

Neurophysiology of Nerve Impulses

Marieb Ex 18A

Biol 2402 Lab

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Preparation:

Read the introductory material in your lab manual, Ex 18A to prepare for this lab exercise.

Introduction:

A large nerve such as the sciatic nerve consists of 1000's of individual nerve fibers or **neurons**. The conduction of an electrical impulse along any one neuron results in the production of a small electrical current. When most fibers in a nerve are conducting nerve impulses their combined activity generates a single impulse called a **compound action potential** which is the sum of the individual voltages from all the firing neurons in the nerve. This compound action potential is of considerably larger voltage than that produced by an individual neuron and can therefore be more easily observed and measured.

Each neuron requires a stimulus of sufficient intensity before it will initiate an impulse. This stimulus can be naturally induced by various neurotransmitter chemicals or artificially induced by an electrode. The compound action potential likewise requires a minimal stimulus to be able to detect a response on the recording equipment. The minimum stimulus needed to see a response in a nerve (a group of neurons) is its **threshold**. The stimulus can be increased until all functional neurons in the nerve are firing, thus producing a **maximal compound action potential**. The amount of stimulus needed to achieve maximal stimulation of the nerve is referred to as the **maximal stimulus**.

Dissection: Isolating the frog sciatic nerve:

Changes

To dissect the sciatic nerve, follow the procedure as outlined below rather than that in your lab manual but you may want to refer to fig 18.2 in your lab manual

Rinse the pithed frog in the sink and place the frog on an uncontaminated dissecting pan. Be sure that neither the pan nor the dissecting instruments have been contaminated with preservatives such as formaldehyde. **The instructor will provide you with pans and tools reserved specifically for the dissection of fresh animals.**

It is not necessary to tie down a double-pithed frog. As the sciatic nerve is dissected assign one of your coworkers the task of frequently flooding the surgical area with frog ringers using a small beaker and dropper. The tissue must not dry out or it will be useless.

Lift the skin around the frog's midsection and cut completely around the torso of the frog. fold the edge of skin back and gently peel from the posterior half of the frog. Be careful not to touch the muscles or nerves with contaminated fingers while exposing them or the poisons in the frog's skin secretions may damage some of the cells.

Once the lower half of the frog has been skinned, Rinse the frog and place it in the dissecting pan, dorsal side up. Gently tease the thigh muscles away from the bone with *wooden* probe or toothpicks or plastic forceps. **Do not use metal instruments!!!**

Note: Nerves are extremely delicate and sensitive and can be easily injured by stretching or touching with metal objects or contaminated fingers. Do as much dissecting as you can using non-metal instruments. Use scissors only to cut nerve branches or to remove the nerve once it has been completely exposed and the ends ligated.

The nerve will appear as a white or cream colored thread within the connective tissues between the muscles and bone of the thigh (Fig. 1). It is essential to obtain as long a nerve as possible to complete the experiment; ideally, about 5 cm long.

Follow the nerve anteriorly to its junction with the trunk then alongside the spinal cord to about midway up the back. Carefully, without stretching the nerve, tease it away from the surrounding tissues with toothpicks. You will need to trim ligaments and tendons particularly near the bottom of the urostyle or tailbone. In a like manner, follow the nerve posteriorly from the thigh to the lower leg behind the gastrocnemius muscle and down to the foot. The knee is particularly difficult to dissect because of the large number of ligaments through which the sciatic nerve passes and the many branches of the nerve itself. Carefully cut these ligaments to expose the nerve. Cut away minor nerves that branch off of the main sciatic trunk. As you move posteriorly, the main body of the nerve becomes smaller as more branching occurs. Dissect as far down the lower leg as you can trace the nerve attempting to get up to a 10 cm section of sciatic nerve.

Once the nerve has been completely exposed and freed along its length, take two pieces of thread and tie each end securely being careful not to stretch the nerve. Make note of proximal and distal ends. Cut the nerve beyond each ligature and carefully lift it out of the frog being especially careful not to stretch it. You should end up with a 6 – 10 cm length of nerve with thread attached at both ends (Fig. 2).

Place the nerve across the electrodes in the nerve chamber so that the proximal end (the end nearest the spinal cord) is over the stimulating electrodes and the distal end over the recording electrodes (Fig 3-6).

Make sure the nerve is in direct contact with the stimulus and recording electrodes.

Place a few drops of frog ringers solution into the nerve chamber . **THE LEVEL OF THE FLUID MUST NEVER CONTACT ANY OF THE WIRES IN THE CHAMBER.** If necessary, use a paper towel to carefully remove any large drops of fluid from the electrodes.

