Laboratory Exercise S-2

Nerve Impulse Stimulation

Purpose of the Exercise

To review the characteristics of a nerve impulse and to investigate the effects of certain stimuli on a nerve.

Materials Needed

Live frog
Dissecting tray
Dissecting instruments
Frog Ringer's solution
Electronic stimulator
Filter paper
Glass rod
Glass plate
Ring stand and ring
Microscope slides
Bunsen burner
Ice
1% HCl
1% NaCl
Ph.I.L.S. 4.0

For Learning Extension Activity:
2% Novocain solution (procaine hydrochloride)

Safety

- Wear disposable gloves when handling the frogs and chemicals.
- Keep loose hair and clothes away from the Bunsen burner.
- Wear heat-resistant gloves when heating the glass rod.
- Dispose of gloves, frogs, and chemicals as instructed.
- Wash your hands before leaving the laboratory.

Learning Outcomes

After completing this exercise, you should be able to

1. Determine the threshold voltage to stimulate a nerve impulse.
2. Test the effects of various factors on a nerve-muscle preparation.
3. Summarize four types of factors that can stimulate a nerve impulse.
4. Define a compound action potential.
5. Diagram and label a compound action potential.
6. Illustrate *recruitment* by observing the change in amplitude in nerve response.
7. Determine *maximum compound action* by observing no further increase in the amplitude of the compound action potential.
8. Measure the amplitude of an action potential.
9. Integrate the concepts of threshold, recruitment, and maximum stimulation to the nerves of the body.

Pre-Lab

1. Carefully read the introductory material and examine the entire lab.
2. Be familiar with the characteristics of action potentials from lecture or the textbook.

A nerve cell usually is polarized due to an unequal distribution of ions on either side of its membrane. When such a polarized membrane is stimulated at or above its threshold intensity, a wave of action potentials is triggered to move in all directions away from the site of stimulation. This wave constitutes a nerve impulse, and if it reaches a muscle, the muscle may respond by contracting.

A nerve, such as the sciatic nerve, is composed of bundles of many axons of nerve cells. It is possible to regulate the amount of neural stimulation that is sent by a nerve by varying the number of axons within the nerve that are stimulated. This can be observed experimentally by applying electrical stimulation, of increasing voltage, to a nerve (sciatic nerve). The collective voltage change produced by the individual nerve cells of the nerve can be measured and is referred to as the *combined action potential* (CAP). Three physiological events can be viewed when a nerve is stimulated with increasing voltage, including: (1) *threshold* (the most responsive axons are stimulated, resulting in the initial production of a combined action potential), (2) *recruitment* (additional axons in the nerve are stimulated, resulting in a larger combined action potential), and (3) *maximum combined action potential* (all axons in the nerve are stimulated, resulting in the largest amplitude of the combined action potential).

Procedure A—Live Frog Exercise

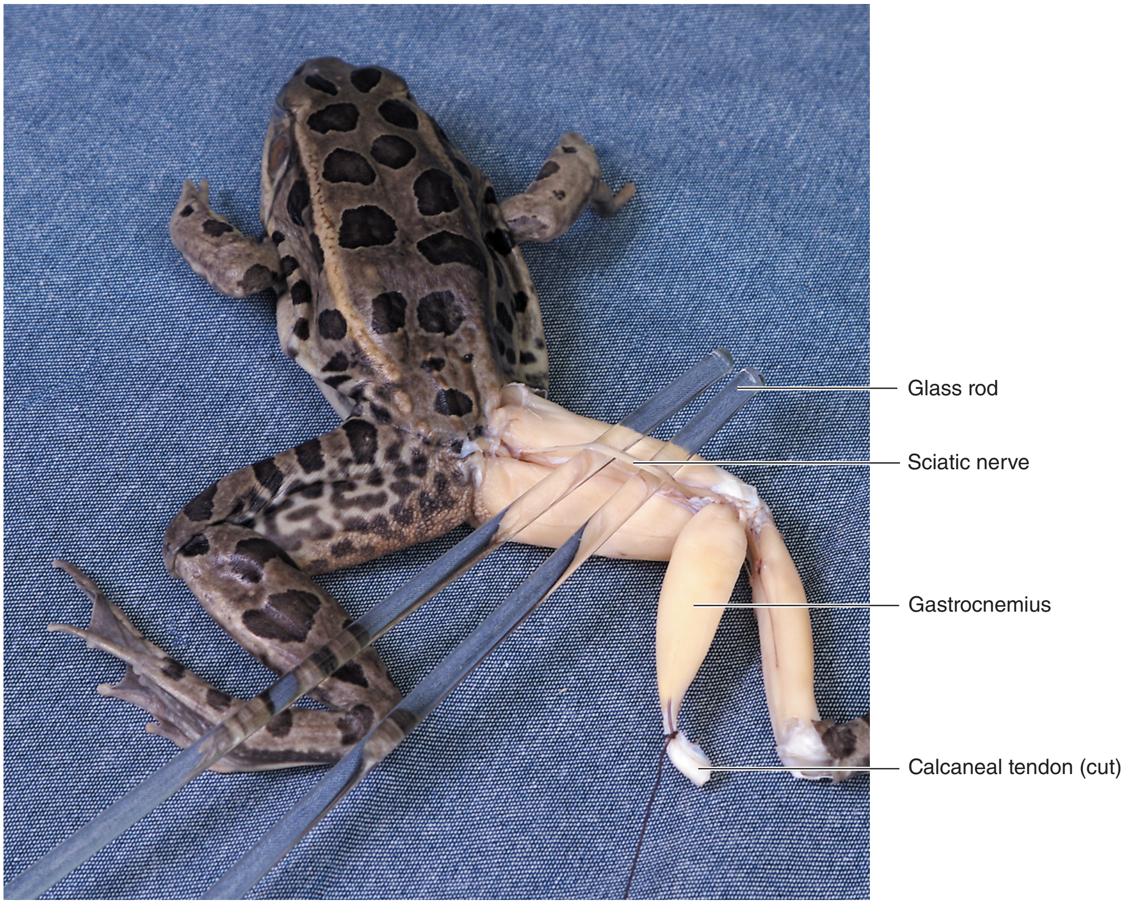
1. Obtain a live frog, and pith its brain and spinal cord as described in Procedure B of Laboratory Exercise S-1.

Alternative Procedure

An anesthetizing agent, tricaine methane sulfonate, can be used to prepare frogs for this lab. This procedure eliminates the need to pith frogs.

2. Place the pithed frog in a dissecting tray and remove the skin from its hindlimb, beginning at the waist, as described in Procedure B of Laboratory Exercise S-1. (As the skin is removed, keep the exposed tissues moist by flooding them with frog Ringer's solution.)
3. Expose the frog's sciatic nerve. To do this, follow these steps:
   a. Use a glass rod to separate the gastrocnemius muscle from the adjacent muscles.
   b. Locate the calcaneal (Achilles) tendon at the distal end of the gastrocnemius, and cut it with scissors.
   c. Place the frog ventral surface down, and separate the muscles of the thigh to locate the sciatic nerve. The

FIGURE S-2.1 The sciatic nerve appears as a silvery white thread between the muscles of the thigh.



nerve will look like a silvery white thread passing through the thigh, dorsal to the femur (fig. S-2.1).

   d. Dissect the nerve to its origin in the spinal cord.
   e. Use scissors to cut the nerve at its origin, and carefully snip off all of the branch nerves in the thigh, leaving only its connection to the gastrocnemius muscle.
   f. Use a scalpel to free the proximal end of the gastrocnemius.
   g. Carefully remove the nerve and attached muscle, and transfer the preparation to a glass plate supported on the ring of a ring stand.
   h. Use a glass rod to position the preparation so that the sciatic nerve is hanging over the edge of the glass plate. (Be sure to keep the preparation moistened with frog Ringer's solution at all times.)

4. Determine the threshold voltage and the voltage needed for maximal muscle contraction by using the electronic stimulator, as described in Procedure C of Laboratory Exercise S-1.
5. Expose the cut end of the sciatic nerve to each of the following conditions, and observe the response of the gastrocnemius muscle. Add frog Ringer's solution after each of the experiments.
   a. Firmly pinch the end of the nerve between two glass microscope slides or pinch using forceps.
   b. Touch the cut end with a glass rod that is at room temperature.
   c. Touch the cut end with a glass rod that has been cooled in ice water for 5 minutes.
   d. Touch the cut end with a glass rod that has been heated in the flame of a Bunsen burner. Wear heat-resistant gloves for this procedure.
   e. Dip the cut end in 1% HCl.
   f. Dip the cut end in 1% NaCl.
6. Complete Part A of Laboratory Assessment S-2.

Learning Extension Activity

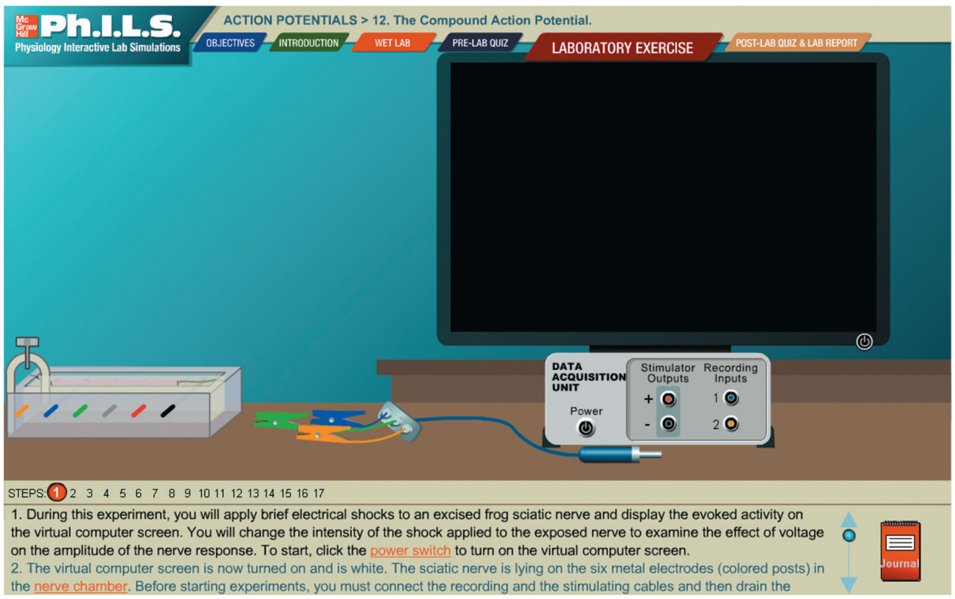
Test the effect of Novocain on a frog sciatic nerve. To do this, follow these steps:

1. Place a nerve-muscle preparation on a glass plate supported by the ring of a ring stand, as before.
2. Use the electronic stimulator to determine the voltage needed for maximal muscle contraction.
3. Saturate a small piece of filter paper with 2% Novocain solution, and wrap the paper around the midsection of the sciatic nerve.
4. At 2-minute intervals, stimulate the nerve, using the voltage needed for maximal contraction until the muscle fails to respond.
5. Remove the filter paper, and flood the nerve with frog Ringer's solution.
6. At 2-minute intervals, stimulate the nerve until the muscle responds again. How long did it take for the nerve to recover from the effect of the Novocain?

Procedure B: Ph.I.L.S. Lesson 12 Action Potentials: The Compound Action Potential

1. Open Exercise 12, Action Potentials: The Compound Action Potential.
2. Read the objectives and introduction and take the pre-lab quiz.
3. After completing the pre-lab quiz, read through the wet lab.
4. The lab exercise will open when you have completed the wet lab (fig. S-2.2).

FIGURE S-2.2 Opening screen for the laboratory exercise on Action Potentials: The Compound Action Potential.



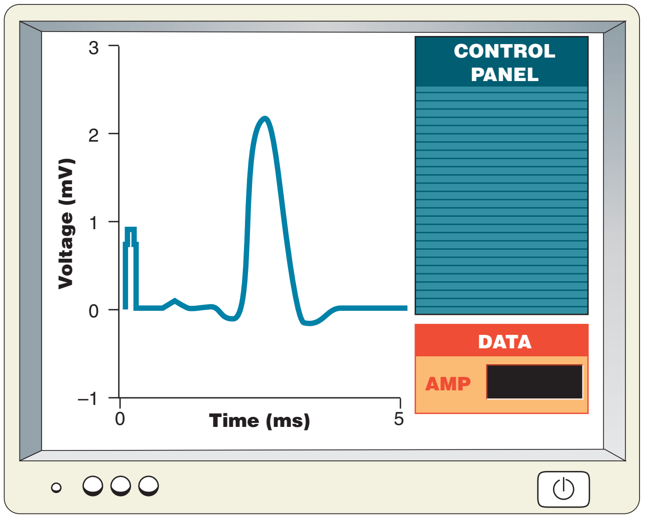
Setup

5. Follow the instructions at the bottom of the screen to turn on the power of the Virtual Computer Screen, the Data Acquisition Unit, to connect the recording cables, and connect the stimulating cables.
6. Drain the saline from the chamber as instructed on the screen.

