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, compound nerve action potential, and 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 Nerve Impulse

Neurons have two major physiological 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 (positively charged ion) outside the cell in the extracellular fluid is sodium, while the major cation inside 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 with a reference, or ground, electrode outside the membrane (see Figure 3.1). 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 $-70$ millivolts (mV). (In humans, the resting membrane potential typically measures between $-40$ mV and $-90$ mV.)

![Figure 3.1](image-url)
Figure 3.2 The nerve impulse. (a) Resting membrane potential (~85 mV). There is an excess of positive ions outside the cell, with Na⁺ the predominant extracellular fluid ion and K⁺ the predominant intracellular ion. The plasma membrane has a low permeability to Na⁺. (b) Depolarization—reversal of the resting potential. Application of a stimulus changes the membrane permeability, and Na⁺ ions are allowed to diffuse rapidly into the cell. (c) Generation of the action potential or nerve impulse. If the stimulus is of adequate intensity, the depolarization wave spreads rapidly along the entire length of the membrane. (d) Repolarization—reestablishment of the resting potential. The negative charge on the internal plasma membrane surface and the positive charge on its external surface are reestablished by diffusion of K⁺ ions out of the cell, proceeding in the same direction as in depolarization. (e) The original ionic concentrations of the resting state are restored by the sodium-potassium pump. (f) A tracing of an action potential.

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 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 (see Figure 3.2b). When depolarization reaches a certain point called threshold, an action potential is initiated (see Figure 3.2c) 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 start to close and potassium ion channels open. By the time the sodium ions channels finally close, the membrane potential
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 (see Figure 3.3). 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, the vertical box on the left side. 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 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?

\[ \text{No} \]

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, that is, the voltage at which you first saw an action potential?

\[ 3 \text{ V} \]

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 you generate 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.)

\[ 3.5 \text{ V produced a slightly stronger action potential} \]
What reason can you give for your answer?

**With increasing voltage, nerve**

**skin nerves are stimulated**

**adding to the compound action potential**

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

5. Continue to increase the voltage by 0.5 V and to click **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: \( \frac{4}{4} \) V

**Why is this considered a maximal voltage?**

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.

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**Activity 2: Mechanical Stimulation**

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

2. Using the mouse, click the glass rod located on the bottom shelf on the left side of the screen, and drag it over to the nerve. When the glass rod is over the nerve, release the mouse button to indicate that the rod is now touching the nerve. What do you see on the oscilloscope screen?

   **an action potential at threshold**

How does this tracing compare with the other tracings that you have generated?

**not threshold for the entire nerve**

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, 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?

pink = cold rod, green = hot rod, white = anesthetic, black = action potential

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

heated glass rod produced a stronger action potential

What explanation can you provide for this?

heat increases membrane permeability for Na\(^+\) & K\(^+\)

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? yes

↑x-cell Na\(^+\) ↓ Na\(^+\) leakage into cells

2. Using your threshold setting, stimulate the nerve.

Click Record Data

Does this tracing differ from the original threshold stimulus tracing?

no

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 (nonsalted) 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? yes

↓ intracellular pH causes proteins to become + charged, raising resting membrane potential + threshold

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?

flat line

What has happened to the nerve? it has been inhibited by ether

Click Record Data to record your results.
Figure 3.4 Opening screen of the Inhibiting a Nerve Impulse experiment.

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). 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 until the timer is set for 2.0 minutes. This will set the stimulus to stimulate the nerve every two minutes. Click on Stimulation to start the stimulations. Watch the Elapsed Time display.

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

6 seconds

5. Click on the Stop button to stop this action and to return the Elapsed Time 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 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 will prevent the acetylcholine from acting. Curare blocks synaptic transmission by preventing the flow of neural impulses 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.
Set the stimulator at the threshold voltage and stimulate the nerve. What effect on the action potential is noted?

What explains this effect? (transmission by injury to ionic channels - this would have no effect on a single nerve during stimulation electrically)

What do you think would be the overall effect of curare on the organism? (neurons would cease communication between many neurons)

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 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?

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. Click Tools → Print Data to print your data.

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

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.

To see the setup for this experiment, click the Experiment pull-down menu and select Nerve Conduction Velocity (Figure 3.5). 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 mV to about +40 mV. 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 will be so minuscule 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. The design is basically 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, 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. 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 3.5. 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 actually 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 cham-
ber. 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 8:**

**Measuring Nerve Conduction Velocity**

1. On the stimulator, click 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 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 it 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 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 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 fit, 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?

\[ 5 \text{ V} \]

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 the elapsed time on the data collection graph. PhysioEx will automatically compute the conduction velocity based on this data. Note that the data collection box includes a Distance (mm) column and that the distance is always 43 mm. This is the distance from the red stimulating wire to the red recording wire. In a wet lab, you would have to measure the distance yourself before you could proceed with calculating the conduction velocity.

It is 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 chart below:

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

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

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

Which nerve in the group has the slowest conduction velocity?

\[ \text{earthworm} \]

What was the speed of the nerve? 8.85 m/sec

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

\[ \text{rat 2 - myelinated} \]

What was the speed of the nerve? 45.74 m/sec

What is the relationship between nerve size and conduction velocity? small diameter is slower than medium diameter is slower than large diameter

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

\[ \text{myelinated fibers are faster than unmyelinated fibers} \]
What is the major reason for the differences seen in conduction velocity between the myelinated nerves and the unmyelinated nerves?

Myelinated = saltatory conduction
Unmyelinated = continuous conduction

History Review Supplement
Turn to p. P-143 for a review of nervous tissue.