

Experiment AN-2: Compound Action Potentials

Equipment Required

PC Computer

IXTA, USB cable, IXTA power supply

NBC Nerve Chamber

iWire-B3G input cable

C-ISO-P5 pin connector-pinjack lead wires (3)

Glass hooks

Pasteur pipettes and bulbs

C-BNC-P2 BNC-dual pinjack stimulator cable

Pinjack-male banana ground cable

Room Temp & Chilled amphibian Ringer's solution

Note – the iWire-B3G needs to be plugged into the IXTA prior to turning it on.

NBC-501 or 402 Nerve Bath Chamber Setup

1. Locate the following items: nerve bath chamber and the C-BNC-P2 stimulator cable.
2. Also, locate the iWire-B3G recording cable and C-ISO-P5 nerve chamber lead wires.

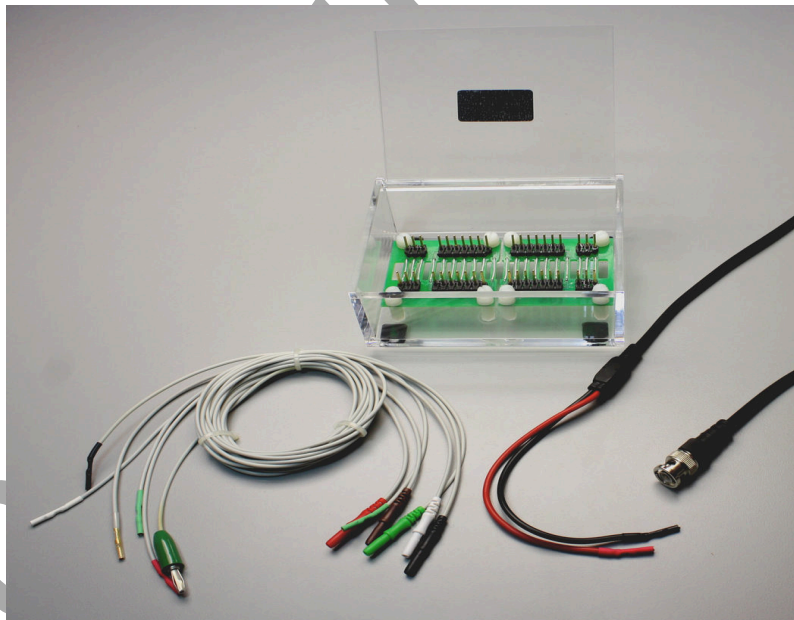


Figure AN-2-S1: The NBC-501 nerve bath chamber.



Figure AN-2-S2: The C-BNC-P2 stimulator cable.



Figure AN-2-S4: The iWire-B3G recording cable.

3. Attach the BNC connector of the C-BNC-P2 stimulator cable to the IXTA Stimulator 1. Place the sockets, at the other end of the stimulator cable, on the closely-spaced electrodes at one end of the NBC-501 or 402 nerve bath chamber. The red socket goes on the positive stimulating electrode (+S), which is the electrode closest to the end of the chamber. The black socket goes on the negative stimulating electrode (-S), on the opposite side of the chamber to avoid the possibility of the short circuit between the two stimulator cables.
4. Insert the connector on the end of the iWire-B3G cable into the iWire 1 input on the front of the IXTA.
5. Attach the red, black, and green C-ISO-P5 nerve chamber lead wires to the corresponding sockets on the iWire-B3G cable. Place the sockets on the ends of the lead wires, on the appropriate electrodes of the nerve bath:
 - The green (C) lead is attached to the electrode (G) between the negative stimulating electrode (-S) and proximal recording electrode (-R).
 - The black (-1) lead is attached to the proximal recording electrode (-R), the recording electrode closest to the ground electrode (G).

- The red (+1) lead is attached to the distal recording electrode (+R) on either the distal end of the nerve or the thread holding the distal end of the nerve in place.



Figure AN-2-S5: The stimulator cable and iWire-B3G cable with three lead wires connected to the IXTA.

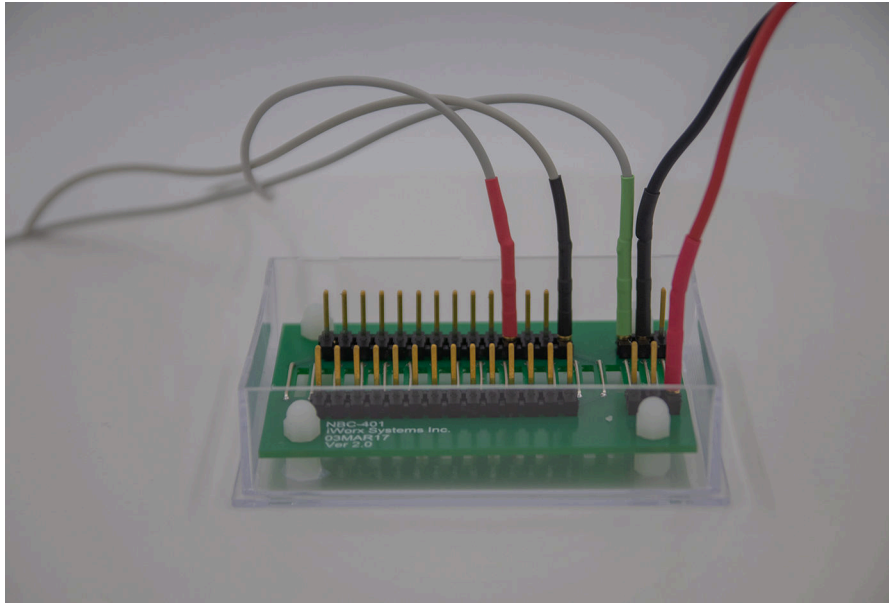


Figure AN-2-S6: The NBC-501 nerve bath chamber with stimulator and recording lead wires attached. Notice that the stimulator leads are attached to the electrodes that are spaced closer together.

Note: When the positive and negative recording electrodes are on the same healthy section of a nerve, a biphasic compound action potential (CAP) is recorded. The upward deflection of the biphasic CAP occurs when the potentials propagating down the axons in the nerve pass the first (negative) recording electrode, and are recorded in reference to the voltage at the second (positive) recording electrode. The downward deflection of the biphasic CAP occurs when the potentials propagating down the axons pass the second (positive) recording electrode, and are recorded in reference to the voltage at the first (negative) recording electrode. When a biphasic CAP is recorded, the A α fibers are usually the only fiber type seen in the CAP. When a monophasic CAP is recorded, other fiber types (A β , A γ , A δ , C) may appear in the recording in addition to the A α fibers.