Compound Action Potential

7. With the stimulus voltage already set at 1.0 volts, click the Start button. (A graph will appear with a red line.)
8. Measure the voltage of the combined action potential by positioning the crosshairs (using the mouse) at the top of the wave. If it is not in the correct location, reposition by dragging it to a new position. Now position the crosshairs at the bottom of the deflection (after the wave or 0 volts) and click. The result will display in the (blue) data panel. Voltage (mV) (on the y-axis) is the change in voltage of all stimulated nerve cells in the sciatic nerve.
9. Click the Journal panel (red rectangle at bottom right of screen) to enter your value into the Journal. A table with volts and amplitude (amp) and a graph will appear. Note the range in values for volts from 0 to 1.6 volts.
10. Close the Journal window by clicking on the X in the right-hand corner.
11. To complete the table and produce the graph,
    a. Click Erase.
    b. Set voltage (for example, 1.1 volts).
    c. Click Shock.
    d. Measure.
    e. Click Journal.
    f. Repeat letters a–e (Ph.I.L.S. steps 13–16), except set voltage at a new value. Complete all values in the table (range from 0 to 1.6 in increments of 0.1 volts).
12. After finishing the laboratory exercise you can print the line tracing by clicking on the P in the bottom left of the monitor screen (fig. S-2.3).

FIGURE S-2.3 An example of a graph to show the effect of stimulus voltage on the amplitude of the evoked compound action potential (CAP) recorded from a frog sciatic nerve.



Interpreting Results

13. *With the Journal still open on the screen,* answer the questions in Part B of the laboratory assessment. If you accidentally closed the graph, click on the Journal panel (red rectangle at bottom right of screen).
14. Complete the post-lab quiz (click open Post-Lab Quiz & Lab Report) by answering the ten questions on the computer screen.
15. Read the conclusion on the computer screen.
16. You may print the lab report for Action Potentials: The Compound Action Potential.

Laboratory Assessment

S-2

Name ______________________

Date ______________________

Section ______________________

The A corresponds to the indicated outcome(s) found at the beginning of the laboratory exercise.

Nerve Impulse Stimulation

Part A Assessments

Complete the following:

1. What was the threshold voltage for the frog sciatic nerve? 1 (experimental results)

2. What was the voltage needed for maximal contraction of the gastrocnemius muscle? 1 (experimental results)

3. Complete the following table: 2

Factor Tested	Muscle Response	Effect on Nerve
Pinching	(experimental results)	
Glass rod (room temperature)		
Glass rod (cooled)		
Glass rod (heated)		
1% HCl		
1% NaCl		

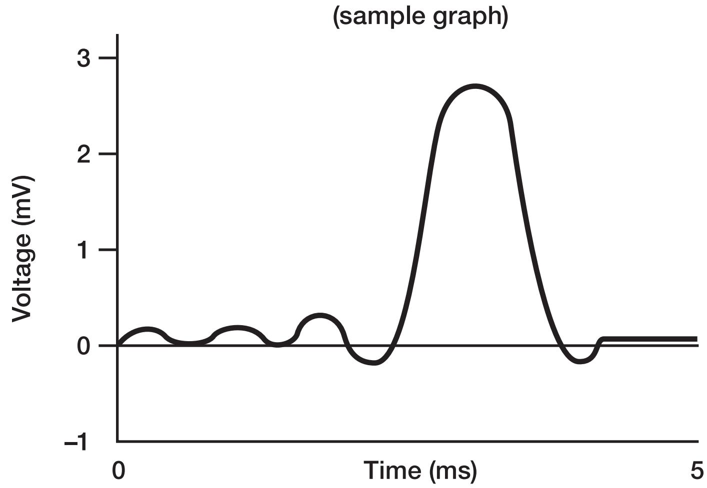
4. Summarize the results of these tests. 3

Answers will vary.

Part B Ph.I.L.S. Lesson 12, Action Potentials: The Compound Action Potential Assessments

1. Define a compound action potential. [4] It is the summation of the action potential of all neurons within a nerve.

2. Diagram a graph of a compound action potential. [5]



3. What was the threshold voltage for stimulation of the sciatic nerve? [1] [8] 0.3 volts

4. What voltage produced the maximum compound action potential? [7] [8] ~1.1 volts

5. What is occurring between the threshold and the maximum compound action potential? [6] [8] recruitment

6. Explain the relationship between increasing voltage stimulation and the recruitment of nerve fibers. [1] [6] [7]

As the voltage increases, the number of neurons stimulating and conducting an action potential increases, causing a larger compound action potential to be generated.

Critical Thinking Assessment

Multiple sclerosis is an autoimmune disorder that involves the destruction of the myelin sheath by the immune system. As a result, the nerve cells affected are no longer able to send impulses. What happens to the maximum combined action potential of the affected nerves? Explain. Since the compound action potential is a summation of action potentials of neurons within a nerve, as the number of neurons affected by multiple sclerosis increases, the number of functioning neurons decreases. With less functioning neurons, the compound action potential decreases.