exercise

3

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

The nervous system is responsible for most of the functions that characterize higher organisms, such as muscular movement, awareness, thought, learning, and memory. **Neurons** are the functional cellular units of the nervous system. They are "excitable" cells that communicate by transmitting electrical impulses ("excitable" means that they are capable of producing large, rapid electrical signals called **action potentials**). Neurons are specialized for receiving, integrating, and transmitting information to other neurons and/or effector cells. A typical neuron consists of a *cell body*, containing its nucleus and organelles; *dendrite(s)*, responsible for carrying nerve impulses toward the cell body; and an *axon*, responsible for carrying nerve impulses away from the cell. Junctions between cells are called *synapses*, where one cell (the *presynaptic* cell) releases a chemical messenger called a *neurotransmitter* that communicates with the dendrite or cell body of a *postsynaptic* cell. While synaptic transmission is usually thought of as being excitatory (inciting an action potential in the postsynaptic cell), some are *inhibitory*. This is accomplished by causing the postsynaptic cell to become *hyperpolarized*, or having a resting membrane potential that is more negative than the normal resting membrane potential.

Neurons have two major physiologic properties: **irritability,** 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 (that is, a neuron that is neither receiving nor transmitting any signals), 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 three sodium ions out of the cell while moving in two potassium ions. Therefore, the major cation in the extracellular fluid outside of the cell 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 the cell membrane and by placing a reference, or ground, electrode outside of the membrane. In the giant squid axon (where most early neural research was conducted), and in the frog axon that will be used in this exercise, the resting membrane potential is −70 mv. (In humans, the resting membrane potential typically measures between −40 mv to −90 mv).

When a neuron is activated by a stimulus of sufficient 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 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 that 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 their force. The cell is in what is called the **absolute refractory period.** During repolarization, the membrane may be stimulated by a very strong stimulus. 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 an **electronic stimulator.** Nerves can be stimulated by chemicals, touch, or electric shock. The electronic stimulator administers an electric shock that is pure DC, and it allows the 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 an **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 back. The filament is heated and gives off a beam of electrons that passes to the front of the tube. Electronic circuitry allows for the electron beam to be brought across the screen at preset 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

The **excitability** of a neuron—its ability to generate action potentials—is what allows neurons to perform their functions. In the following experiments you will be investigating what kinds of stimuli trigger an action potential. To begin, follow the instructions for starting PhysioEx in the "Getting Started" section at the front of this manual. From the main menu, select **Neurophysiology of Nerve Impulses.** The opening screen will appear in a few seconds (see Figure 3.1). 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, and 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 1: 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 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.

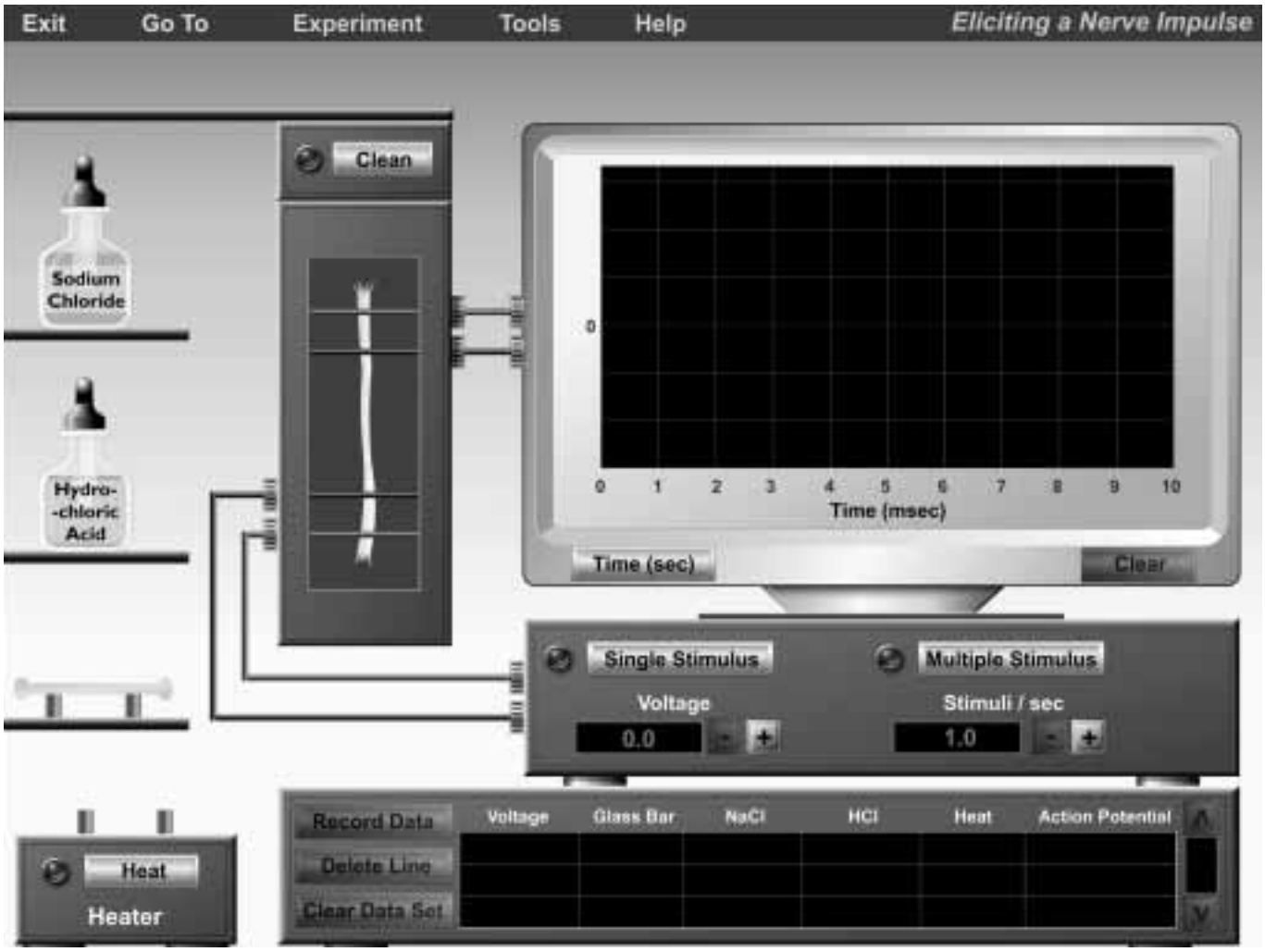

Figure 3.1 Opening screen of the Eliciting a Nerve Impulse experiment.

5. Continue increasing 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 you have seen that an electrical impulse can cause an action potential, let's try some other methods of stimulating a nerve. ■

Activity 2:
Mechanical Stimulation

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

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

_____

How does this tracing compare with the other tracings 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 3:
Thermal Stimulation

1. Click on the glass rod and drag it to the heater, and then release the mouse button. Click 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 4:
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 voltage setting, stimulate the nerve. Does this tracing differ from the original threshold stimulus tracing? If so, how?

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 over to the nerve, and release the mouse button to dispense drops. Does this generate an action potential?

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

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

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

7. Click **Tools** → **Print Data** to print your data.

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

The local environment of most neurons is controlled so that the neurons are protected from changes in the composition of the interstitial fluid. However, 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 (Figure 3.2). To the left are bottles of three agents that we will test on the nerve. Keep the tracings you printed out from the first activity close at hand for comparison.

Activity 5:
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 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 represents 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 to set the interval to 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 the **Stop** button to stop this action and to return the elapsed time to 0.00.