Cover the nerve chamber with glass microscope slides.

Equipment Setup (Frog Nerve Physiology)

1. Make sure computer and iWorx interface (black box) are unplugged)
2. Attach the free end of the AMI cable into the Channel 1&2 socket on the iWorx box
3. Plug the free end of the “stimulator cable” into the free outlet of the double banana plug in the stimulator section of the iWorx interface
4. Plug the computer power cord into the power outlet.
5. Plug the iWorx power cord into the outlet
6. Turn on the iWorx interface
7. Turn on the computer
8. After the computer has booted up, click on the iWorx icon
9. Use cursor to select “settings” → choose “load group” in the drop down menu that appears
10. Use cursor to select “AK204” in the box that appears and choose “load” in the same box
11. Use cursor to select “settings” again → choose “Membrane #2”
12. Press the “mode” button on the Channel 1&2 section of the iWorx box until the EMG light is illuminated

Your equipment is now ready for the experiment

Activity: Eliciting a Nerve Impulse:

The iWorx station has been set up for you (settings: AK204; Membrane #2; emg in ch 1)

1. Click the <stimulator> icon in the LabScribe toolbar (Fig 2.14) to display the stimulator panel. check that the amplitude of the shock is 0.25 volts and the pulse width is 0.1 ms.
2. Click <start> then press <enter> on the keyboard to stimulate the nerve with a single shock and to mark your tracing. You should see the stimulus artifact, a compressed “S” shaped curve, at the beginning of the trace and before the compound action potential.
3. click <stop>

If everything is set up properly and the sciatic nerve is in good condition you should see an action potential as in Fig 3-8. Continue with the exercise.

5. If the tracing is upsidedown, rightclick and invert.

Activity: Visualizing the Compound Action Potential:

A. Relation between stimulus Intensity and Compound Action Potential and Maximal Stimulus

1. Click edit → preferences → stimulator in the toolbar to display the stimulator panel and set the amplitude of the stimulus to 0 volts.
2. click <start> on the keyboard to stimulate the nerve with 0 volts.
3. Click <stop>
4. Click edit → preferences → stimulator on the toolbar to change the parameters of the stimulus to 0.05 volts.
5. Repeat steps 2 through 4 above
6. Continue increasing the voltage to the voltages listed in the table on your data sheet and notice that the compound action potential increases in size until it reaches a maximum.
7. When the compound action potential does not appear to increase in amplitude with increasing voltage of stimulus, stop the experiment.
8. Starting with your last recording, click the <2 cursor> icon in the toolbar, click and drag them horizontally to place one on the flat portion of the trace and the second on the peak of the compound action potential as shown in Fig. 3-8.
9. Measure the absolute voltage difference (ignore negative sign if present) between the two cursors in the title bar to the upper right of the window (for example, the value on fig. 3-8 is $V_2 - V_1 = 0.508$ Volts).
10. Scroll back through your recordings repeating the above process, matching the measured amplitude of the compound action potential with the voltage applied. Record your results on the table on your data sheet.
11. Plot the data on a piece of graph paper with the Compound Action Potential voltage on the y-axis and the stimulus voltage on the x-axis.
12. Draw a smooth “best fit” line through your data points
13. Determine the maximal stimulus using this graph.

B. The Velocity of a Nerve Impulse:

The speed of a nerve impulse varies from a few miles per hour to several hundred miles per hour. the velocity of the impulse is a function of neuronal diameter and the amount of insulation , among other factors.

1. Click the stimulator icon in the lab scribe toolbar to display the stimulator panel; set the amplitude to a voltage that produces a compound action potential with a maximum amplitude.
2. Click <1-cursor icon>
3. Type “long pathway” into the Marks field and hit <enter> on the keyboard.
4. Click <start > to stimulate the nerve and observe the compound action potential
5. Click <stop>
6. Remove the clips on the stimulating electrodes and move each by an increment of three electrode bars *toward* the recording electrodes.
7. Click <1-cursor> icon
8. Type “short pathway” into the marks field and hit <enter> on the keyboard.
9. click <start> to stimulate the nerve and observe the compound action potential.
10. Click <stop>.
11. click the <Scopeview > icon in the toolbar, set precision to 5, and *deselect* all sweeps except the last two
12. select “stacked”
13. Your traces should appear similar to fig 3-9 below:

14. Click the <2 cursor> icon in the toolbar; click and drag cursors to the peaks of each response (Fig 3.9) and read the T2-T1 value (in fig 3.9 it is 0.0052s or 52 ms)
15. With a millimeter ruler measure the distance that you moved the stimulator wires from the “long pathway” to the “short pathway” on the nerve chamber (it should be about 15 mm)
16. Calculate the conduction velocity in meters per second:

$$\text{velocity} = (\text{mm stimulus wires moved})/[\text{T2-T1}]$$

16. Convert this value to meters/sec
17. Record this value on your data sheet.

C. Bidirectionality of a nerve.

1. Return the electrodes to their original positions
2. Gently lift the sciatic nerve out of the nerve chamber by using two pairs of forceps to grasp the threads and reverse the ends 180°.
3. Click the <stimulator> icon in the toolbar to display the stimulator panel and set the amplitude of the stimulus to 0 volts.
4. Type “0 volts” in the marks area and press <enter> on the keyboard.
5. click <start> to stimulate the nerve.
6. Click <stop> once the tracing is produced.
7. Determine its threshold and maximal stimulus in the same way that you did earlier and record these results on the data sheet.
8. Use a different color or symbol to graph this data onto the **same graph** that you made in Part A.
9. Describe any similarities and differences between the two graphs

D. Mechanical and chemical stimulation of a nerve

1. Return the nerve to its original orientation in the nerve chamber
2. To investigate mechanical and chemical stimulation unplug the stimulator electrodes from the nerve chamber
3. press <start> then with scissors cut a small portion of the proximal end including the thread ligature off of the nerve.
4. press <stop>
5. Was a nerve impulse generated? If so measure its amplitude as above and record this value on your data sheet
6. press <start> then pinch the free end of the nerve by firmly pinching it with plastic forceps.
7. Press <stop> and describe your results on the data sheet. Again, if a nerve impulse was generated, measure and record its amplitude on the data sheet
8. Chemical stimulation can be tested by applying a drop of 0.01% Hydrochloric acid solution to the free end of the nerve after pressing <start>.
9. Press <stop> and record your results on the data sheet
10. Dip a moistened toothpick into some grains of salt then touch it to the free end of the nerve after pressing <start>.
11. Press <stop> and record your observations on your data sheet.

Activity: Inhibiting the Nerve Impulse:

(skip)

Completing your Lab Exercise

Print and attach a representative example of your compound action potential and write your group number on it. Turn this in with your data sheet

Cleanup and Disposal

Wrap frog and nerve in paper towels and discard in trash can.

Clean all dissecting equipment with soap and water, blot dry with paper towels and return to tray

Disconnect the nerve chamber from your station, pour off the saline and rinse with DI water, place the cleaned nerve chamber on a paper towel at your station.

Turn off the iWorx station.

Remember to spray your work area with disinfectant before leaving the labroom.

Name: _____

Group: _____

Due Date: _____

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Data Sheet

Activity: Visualizing the Compound Action Potential:

Stimulus Voltage versus amplitude of compound action potential (CAP)

Stimulus voltage	0	.05	.10	.20	.40	.60	.80	1.0	1.2	1.4	1.6	1.8	2.0	2.5	3.0	3.5
CAP voltage																

A. Relation between stimulus Intensity and Compound Action Potential and Maximal Stimulus

Stimulus Threshold: _____

Maximal Stimulus Voltage: _____

Maximal CAP: _____

B. The Velocity of a Nerve Impulse:

$T_2 - T_1$: _____ mm between electrodes: _____

Velocity of a nerve impulse (show your work):

How does this compare with values given in lecture or text?

C. Bidirectionality of a nerve.

Stimulus voltage	0	.05	.10	.20	.40	.60	.80	1.0	1.2	1.4	1.6	1.8	2.0	2.5	3.0	3.5
CAP voltage																

Threshold Stimulus Voltage: _____

Maximal Stimulus Voltage: _____

Maximal CAP: _____

Describe and explain the effects of turning the nerve in the nerve chamber compared to your original recordings:

D. Mechanical and chemical stimulation of a nerve

Maximal CAP (from 1st experiment): _____

Treatment	CAP Generated? (yes or no)	Amplitude of CAP (if 'yes')
Cutting Nerve		
Pinching Nerve		
Acid		
Salt		

Describe the similarities and differences between the maximal Compound Action Potential you originally produced and those produced by the following methods:

1. Cutting Nerve

2. Mechanical 'pinching'

3. Acid Treatment:

4. Salt: