

Experiment AN-3: Neuromuscular Studies

Exercise 1: Maximal Muscle Response

Aim: To apply stimulus pulses of increasing amplitude to the proximal end of the nerve, and record the compound action potentials evoked from the nerve and the muscle.

Approximate Time: 30 minutes

Procedure

1. Click the Stimulator Preferences icon on the LabScribe toolbar ([Figure AN-3-L1](#)) to open the stimulator control panel ([Figure AN-3-L2](#)) on the Main window.

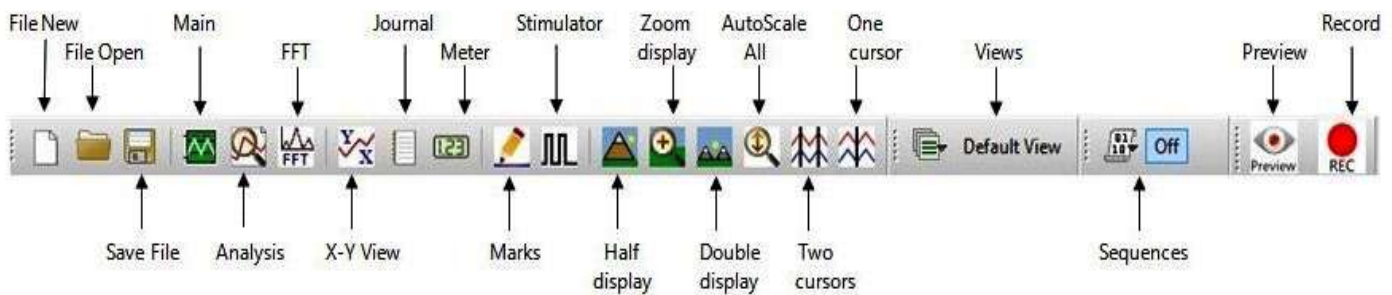


Figure AN-3-L1: The LabScribe toolbar.

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 0.1 ms. The value for a stimulus parameter can be changed by either of two methods: click on the arrow buttons to the right of the window that displays the value of the parameter to increase or decrease the value; or, type the value of the parameter in the window next to the label of the parameter. Click the Apply button to finalize the change in any stimulus parameter.

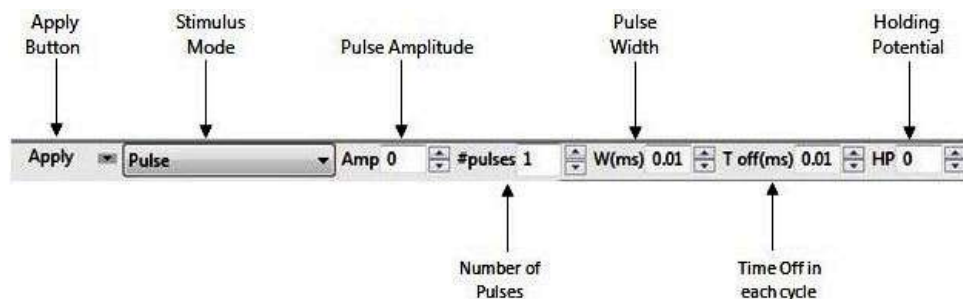


Figure AN-3-L2: The stimulator control panel.

- 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 still in the chamber. 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.

- Click the Record button to stimulate and record from the nerve. The recording stops automatically after one sweep that is 30 milliseconds wide. Type 0.250 V in the Mark box to the right of the Mark button. Press the Mark button to attach this notation regarding the stimulus amplitude to the recording. Click the AutoScale All button. The recording should be similar to the one in [Figure AN-3-L3](#).

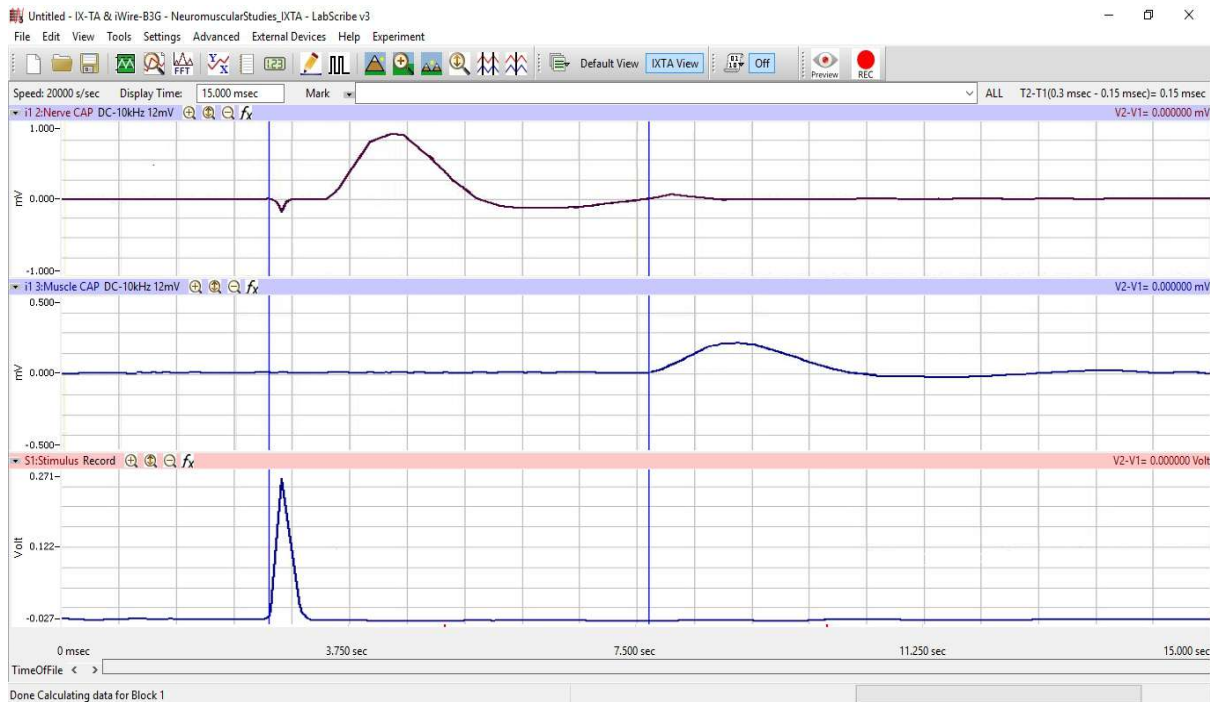


Figure AN-3-L3: The compound action potential (CAP) from the sciatic nerve is displayed on the upper channel. The CAP from the tibialis anterior muscle is displayed on the middle channel. The time between the cursor at the onset of the stimulus artifact and the cursor at the onset of the muscle action potential is the muscle latency.

- Change the stimulus amplitude (Amp) to 0.300 V by clicking on the arrow buttons next to the value for this parameter as displayed on the stimulator control panel. The value for the stimulus amplitude (Amp) can also be typed into the window next to the label Amp. Click the Apply button to finalize the change in the stimulus amplitude.

6. Click Record to stimulate the nerve with 0.300 V. Type 0.300 V in the Mark box to the right of the Mark button. and press the Mark button to attach a comment to the recording.
7. Increase the stimulus amplitude (Amp) by an increment of 0.050 V using one of the techniques explained in Step 5. Remember to click the Apply button each time to finalize the change in the stimulus amplitude. Record and mark the response of the nerve.
8. Repeat Step 7 until the maximum compound action potential from the muscle is produced.
9. Select Save As in the File men and type a name for the file. Click on the Save button to save the data file.
10. Fill the nerve/muscle chamber with fresh Ringer's solution to prevent the nerve and muscle from drying out.
11. Increase the stimulus amplitude by another 10% before proceeding to the next exercise. Click the Apply button to finalize the change in the stimulus amplitude. This level of stimulation ensures a supramaximal response from the nerve and muscle.

Exercise 2: Synaptic Delay

Aim: To measure the time taken to transmit a signal across the synapse from the end of the nerve to the surface of the muscle fibers.

Approximate Time: 10 minutes

Note: *The time between the nerve action potential and the muscle action potential depends on three major factors: the conduction time down the nerve; the synaptic delay; and, the conduction time of the muscle action potential from the neuromuscular junction (NMJ) to the muscle-recording electrodes. Assume that the third factor is negligible. The synaptic delay can be determined by subtracting the conduction time along the nerve from the time interval between the nerve and muscle action potentials.*

Procedure

1. Make sure the stimulus amplitude (Amp) is set to the supramaximal voltage determined at the end of Exercise 1. If any change was made to the stimulus parameters, click the Apply button to finalize the changes.
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. Click Record to stimulate the nerve with the supramaximal stimulus. Type <Supramaximal> V in the Mark box and press the Mark button to attach a comment to the recording.
4. Select Save in the File menu.
5. Fill the nerve/muscle chamber with fresh Ringer's solution to prevent the nerve and muscle from drying out.

Exercise 3: Facilitation of the Muscle Response

Aim: To demonstrate facilitation in a muscle by stimulating the nerve/muscle preparation with a succession of pulses that occur more frequently. Facilitation is an increase in the amplitude of the sub-maximal muscle action potentials that occur when there is less time between identical stimulus pulses.

Approximate Time: 30 minutes

Procedure

1. Adjust the Display Time and Timed Stop of the sweep to allow more action potentials to be displayed on the computer screen.
 - Open the Edit menu on the Main window and select Preferences.
 - On the Channels window of the Preferences Dialog, change the Display Time and the Timed Stop to 0.300 sec.
 - Click the OK button at the bottom of the Preferences Dialog.
2. Adjust the critical stimulus parameters to the values listed in [Table AN-3-L1](#) using the same techniques used in Exercise 1. The stimulus amplitude must be set to a voltage that will generate compound action potentials in the muscle with amplitudes that are 50% to 70% of the maximal muscle CAP. Click the Apply button to finalize the changes to these stimulus parameters.

Table AN-3-L1: Stimulus Parameters Required for Measuring the Facilitation of the Muscle.

Stimulus Parameter	Value
Stimulus Amplitude (Amp)	Volts for 50-70% of Max Muscle CAP
Number of Pulses (#pulses)	5
Pulse Width (W)	0.1 msec
Time Between Pulses (T Off)	20 msec
Holding Potential (HP)	0

3. 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.
4. Click Record to stimulate the nerve. Type 20 msec Interval in the Mark box to the right of the Mark button. Press the Mark button to attach this notation to the recording.
5. Change the time interval between stimulus pulses (T Off) to 15 msec. Click the Apply button to finalize this change.
6. Click Record to stimulate the nerve. Type 15 msec Interval in the Mark box. Press the Mark button to attach this notation to the recording.

7. Repeat Steps 5 and 6 to record the amplitudes of the muscle compound action potentials in the series as the time interval between the stimuli is decreased. Change the time interval between pulses (T Off) to 10, 5, and 2 msec. Record and mark the recordings at each new time interval.
8. Select Save in the File menu.
9. Fill the nerve chamber with fresh Ringer's solution to prevent the nerve from drying out.

Warning: Do not test the response of the muscle more than two times at each interpulse time interval. The muscle may fatigue with frequent stimulation and may never recover.

Warning: Wear gloves when handling any drug solution, any tissue treated with drugs, or any other object that has come in contact with a drug solution. Some of these drugs are very effective poisons.

Exercise 4: Effects of Eserine

Aim: To measure the changes in the compound action potential of muscle whose neuromuscular junction was exposed to the anticholinesterase, eserine.

Approximate Time: 45 minutes

Procedure

1. Use the same Display Time and Timed Stop that was used in Exercise 3.
2. Adjust the critical stimulus parameters to the values listed in [Table AN-3-L2](#) using the same techniques used in Exercise 1.
3. Click the Apply button to finalize the changes to these stimulus parameters.

Table AN-3-L2: Stimulus Parameters Required for Measuring the Effects of Eserine and Other Drugs on the Nerve/Muscle Preparation.

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

4. 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.
5. Click Record to stimulate the nerve. Type 0.100 V in the Mark box to the right of the Mark button. Press the Mark button to attach this notation to the recording.
6. Change the stimulus amplitude (Amp) to 0.200 V. Click the Apply button to finalize this change.
7. Click Record to stimulate the nerve. Type 0.200 V in the Mark box. Press the Mark button to attach this notation to the recording.
8. Repeat Steps 6 and 7 to record the amplitudes of the compound action potentials on the nerve and muscle up to a maximal response from the muscle. Change the stimulus amplitude by increments of 0.100 V. Record and mark the recordings for each new stimulus amplitude.
9. After recording compound action potentials generated by stimulus pulses of different amplitudes, put 5 drops of the eserine solution (0.01 mg/ml in Ringer's) on the surface of the muscle. Put another 5 drops of eserine solution on the muscle at one-minute intervals for a total of 30 drops.
10. Reset the stimulus amplitude (Amp) to 0.100 V. Click the Apply button to finalize this change.
11. Click Record to stimulate the nerve. Type 0.100 V-Eserine in the Mark box to the right of the Mark button. Press the Mark button to attach this notation to the recording.
12. Change the stimulus amplitude (Amp) to 0.200 V. Click the Apply button to finalize this change.
13. Click Record to stimulate the nerve. Type 0.200 V-Eserine in the Mark box. Press the Mark button to attach this notation to the recording.
14. Repeat Steps 12 and 13 to record the amplitudes of the compound action potentials from the nerve and muscle. Use the same stimulus amplitudes as the ones applied to the untreated nerve/muscle preparation. If there is a significant difference between muscle compound action potentials recorded before and after the eserine treatment, proceed to Step 16.
15. If there is no significant difference between muscle compound action potentials recorded before and after the eserine treatment:
 - Repeat the application of eserine in the same manner as it was applied in Step 9.
 - Test the response of the muscle using the procedures used in Steps 10 through 14.
 - If there is a difference between the muscle action potentials recorded before the first eserine treatment and after the second eserine treatment, proceed to Step 16.
 - If there is no difference between the muscle action potentials recorded before the first eserine treatment and after the second eserine treatment, apply a third treatment of eserine. Test the response of the muscle after the third treatment of eserine using the procedures used in Steps 10 through 14. Proceed to Step 16, whether there is a change in the compound action potentials of the muscle or not.
16. Reverse the effects of eserine on the muscle:
 - Remove the Ringer's solution containing eserine from the bottom of the bath chamber.

- Rinse the inside of the bath chamber and the surface of the muscle and nerve with fresh Ringer's solution.
 - Submerge the nerve/muscle preparation in fresh Ringer's solution for 5 minutes, changing the Ringer's solution every minute.
17. 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.
 18. Record the compound action potentials from the preparation using the same procedures used in Steps 10 through 14. Mark each recording to indicate the stimulus voltage used while testing the reversibility of the effects of eserine. If the muscle compound action potentials have returned to normal, proceed to the next exercise.
 19. If the muscle compound action potentials have not returned to their pretreatment levels:
 - Submerge the nerve/muscle preparation in fresh Ringer's solution for another 5 minutes, changing the Ringer's solution every minute.
 - Retest the response of the muscle using the same procedures used in Steps 10 through 14.
 - If the muscle compound action potentials have returned to normal, proceed to the next exercise.
 - If the muscle compound action potentials have not returned to normal, continue rinsing the preparation with fresh Ringer's solution until the muscle response recovers. If the muscle has not fully recovered after 30 minutes of rinsing, consult your instructor.
 20. Select Save in the File menu.
 21. Fill the nerve/muscle chamber with fresh Ringer's solution to prevent the nerve and muscle from drying out.

Exercise 5: Effects of Other Agents

Aim: To test the effects of other reagents on the compound action potentials of the nerve and the muscle in the preparation. Each group will be assigned one of the following substances to test: curare, atropine, acetylcholine (high concentration), nicotine, dantrolene, magnesium (high concentration), and calcium (high concentration). Each reagent is dissolved in Ringer's solution.

