential, picked up by the red wire, is then amplified by the bio-amplifier and the output is carried to the oscilloscope. The oscilloscope then shows the trace of the nerve action potential.

**Activity: Measuring Nerve Conduction Velocity**

1. On the stimulator, click on the **Pulse** button.

2. Turn the bio-amplifier on by clicking the button in the lower left corner of the bio-amplifier and dragging it to the **On** setting.

On the left side of the screen are the four nerves which will be studied. The nerves included are the earthworm, a frog nerve, and two rat nerves of different sizes. The earthworm as a whole is used because it has a nerve running down its ventral surface. A frog nerve is used as the frog has long been the animal of choice in many physiology laboratories. The rat nerves are used so that you may
compare a) the conduction velocity of different sized nerves, and b) the conduction velocity of a myelinated versus unmyelinated nerve. Remember the frog nerve is myelinated, and that rat nerve #1 is the same size as the frog nerve, but unmyelinated. Rat nerve #2, the largest nerve of the bunch, is myelinated.

3. Using the mouse, click and drag the dropper from the bottle labeled Ethanol over the earthworm and release the mouse button to dispense drops of ethanol. This will narcotize the worm so it does not move around during the experiment, but will not affect nerve conduction velocity. The alcohol is at a low enough percentage that the worm will be fine and back to normal within 15 minutes.

4. Click and drag the earthworm into the nerve chamber. Be sure the worm is over both of the stimulating electrodes and all 3 of the recording electrodes.

5. Using the (+) button next to the Voltage display, set the voltage to 1.0 V. Then click Stimulate to stimulate the nerve. Do you see an action potential? If not, increase the voltage by increments of 1.0 V until a trace is obtained. At what threshold voltage do you first see an action potential generated? __________ V

6. Next, click on the Measure button located on the stimulator. You will see a vertical yellow line appear on the far left edge of the oscilloscope screen. Now click the (+) button under the Measure button. This will move the yellow line to the right. This line lets you measure how much time has elapsed on the graph at the point that the line is crossing the graph. You will see the elapsed time appear on the Time (msec) display on the stimulator. Keep clicking (+) until the yellow line is right at the point in the graph where the graph ceases being a flat line and first starts to rise.

7. Once you have the yellow line positioned at the start of the graph’s ascent, note the time elapsed at this point. Click Record Data to record this time on the data collection graph. PhysioEx will automatically compute the conduction velocity for you, based on this data. Note that the data collection box includes a Distance (mm) column and that this distance is always 43mm. This is the distance between the red stimulating wire to the red recording wire. In a wet lab, you would have to measure this distance yourself before you could proceed with calculating the conduction velocity.

It is very important that you have the yellow vertical measuring line positioned at the start of the graph’s rise before you click Record Data-- otherwise, the conduction velocity calculated for the nerve will be inaccurate.

8. Fill in the data under the Earthworm column on the following chart:
<table>
<thead>
<tr>
<th>Nerve</th>
<th>Earthworm (small nerve)</th>
<th>Frog (medium nerve, myelinated)</th>
<th>Rat Nerve #1 (medium nerve, unmyelinated)</th>
<th>Rat Nerve #2 (large nerve, myelinated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threshold voltage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elapsed time from stimulation to action potential</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conduction Velocity</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

9. Click and drag the earthworm to its original place. Click **Clear** to clear the oscilloscope screen.

10. Repeat steps 4-9 for the remaining nerves. Remember to click **Record Data** after each experimental run and to fill in the chart for question #8.

Which nerve in the group has the slowest conduction velocity?

What was the speed of the nerve?

Which nerve in the group of 4 has the fastest conduction velocity?

What was the speed of the nerve?

What is the relationship between nerve size and conduction velocity?
Based on the results, what is your conclusion regarding conduction velocity and whether the nerve is myelinated or not?

What is the major reason for the differences seen in conduction velocity between the myelinated nerves and the unmyelinated nerves?

**Activity: Printing Your Data**

When you have finished with all experiments, you can print your recorded data sets and turn them in if your instructor requests them. Simply go to **Tools**, and select **Print Data**. Remember, you may also print the graphs you generate on the oscilloscope screen by going to **Tools** and then selecting **Print Graph**.