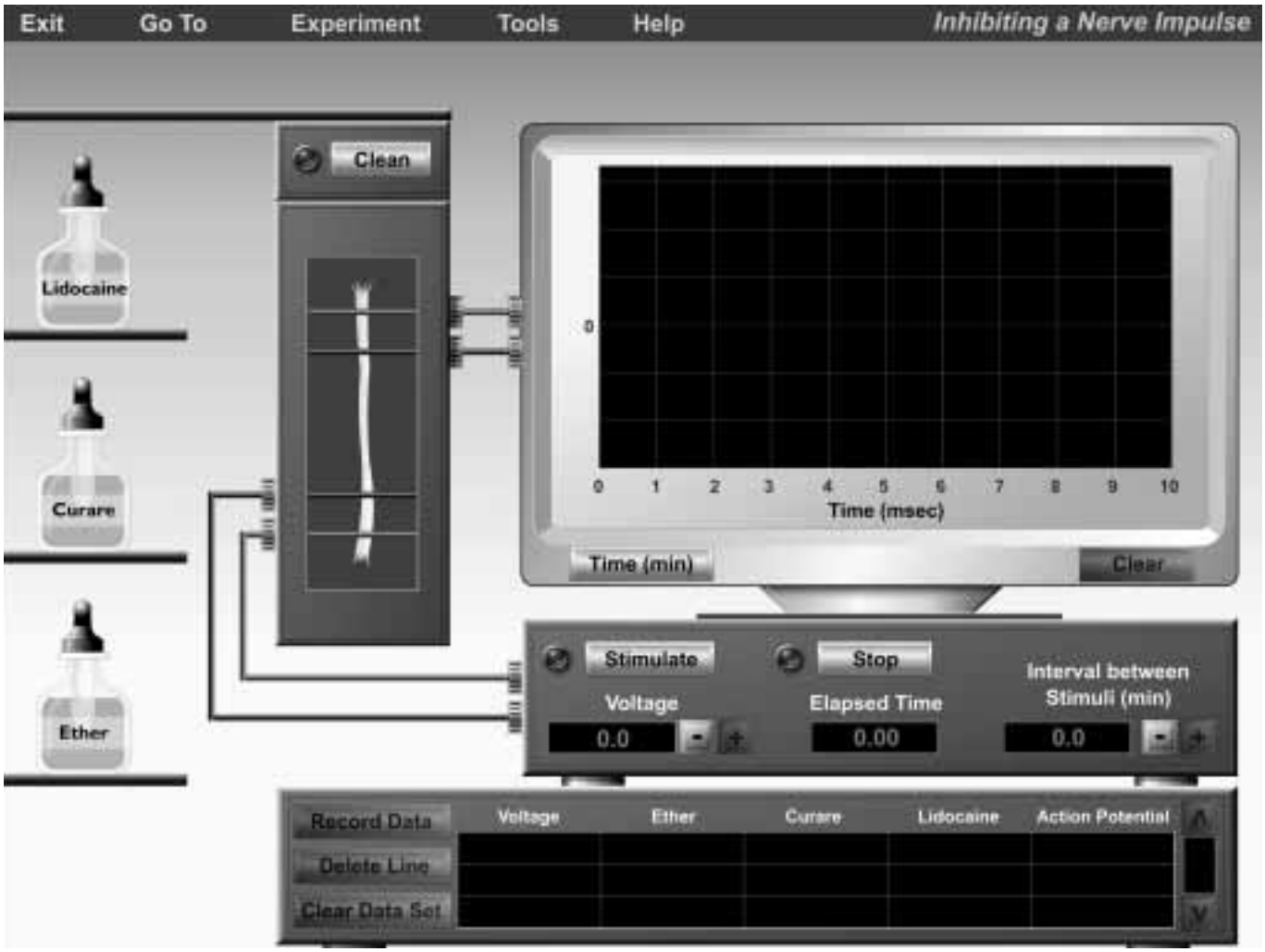

Figure 3.2 Opening screen of the Inhibiting a Nerve Impulse experiment.

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 0.0.

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

Activity 6:
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 that binds to acetylcholine binding sites on the postsynaptic cell membrane, which prevents 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, 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 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 7:
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?

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2. Stimulate the nerve at the threshold voltage. What sort of tracing is seen?

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Why does lidocaine have this effect on nerve fiber transmission?

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

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

4. Click **Tools** → **Print Data** to print your data.

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

The speed of transmission of information in the nervous system depends in part on the conduction velocity of the axon, carrying information from the cell body to the next cell. Conduction velocity depends on the size, or diameter, of the axon, and whether or not it is myelinated. 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 an action potential occurs, 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 that is being observed. The change that occurs here during depolarization is 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 electrodes will be used for recording the membrane depolarization.

In this experiment we will measure and compare the conduction velocities of different types of nerves. We will examine four nerves: an earthworm nerve, a 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. Rat 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 Figure 3.3. 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 bottom left side 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 measured in milliseconds, is critical for determining conduction velocity.

Look closely at the screen. The wiring of the circuit may seem complicated, but it really is not. First, look at the stimulator, located 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.

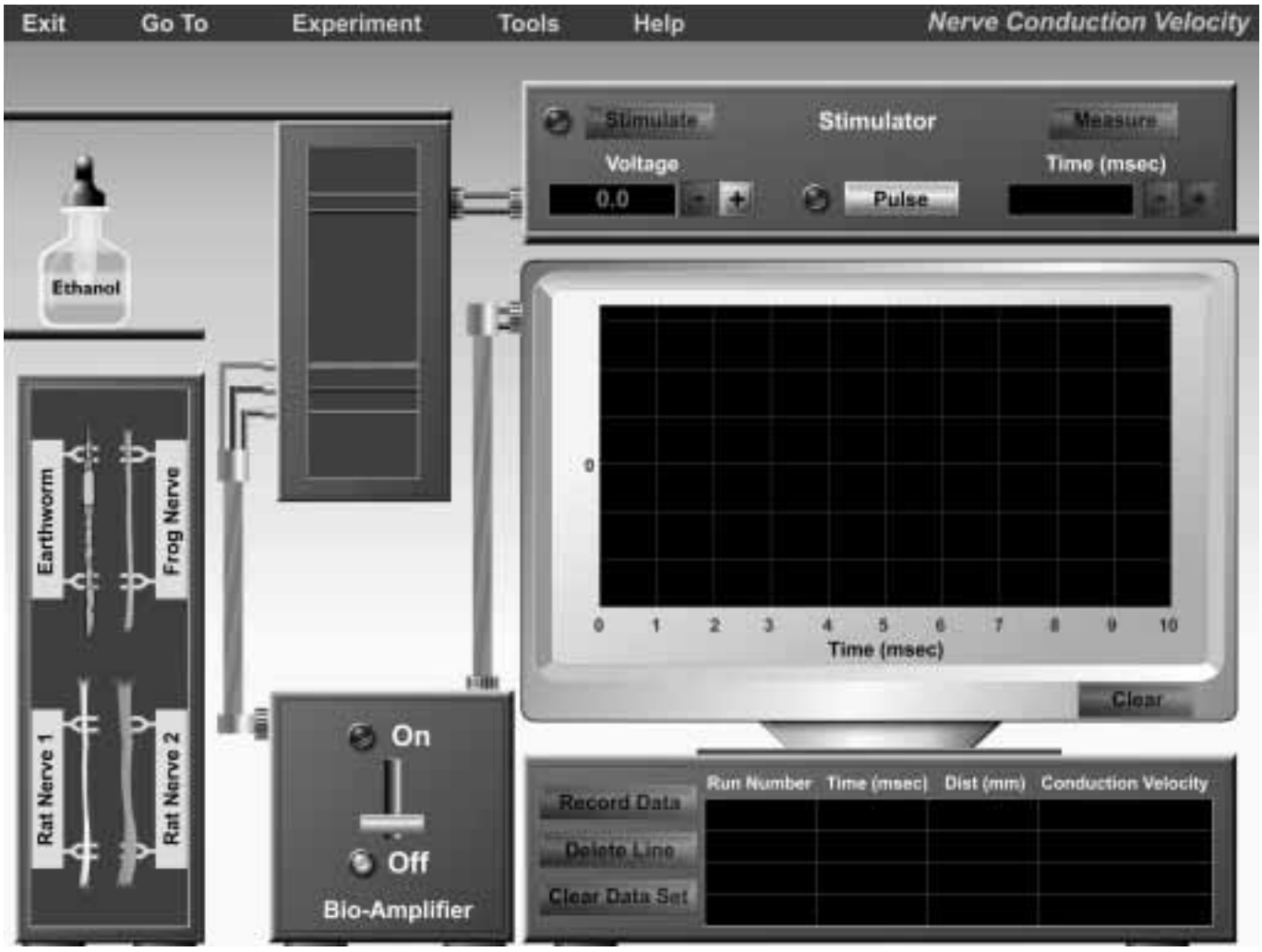

Figure 3.3 Opening screen of the Nerve Conduction Velocity experiment.

Activity 8:
Measuring Nerve Conduction Velocity

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

2. Turn the bio-amplifier on by clicking the horizontal bar on the bio-amplifier and dragging it to the **On** setting.

On the left side of the screen are the four nerves that will be studied. The entire earthworm is used because it has a nerve running down its ventral surface. A frog nerve is used because 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 an unmyelinated nerve. Remember that 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 to 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 it not affect nerve conduction velocity. The alcohol is at a low enough concentration 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 three 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 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 43 mm. This is the distance between the red stimulating wire and 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 in the chart below:

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 below.

11. Click **Tools** → **Print Data** to print your data.

Which nerve in the group has the slowest conduction velocity?

_____

What was the speed of the nerve? _____

Which nerve of the four has the fastest conduction velocity?

_____

What was the speed of the nerve? _____

What is the relationship between nerve size and conduction velocity? What are the physiological reasons for this relationship?

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Based on the results, what is your conclusion regarding the effects of myelination on conduction velocity? What are the physiological reasons for your conclusion?

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What are the evolutionary advantages achieved by the myelination of neurons?

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Histology Review Supplement

Turn to p. 153 for a review of nervous tissue.

Nerve	***Earthworm*** **(small nerve)**	***Frog*** **(medium nerve, myelinated)**	***Rat Nerve #1*** **(medium nerve, unmyelinated)**	***Rat Nerve #2*** **(large nerve, myelinated)**
Threshold voltage				
Elapsed time from stimulation to action potential				
Conduction velocity				