To record a monophasic CAP move the positive recording electrode from the healthy section of nerve to the thread holding the nerve in place. B fibers, which are preganglionic autonomic types, are not found in the sciatic nerve.

If there is an issue with electrical noise – please see the appendix.

The Dissection

1. Place a frog in ice water for 15 minutes. Double pith the frog as soon as it is removed from the ice water.
2. Remove the skin from the legs by making an incision through the skin around the entire lower abdomen. Cut the connections between the skin and the body—especially around the base of the pelvic girdle. Use stout forceps to pull the skin off the frog in one piece (like a pair of pants).

3. Place the frog in a dissection tray with its dorsal side up. Moisten the exposed limbs of the frog with Ringer's solution every five minutes or so.
4. Separate the muscles of the upper leg to expose the sciatic nerve. Muscles are surrounded by connective tissue called fascia, and the large medial and lateral muscles on the dorsal side of the upper leg are joined to each other by a fusion of their fascia along a thin "white line". Grab the muscle groups on either side of the "white line" with a forceps, and firmly pull the muscle groups apart. The fascia will tear.
5. Deflect the muscles away from each other to expose the cream-colored sciatic nerve lying deep between the muscles. The sciatic nerve is covered with fascia, which also includes some blood vessels.
6. Use a glass hook, made by flaming the tip of a Pasteur pipet, to separate the nerve from the fascia and the vessels. If possible, avoid cutting the blood vessels. If bleeding does occur, rinse away the blood with lots of Ringer's solution. Free the nerve from the knee joint to the pelvis.
7. Use the glass hook to place a thread under the nerve. Move the thread as close to the knee joint as possible. Ligate the nerve; the foot should jump as the knot is tied tightly.
8. Carefully separate the muscles of the pelvis to expose the sciatic nerve. Remember to rinse any blood away with Ringer's solution. The sciatic nerve enters the abdomen of the frog through an opening at the end of the urostyle, a bone that forms part of the pelvis.
9. Carefully expose the remainder of the nerve through an opening along the lateral side of the urostyle. To avoid cutting the nerve, lift the end of the urostyle with forceps as the muscle is cut away from the urostyle with a blunt scissors. Cut along the urostyle from its tip to the vertebral column.
10. Deflect the muscle away from the urostyle to expose the sciatic nerve. Use a glass hook to separate connective tissue from the nerve and to place a piece of thread under the nerve. Move the thread as close to the vertebral column as possible. Ligate the nerve; the leg should jump as the knot is tied tightly.
11. Prior to cutting the nerve, measure the in situ length of the nerve between the two ligatures.
12. Cut the upper end of the nerve between the knot and the vertebral column. Cut the lower end of the nerve between the knot and the knee joint. Keep the exposed nerve moist with Ringer's solution at all times.
13. Use the thread to lift the proximal end of the nerve from the abdomen of the frog. Do not pinch or stretch the nerve. Remove any connective tissues, blood vessels, or nerve branches that may still keep the nerve attached to the frog. Continue to lift the nerve out of the frog until it is clear of the abdomen, the pelvis, and the thigh.
14. Grasp the threads at either end of the nerve, and carefully place the nerve in the bath chamber: Place the proximal end of the nerve, that was connected to the spinal column, over the stimulating electrodes. The distal end of the nerve, that was near the knee joint, should be over the recording electrodes.
15. To improve contact between the nerve and the electrodes:
 - Place the thread on the proximal end of the nerve under the outermost stimulating (positive) electrode.

- Place the thread on the distal end of the nerve under the outermost recording electrode at the other end of the chamber.
 - Gently pull the thread on the proximal end of the nerve to position the section of nerve that is just inside the knot under the outermost stimulating electrode. Secure the thread on the wall of the chamber with soft wax or dental wax.
 - Gently pull the thread on the distal end of the nerve until the nerve is at its in situ length. Secure the thread on the wall of the chamber with soft wax.
16. Fill the nerve bath chamber with Ringer's solution to immerse the nerve. Cover the nerve bath chamber to prevent evaporation of the Ringer's solution when the nerve is bathing, or dehydration of the nerve when the Ringer's solution has been removed.

Warning: The nerve preparation used in this experiment is functional for a limited period of time. If the nerve is bathed periodically in Ringer's solution, it will work for about four hours. To conserve time, complete all the exercises in the experiment before analyzing the data.

Experiment AN-2: Compound Action Potentials

Exercise 1: The Compound Action Potential

Aim: To apply a brief stimulus at the proximal end of the nerve and record a compound action potential from the distal end as a test of the viability of the nerve.

Approximate Time: 20 minutes

Procedure

1. Click the Stimulator Preferences icon on the LabScribe toolbar to open the stimulator control panel if it does not open automatically.

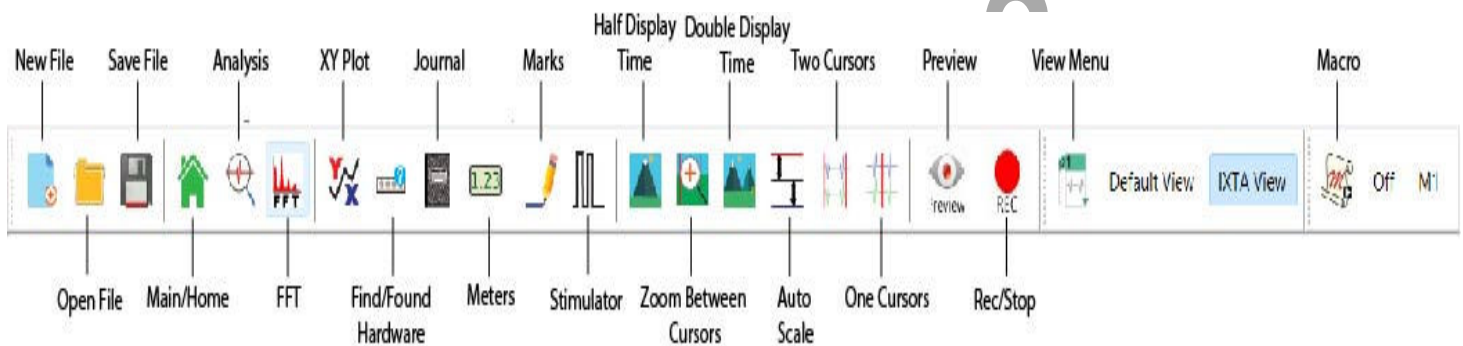


Figure AN-2-L1: The LabScribe toolbar.

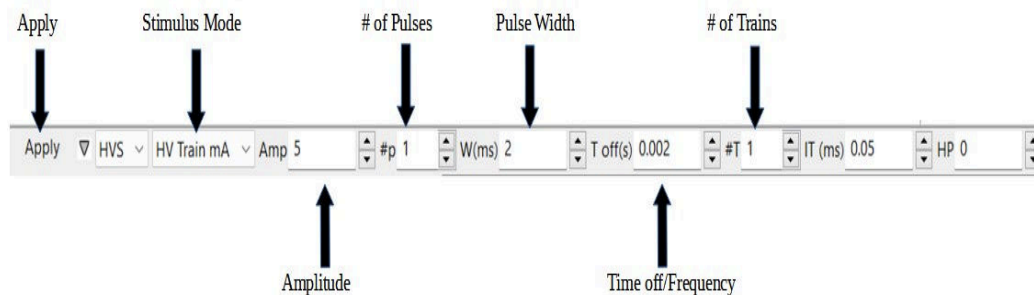


Figure AN-2-L2: The stimulator control panel

2. Check the values for the stimulus parameters that are listed in the stimulator control panel on the Main window: the pulse amplitude (Amp) should be set to 0.250 V; the number of pulses (#pulses) to 1; and, the pulse width (W) to 0.1ms. The value for a stimulus parameter can be changed by typing the value of the parameter in the window next to the label of the parameter. Click the Apply button to finalize any changes.
3. Use a Pasteur pipette to lower the level of Ringer's solution in the nerve bath chamber below the nerve. Make sure that no part of the nerve or the thread holding the nerve in place is in contact with the Ringer's solution. If necessary, carefully blot any large drops of solution from the recording electrodes and the nerve with the corner of a laboratory wipe.

Note: The stimulus amplitude and width set for this exercise should be strong enough to cause a CAP in a healthy nerve.

4. Click the Record button to stimulate and record from the nerve. The recording stops automatically after one sweep that is 30 milliseconds wide.
5. Click the AutoScale All button. The recording should be similar to the one below.
6. On extracellular recordings of compound action potentials, a stimulus artifact may appear. The size of the artifact depends on the width and amplitude of the stimulus pulse, and the effectiveness of the ground electrode separating the stimulating electrodes from the recording electrodes. If the end of the stimulus artifact merges with the beginning of the compound action potential, move the black (-1) recording lead wire from the first recording electrode to another recording electrode farther down the nerve. Always make sure the red (+1) recording lead wire is on the outermost recording electrode. Record another compound action potential.
7. If an action potential did not appear on the screen, check:
 - The wiring between the stimulator and the nerve chamber.
 - The wiring between the isolated inputs of the iWorx box and the nerve chamber.
 - The values for the stimulus parameters shown on the stimulator control panel. These values should match the ones listed in Step #2. If they do not match, make the necessary adjustments. Click the Apply button on the stimulator control panel to finalize the stimulus settings.
8. Click the Record button to stimulate and record from the nerve. If the nerve does not respond after the connections and the settings have been verified, increase the stimulus amplitude (Amp) by 0.050V. Remember to click the Apply button to finalize the change.
9. Click the Record button to stimulate and record from the nerve again. If no compound action potential is generated, increase the stimulus amplitude (Amp) by an increment of 0.050V, again.
10. Repeat Step 9 until a moderate-sized CAP is recorded from the nerve.
11. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.

Warning: Do not exceed a stimulus amplitude of 1.000V without consulting your instructor.

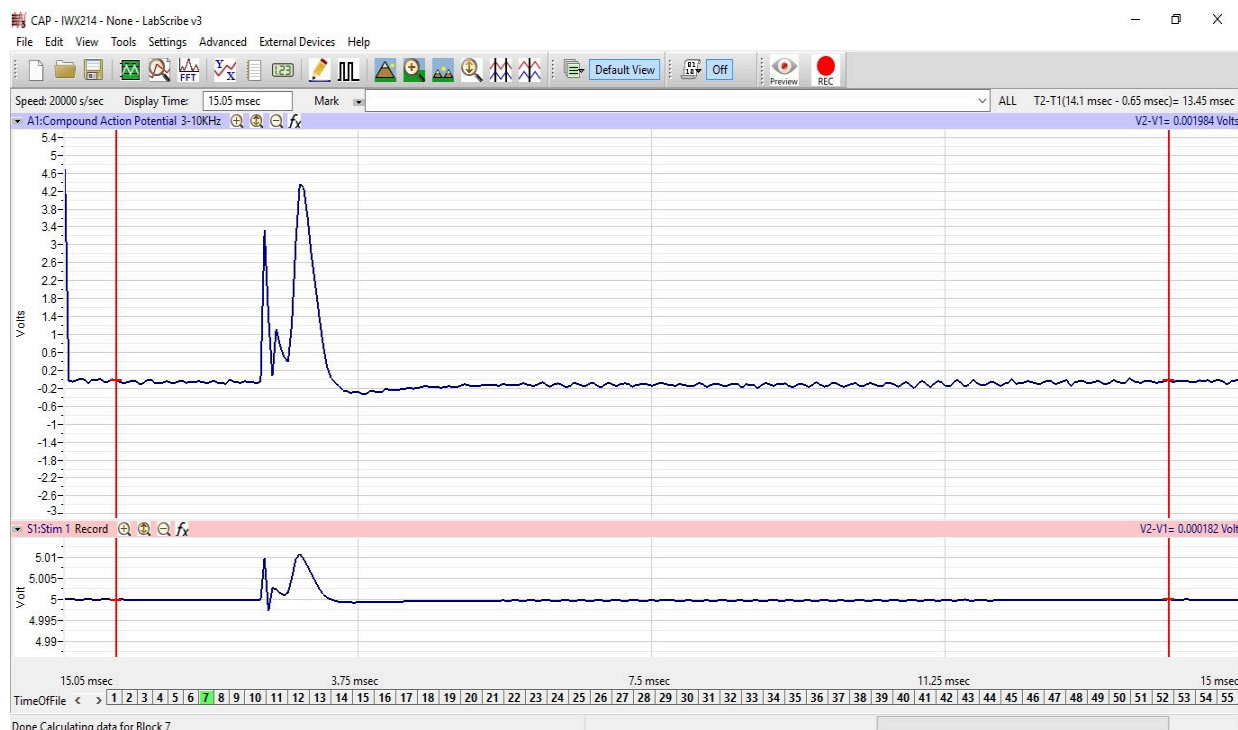


Figure AN-2-L3: The monophasic compound action potential.

Exercise 2: Stimulus and Response

Aim: To quantify the relationship between amplitude of the stimulus and amplitude of the compound action potential.

Approximate Time: 30 minutes

Procedure

1. Change the stimulus amplitude (Amp) to 0.000V by typing into window next the label Amp. Click the Apply button to finalize the change in the stimulus amplitude.
2. If necessary, lower the level of Ringer's solution in the nerve bath chamber below the nerve and blot any drops of the solution from the preparation as described in Exercise 1.
3. Type **0.000V** in the Mark box. The mark will be made automatically when you click Record. Click Record to stimulate the nerve with 0.000V. A flat line should be observed.
4. Change the stimulus amplitude (Amp) to 0.050V using the either of the techniques explained in Step 1. Remember to click the Apply button.
5. Type **0.050V** in the Mark box and then click Record to stimulate the nerve with 0.050V. The mark will be made automatically.
6. Use the Zoom In button on the upper margin of the Compound Action Potential channel to magnify the recording. Look for a peak on the recording that occurs at about the same location as the compound action potential seen in Exercise 1.

Note: Each of the axons in a nerve will generate its own action potentials when the strength of the stimulus exceeds the threshold of that axon. The action potentials, that are generated by the many axons in the nerve, sum to form the compound action potential of the nerve.

7. If a compound action potential is detected at a stimulus amplitude (Amp) of 0.050V, lower the stimulus amplitude to 0.010V, and repeat Steps 5 and 6. Continue to lower the stimulus amplitude by 0.010V and repeat Steps 5 and 6 until the compound action potential disappears.
8. If a compound action potential is not detected at stimulus amplitude of 0.050V, increase the stimulus amplitude (Amp) by an increment of 0.050V. Record and mark the response of the nerve.
9. Repeat Step 8 until the threshold response of the nerve is detected. Note when Threshold is reached.
10. Once the threshold response of the compound nerve is found, continue to increase the stimulus amplitude (Amp) in 0.050V increments until the maximal response of the nerve is observed. Change voltages, record sweeps, and mark recordings in the same manner as before.

Note: C fibers have thresholds and latencies up to 30 times higher than those of A fibers. Therefore, higher stimulus amplitudes and longer display times may be required to see C fibers.

11. Select Save in the File menu.
12. Fill the nerve chamber with fresh Ringer's solution to prevent the dessication of the nerve.

Exercise 3: Conduction Velocity

Aim: To measure the velocity of action potential conduction.

Approximate Time: 20 minutes

Procedure

1. Change the stimulus amplitude (Amp) to the lowest voltage that creates a maximal compound action potential. Click the Apply button to finalize the change in the stimulus amplitude.
2. If necessary, lower the level of Ringer's solution in the nerve bath chamber below the nerve and blot any drops of solution from the preparation as described in Exercise 1.
3. Connect the black (-) recording lead wire to the first recording electrode that is next to the ground electrode. Make sure the red (+) recording lead wire is on the outermost recording electrode.
4. Type **Short Path** in the Mark box. Click Record to stimulate the nerve. The mark will be made automatically.
5. Move the black (-) recording lead wire to another recording electrode that is farther from the ground electrode. Do not move the red (+) recording lead wire.

6. Type **Long Path** in the Mark box and click Record to stimulate the nerve.
7. Measure the distance between the two positions of the negative (-1) recording electrode.
8. Select Save in the File menu.
9. Fill the nerve chamber with cold Ringer's solution and chill the nerve for ten minutes before performing Exercise 4.

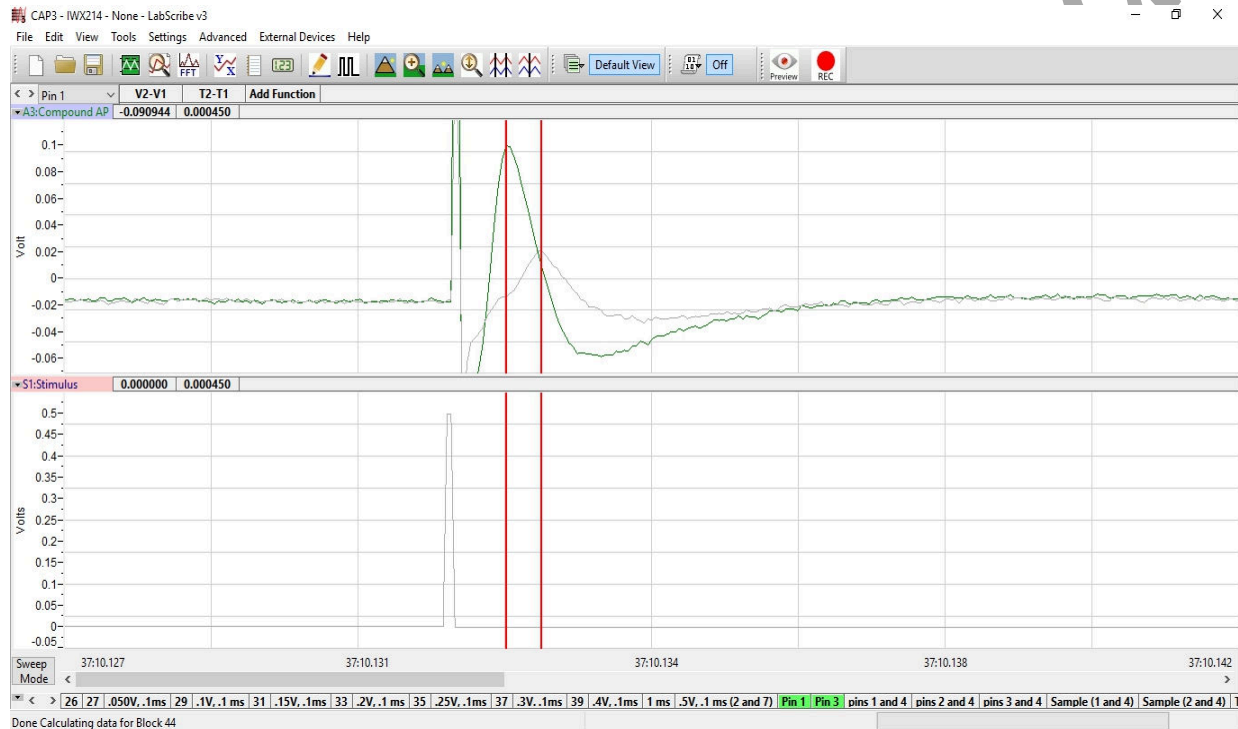


Figure AN-2-L4: Two sweeps used to determine the conduction velocity of the nerve.

Exercise 4: Conduction Velocity and Temperature

Aim: To examine the effects of cooling on the conduction velocity of a cold nerve.

Approximate Time: 20 minutes

Procedure

Note: This part of the experiment must be done quickly since the nerve will begin to warm as soon as the bath is drained.

1. Drop cold Ringer's onto the nerve. Let the nerve chill for a few minutes.
2. Lower the level of cold Ringer's solution in the nerve bath chamber below the nerve and blot any drops of solution from the preparation as described in Exercise 1.
3. As soon as the cold Ringer's has been removed from the nerve bath chamber, measure the conduction velocity of the nerve using the same procedures used in Exercise 3.

4. After data for this exercise is recorded, fill the nerve bath chamber with room temperature Ringer's solution. As you are waiting for the nerve to warm up to room temperature, program the stimulator to the parameters needed in the next exercise.

Exercise 5: Refractory Period

Aim: To measure the effect of stimulus frequency on the amplitudes of the compound action potentials.

Approximate Time: 20 minutes

Note: In the short period of time after an axon generates an action potential, the axon is unable to generate a second action potential. This period of time, in which the axon is not responsive to any strength of stimulus, is known as the absolute refractory period. With additional time to recover, the axon is able to generate a second action potential, but the strength of the stimulus needed is greater than the threshold stimulus that can normally trigger an action potential. This period, in which the responsiveness of the axon is reduced, is known as the relative refractory period. As the axon is given more time to recover, the strength of the stimulus required to trigger a second action potential decreases. In fact, the end of the relative refractory period is defined as the time when the stimulus required to trigger the second action potential is the same as the threshold stimulus that triggered the first action potential.

Nerves are composed of axons of different diameters, conduction velocities, thresholds, and refractory periods. In the short period of time after a nerve generates a compound action potential, none of the axons in the nerve are able to generate a second action potential because all the axons are within their absolute refractory periods. As time passes, the axons with lower thresholds and shorter refractory periods will generate action potentials when the same stimulus pulse is delivered to the nerve. As more time passes, more axons recover and more axons can generate action potentials with the same stimulus pulse.

Procedure

1. Adjust the critical stimulus parameters to the values listed in Table 1 using the same techniques used in earlier exercises. The stimulus amplitude must be set to a voltage that will generate compound action potentials with maximal amplitudes as determined in previous exercises. Click the Apply button to finalize the changes to these stimulus parameters.
2. If necessary, lower the level of Ringer's solution in the nerve bath chamber below the nerve and blot any drops of solution from the preparation as described in Exercise 1.
3. Type **10 msec Interval** in the Mark box and click Record to stimulate the nerve.
4. Change the time interval between stimulus pulses (T Off) to 9 msec. Click the Apply button to finalize this change.
5. Type **9 msec Interval** in the Mark box and click Record to stimulate the nerve.
6. Repeat Steps 4 and 5 to record the change in the amplitude of the second compound action potential as the time interval between the two stimuli in the pair is decreased. Change the time interval between pulses (T Off) to 8, 7, 6, 5, 4, 3, 2, and 1 msec. Record and mark the CAPs at each new time interval.

7. Select Save in the File menu.
8. Fill the nerve chamber with fresh Ringer's solution to prevent the nerve from drying out.

Table AN-2-L1: Stimulus Parameters Required for Measuring the Refractoriness of the Nerve.

Stimulus Parameter	Value
Stimulus Amplitude (Amp)	Volts for Max CAP
Number of Pulses (#pulses)	2
Pulse Width (W)	0.1 msec
Time Between Pulses (T Off)	10 msec
Holding Potential (HP)	0

Exercise 6: Stimulus Strength-Duration

Aim: To demonstrate the relationship between the stimulus amplitude and the stimulus duration needed to generate a compound action potential of a defined amplitude.

Approximate Time: 30 minutes

The stimulus pulse delivered to an axon changes the membrane potential of the axon. If the change in the membrane potential is great enough to exceed a critical level known as the threshold, the axon generates an action potential. The level to which the membrane potential can change is dependent on the stimulus amplitude and the stimulus duration. A stimulus pulse with a low amplitude and a long duration could have the same effect as a stimulus pulse with a high amplitude and a short duration.

Procedure

1. Adjust the critical stimulus parameters to the values listed in Table 2 using the same techniques used in earlier exercises. Click the Apply button.
2. If necessary, lower the level of Ringer's solution in the nerve bath chamber below the nerve and blot any drops of solution from the preparation as described in Exercise 1.
3. Type **0.1 msec, 0.100V** in the Mark box and click Record to stimulate the nerve.
4. Change the stimulus amplitude to 0.200V. Click the Apply button.
5. Type **0.1 msec, 0.200V** in the Mark box and click Record to stimulate the nerve.

Table AN-2-L2: Stimulus Parameters Required for Determining the Stimulus Strength-Duration Relationship of the Nerve.

Stimulus Parameter	Value
Stimulus Amplitude (Amp)	0.100V
Number of Pulses (#pulses)	1
Pulse Width (W)	0.1 msec
Time Between Pulses (T Off)	0.9 msec
Holding Potential (HP)	0

6. Repeat Steps 4 and 5 to record the change in the amplitude of compound action potential as the stimulus amplitude is increased. Change the stimulus amplitude (Amp) by increments of 0.100V until the compound action potential reaches a maximum level. Record and mark the CAPs at each new stimulus amplitude.
7. From the amplitudes of the compound action potentials generated by different stimulus amplitudes, determine the stimulus amplitude that will deliver a mid-sized compound action potential that will be easy to measure. This CAP is designated the criterion to which the compound action potentials generated by stimuli of different amplitudes and durations must be matched.
8. Change the stimulus amplitude to the voltage that generates the criterion. Click the Apply button to finalize this change.
9. Type **0.1 msec, <Criterion Stimulus Amplitude> V** in the Mark box. Click Record to stimulate the nerve.
10. Record the amplitude of the criterion CAP, and the amplitude and duration of the stimulus pulse used to generate the criterion CAP in the Journal.
11. Determine the stimulus amplitudes needed for each of the durations listed to produce a compound action potential with the amplitude of the criterion CAP. The stimulus durations to be used are:
5, 2, 1, 0.5, 0.2, and 0.05 msec.
12. Change the stimulus duration to 5 msec and the stimulus amplitude to 0.100V. Click the Apply button to finalize this change.
13. Type **5 msec, 0.100V** in the Mark box. Click Record to stimulate the nerve.
14. If the amplitude of the CAP is more than the amplitude of the criterion CAP, decrease the stimulus amplitude. If the amplitude of the CAP is less than that of the criterion, increase the stimulus amplitude. After changing the stimulus amplitude click the Apply button.

15. Stimulate the nerve with different stimulus amplitudes until the amplitude that causes the criterion response is found. Record the stimulus amplitude needed for the stimulus duration of 5 msec in the Journal.

Note: *If the stimulus duration is longer, the stimulus amplitude needed to create the criterion is usually smaller.*

16. Repeat Steps 12 through 15 for each of the other stimulus durations.
17. Select Save in the File menu.

Exercise 7: Bidirectionality

Aim: To determine if action potentials can travel in either direction in a nerve, and if they can, is there a difference in the conduction velocity in one direction compared to the other direction.

Approximate Time: 15 minutes

Procedure

1. Reverse the positions of the lead wires attached to the electrodes of the nerve bath chamber. Put the lead wires of the stimulator on the distal end of the nerve where the recordings were made in the other exercises. Put the lead wires of the recording input on the proximal end of the nerve where the stimulus was delivered in the other exercises. Put the lead wire for the ground on an electrode between the stimulating and recording electrodes.
2. If necessary, lower the level of Ringer's solution in the nerve bath chamber below the nerve and blot any drops of solution from the preparation as described in Exercise 1.
3. Adjust the stimulus duration (W) to 0.1 msec. Adjust the stimulus amplitude (Amp) to the voltage used to generate the criterion CAP at a duration of 0.1 msec. Click the Apply button to finalize the changes to these stimulus parameters.
4. Use the same procedures used in Exercises 3 and 4 to measure the conduction velocity of the compound action potential, if one is generated.

Data Analysis

Exercise 2-Stimulus and Response

1. Use the Sweep Selection bar at the bottom of the Main window to display the first sweep recorded in Exercise 2. Click on the tab on the selection bar for that sweep and the sweep will appear on the Main window. The stimulus delivered to the nerve in this sweep had an amplitude of 0.000V and a compound action potential (CAP) should not be seen.
2. To display the second sweep recorded in this exercise, click on the tab on the selection bar for that sweep. The stimulus delivered to the nerve in this sweep had an amplitude of 0.050V. Click the AutoScale button of the Compound Action Potential channel to maximize the size of a compound action potential and determine if a CAP occurred.

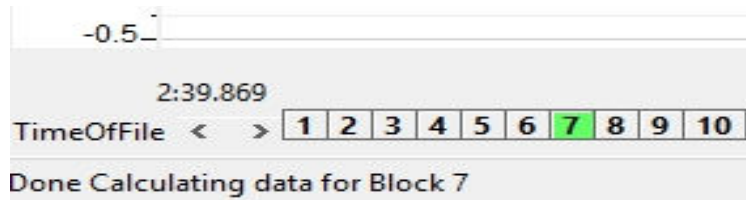


Figure AN-2-L5: The Sweep Selection bar showing the tab for Sweep 7 highlighted.

2. If a CAP does not appear on the second sweep of this exercise, display the next sweep in succession. Continue to move to the next sweep in succession until the sweep that displays the first appearance of a compound action potential is found.
3. Once the sweep displaying the first CAP is found, transfer that sweep to the Analysis window. Click on the Analysis window icon in the toolbar or select Analysis from the Windows menu to transfer the sweep from the Main window to the Analysis window.

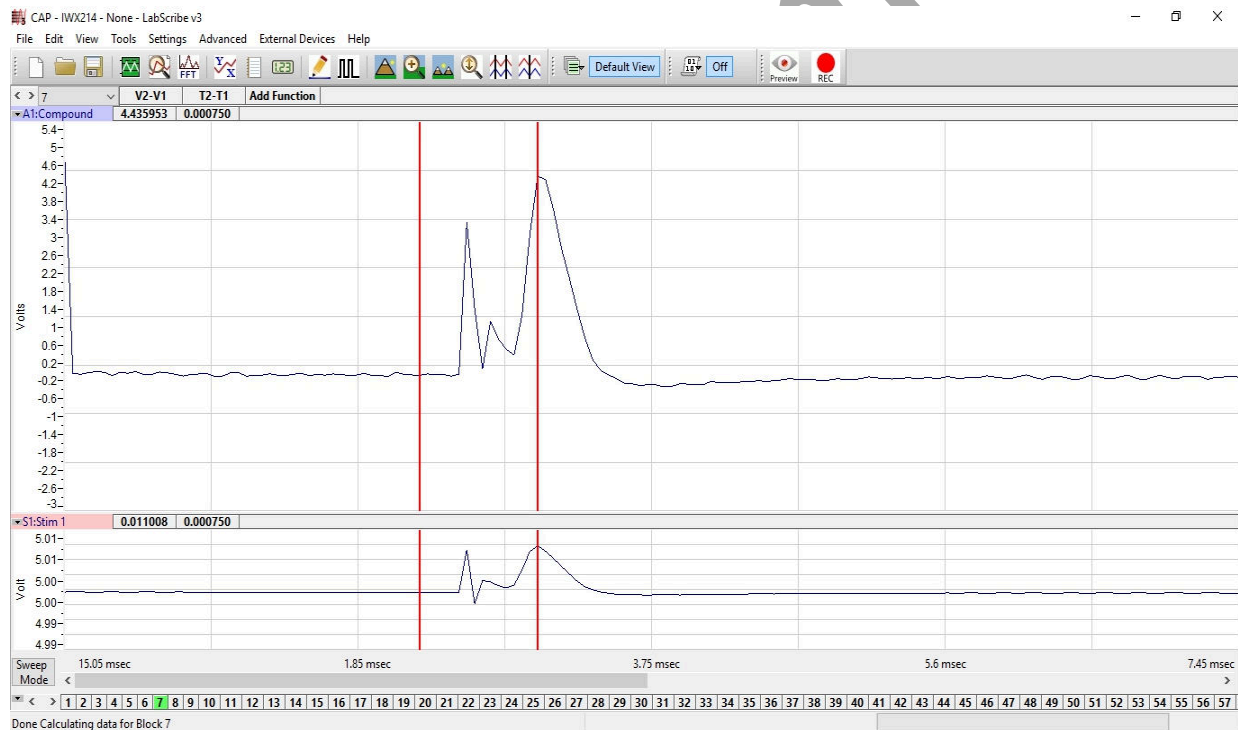


Figure AN-2-L6: Compound action potential (CAP) and stimulus pulse displayed in the Analysis window, with cursors positioned to measure the amplitude of the CAP.

5. Look at the Function Table that is above the uppermost channel displayed in the Analysis window. The mathematical functions, V2-V1 and T2-T1 should appear in this table. The values for V2-V1 and T2-T1 on each channel are seen in the table across the top margin of each channel.

6. Maximize the height of the trace on the Compound Action Potential channel by clicking on the arrow to the left of the title of that channel to open the channel menu. Select Scale from the menu and AutoScale from the Scale submenu to increase the height of the data on that channel.
7. Once the cursors are placed in the correct positions for determining the amplitude of the compound action potential, the amplitude (V2-V1) can be recorded in the on-line notebook of LabScribe by typing its name and value directly into the Journal, or on a separate data table.
8. The functions in the channel pull-down menus of the Analysis window can also be used to enter the name and value of the parameter from the recording to the Journal. To use these functions:
 - Place the cursors at the locations used to measure the amplitude of the compound action potential.
 - Transfer the names of the mathematical functions used to determine the amplitude to the Journal using the Add Title to Journal function in the Compound Action Potential Channel pull-down menu.
 - Transfer the values for the amplitude to the Journal using the Add Ch. Data to Journal function in the Compound Action Potential Channel pull-down menu.
9. To measure the amplitude of a compound action potential, place one cursor on the baseline before the stimulus artifact displayed on the Compound Action Potential Channel. Place the other cursor on the peak of the CAP. The value for the V2-V1 function on the Compound Action Potential Channel is the amplitude of the compound action potential.
10. Record the amplitude and the stimulus amplitude used to generate the CAP in the Journal using the one of the techniques described in Steps 7 or 8, and on Table 3.
11. To display the compound action potentials recorded on other sweeps, click on the tabs for those sweeps in the Sweep Selection bar at the bottom of the Analysis window. The sweeps that are selected will appear together on the Analysis window. Each successive sweep should have a compound action potential with a greater amplitude since the stimulus amplitude was increased in each successive sweep. The compound action potential will reach a maximum amplitude when all the fibers in the nerve are firing. The amplitude of the compound action potential will not increase even if a stimulus with a higher amplitude is applied to the nerve.
12. To take measurements from another sweep displayed on the Analysis window, select its name from the Sweep menu in the upper left margin of the data display window. Repeat Steps 9 and 10 to measure and record the amplitude of the CAP.
13. Measure and record the amplitudes of all the compound action potentials from threshold to maximum level.
14. Select Save in the File menu.

Table AN-2-L3: Amplitudes of Action Potentials Generated by Stimulus Pulses of Different Amplitudes.

	Compound Action Potentials
Stimulus Amplitude (V)	Amplitude (mV)
0.000	0
0.050	
0.100	
0.150	
0.200	
0.250	
0.300	
0.350	
0.400	
0.450	
0.500	

15. Click on the Main Window icon to return to that window.
16. Graph the amplitude of the compound action potential as a function of the stimulus amplitude.

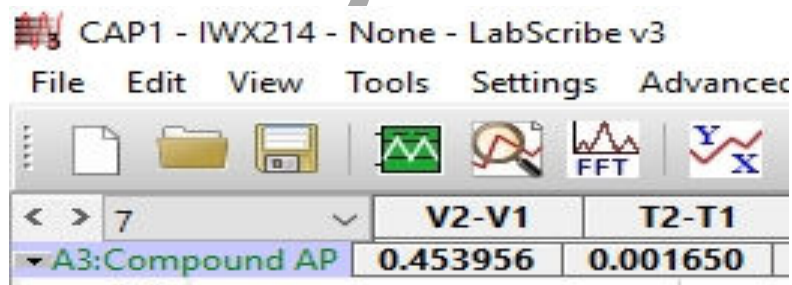


Figure AN-2-L7: The upper left corner of the Analysis window showing the Sweep menu used to select the sweep from which the data is measured.

Questions

1. Does the action potential in a single axon increase in amplitude when the stimulus amplitude is increased?
2. Does the amplitude of the CAP increase because more fibers are firing, or the amplitude of the action potentials from single fibers are increasing, or a combination of both?
3. How many fiber types did you observe in your monophasic recording of compound action potentials?
4. The first peak of the compound action potential is composed of the responses of the A α fibers. How does the threshold and diameter of these fibers compare to A β , A γ , A δ and C fibers?

Exercise 3-Conduction Velocity

1. Use the Sweep Selection bar at the bottom of the Main window to display the first sweep needed to calculate the conduction velocity of the nerve. Click on the tab of that sweep and it will appear on the Main window.
2. Transfer the sweep to the Analysis window using the Analysis window icon in the toolbar or the Analysis listing on the Windows menu.
3. Display the second sweep needed to calculate the conduction velocity by clicking on its tab in the Sweep Selection bar at the bottom of the Analysis window.
4. Measure the time between the peaks of the two compound action potentials displayed on the Analysis Window:
 - Place one cursor on the peak of the compound action potential in the first sweep.
 - Place the second cursor on the peak on the compound action potential in the second sweep.
 - The value for T2-T1 on the Compound Action Potential Channel is the time it takes the CAP to move the distance between the two positions of the negative recording electrode used in this exercise.
 - Record the value for T2-T1 (in msec) in the Journal, and on Table 4, for temperature and direction labeled as Room-Normal.
5. Measure the conduction distance (in mm) between the two positions of the negative recording electrode. Record this distance (in mm) in the Journal, and on the table, as the conduction distance for temperature and direction labeled as Room-Normal.
6. Calculate the conduction velocity (in meters per second). Divide the distance (in mm) between the two positions of the negative recording electrode by T2-T1, which is the time (in msec) between the peaks of the two compound action potentials.

For example, $10 \text{ mm} / 0.2 \text{ msec} = 50 \text{ mm/msec} = 50 \text{ m/sec}$
7. Record the conduction velocity in the Journal, and on the table, for the temperature and direction labeled as Room-Normal.
8. Select Save in the File menu.

Table AN-2-L4: Conduction Velocities of a Nerve at Two Different Temperatures.

Temperature/ Direction	Conduction Distance (mm)	Time (msec)	Conduction Velocity (m/sec)
Room-Normal			
Cold-Normal			
Room-Opposite			

Exercise 4-Conduction Velocity at Low Temperature

1. Use the same techniques used in the analysis of Exercise 3 to take measurements from the data in Exercise 4.
2. Use the same techniques used in Exercises 2 and 3 to record the measurements from this exercise in the Journal, and on Table 4 for temperature and direction labeled as Cold-Normal.
3. Calculate and record the conduction velocity of the nerve at a low temperature in the Journal, and on the table for temperature and direction labeled as Cold-Normal.
4. Select Save in the File menu.

Questions

1. Does the conduction velocity change when the nerve is cooled?
2. What properties of the ion channels may change with temperature?

Exercise 5-Refractory Period

1. Use the Sweep Selection bar at the bottom of the Main window to display the first sweep used to demonstrate the refractoriness of the nerve. Click on the tab of that sweep and it will appear on the Main window. According to the design of the exercise for this sweep, two stimulus pulses are delivered to the nerve at an interval of 10 msec.
2. Transfer the sweep to the Analysis window using the Analysis window icon in the toolbar or the Analysis listing on the Windows menu.
3. Measure the amplitude of the first compound action potential in the pair:
 - Place one cursor on the baseline before the stimulus artifact displayed on the Compound Action Potential Channel.
 - Place the other cursor on the peak of the CAP.
 - The value for the V2-V1 function on the Compound Action Potential Channel is the amplitude of the compound action potential. Record this value in the Journal, and on Table 5.

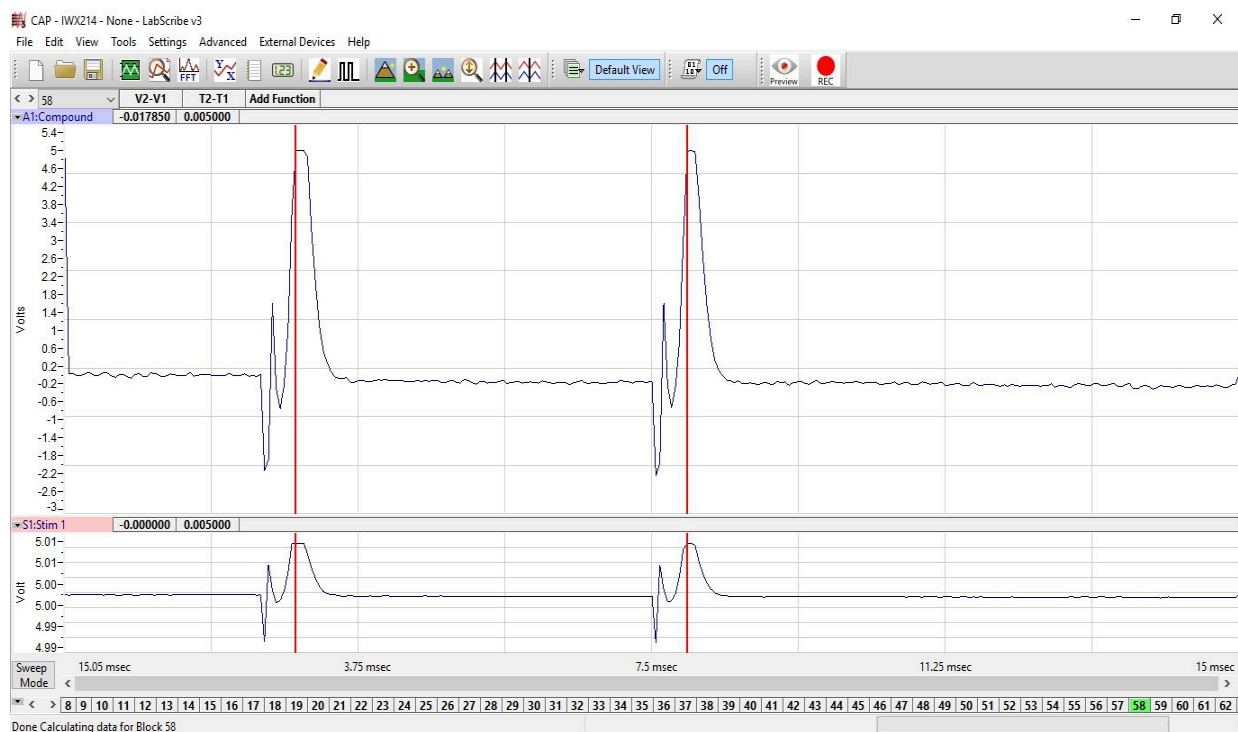


Figure AN-2-L8: Pair of compound action potentials stimulated by a pair of pulses that are 5 msec apart.

4. Repeat Step 3 for the second CAP in the pair.
5. To display the compound action potentials recorded on other sweeps where the intervals between the stimulus pulses are getting shorter, click on the tabs for those sweeps in the Sweep Selection bar at the bottom of the Analysis window. The sweeps that are selected will appear together on the Analysis window.
6. To take measurements from another sweep displayed on the Analysis window, select its name from the Sweep menu in the upper left margin of the data display window. Repeat Steps 3 and 4 to measure and record the amplitude of the CAP.
7. Select Save in the File menu.

Questions

1. Does the first CAP in the pair have the same amplitude at all of the frequencies?
2. Does the second CAP in the pair have the same amplitude at all of the frequencies?
3. At which frequency, or interpulse interval, does the second CAP in the pair start to decline?
4. What causes the change in the amplitude of the second CAP at some stimulus frequencies?
5. What could be done to increase the amplitude of the second CAP if it declined?
6. Is an axon that responds to a stimulus of increased amplitude in the absolute or relative portion of its refractory period?

Table AN-2-L5: Amplitudes of Compound Action Potentials Occurring at Different Frequencies.

Interpulse Interval (ms)	Effective Frequency (Hz)	Amplitudes (mV)	
		First CAP	Second CAP
10	100		
9	111		
8	125		
7	143		
6	167		
5	200		
4	250		
3	333		
2	500		
1	1000		

Exercise 6-Stimulus Strength-Duration

1. Graph the stimulus amplitude needed to create the criterion CAP as a function of the stimulus duration.
2. Place the values for the stimulus amplitudes on the Y-axis, and the values for the stimulus durations on the X-axis.
3. The minimum stimulus amplitude which will elicit action potentials at an infinitely long stimulus duration is a value known as the rheobase. Another value, chronaxie, is the stimulus duration where the stimulus amplitude is twice the value of the rheobase. Chronaxie values are used as measures of the excitability of nerves. The most excitable nerves have the smallest chronaxies. Using the graph created in Step 1, determine rheobase and chronaxie for the nerve in your chamber.

Questions

1. How does the excitability of your nerve compare to the nerves used by other groups in your lab section?
2. Collect conduction velocity and chronaxie data from the other groups in your lab section. Is there a relationship between conduction velocity and nerve excitability? If so, what is it?

Exercise 7-Bidirectionality

1. Use the same techniques used in the analysis of Exercise 3 to take measurements from the data in Exercise 7.
2. Use the same techniques used in Exercises 2 and 3 to record the measurements from this exercise in the Journal, and on Table 4 for temperature and direction labeled as Room-Opposite.
3. Calculate and record the conduction velocity of the nerve at room temperature and in the opposite direction in the Journal, and on the table for temperature and direction labeled as Room-Opposite.
4. Select Save in the File menu.

Questions

1. Why do you record an action potential from the proximal end of the nerve?
2. What is the conduction velocity for the nerve when stimulated in reverse direction? Is this similar to the value recorded when the nerve was stimulated from the proximal end to the distal end?
3. How can an axon conduct action potentials in both directions?

Note: *Where are the cell bodies and synapses in this preparation?*