Approximate Time: 45 minutes

Procedure

1. Begin the study of the reagent that your group is assigned only after the effects of eserine have been reversed, or if the response of the muscle is measurable and stable.
2. Use the same procedures employed in Exercise 4 to test the effects of your assigned reagent on the compound action potentials of the nerve and the muscle.
3. Select Save in the File menu.
4. Follow the directions provided by your instructor for the cleaning of the equipment and the

disposal of tissue and reagents.

Data Analysis

Exercise 2: Synaptic Delay

1. Use the Sweep Selection bar at the bottom of the Main window ([Figure AN-3-L4](#)) to display the 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.

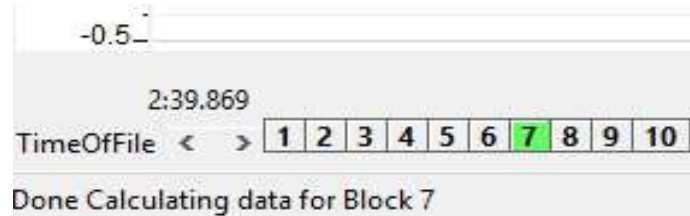


Figure AN-3-L4: The Sweep Selection bar showing the tab for Sweep 7 highlighted.

2. Transfer the 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.
3. 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.
4. Maximize the height of the trace on both channels by clicking on the arrow to the left of the title of each 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.
5. Once the cursors are placed in the correct positions for determining the latency of the nerve, the time (T2-T1) 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.
6. 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 latency of the nerve.
 - Transfer the names of the mathematical function used to determine the latency to the Journal using the Add Title to Journal function in the Nerve CAP channel pull-down menu.
 - Transfer the values for the amplitude to the Journal using the Add Ch. Data to Journal function in the Nerve CAP channel pull-down menu.
7. Measure the latency of the nerve compound action potential:
 - Place one cursor on the onset of the stimulus artifact displayed on the Nerve CAP channel.

- Place the other cursor on the onset of the nerve compound action potential displayed on the same channel.
 - The value for T2-T1 function on the Nerve CAP channel is the latency of the nerve, which is the time it takes the action potential to move from the negative stimulating electrode to the negative nerve recording electrode.
 - Record the nerve latency in the Journal using the one of the techniques described in Steps 5 or 6, and on [Table AN-3-L3](#).
8. Measure the nerve conduction distance (in mm) between the negative stimulating electrode to the negative nerve recording electrode. Record the nerve conduction distance in the Journal using the one of the techniques described in Steps 5 or 6, and on the data table.
 9. Calculate the nerve conduction velocity (in millimeters per millisecond) of the nerve. Divide the nerve conduction distance (in mm) that was measured in Step 9 by the nerve latency (in msec) that was measured in Step 7.

For example, $10 \text{ mm} / 0.2 \text{ msec} = 50 \text{ mm/msec}$

Record the nerve conduction velocity in the Journal, and on the data table.

Note: *If the nerve and the nerve/muscle chamber are long enough that the nerve crosses three or more electrodes that can be used for recording nerve action potentials, a more accurate method of determining the nerve conduction velocity can be utilized. This recording can be done monophasically (without a positive nerve recording electrode) or biphasically (with both a positive and a negative recording electrode). If the positive (second) nerve recording electrode is used, it should be placed on electrode (F). Record the compound nerve action potential with the negative (first) nerve recording electrode in position (D). Move this negative nerve recording electrode to a new position (E) and record the compound nerve action potential, again. Measure the distance (in mm) between electrode (D) and electrode (E). Divide this distance (D to E) by the time interval (in msec) between the peak of the action potential recorded at (D) and the peak of the action potential recorded at (E).*

10. Measure the muscle conduction distance (in mm) between the negative stimulating electrode and the negative recording electrode on the muscle. Record this distance in the Journal, and on the data table.
11. Calculate the conduction time (in msec) of the nerve compound action potential from the point of stimulation of the nerve, which is the negative stimulating electrode, to the point of recording on the muscle fibers, which is the negative recording electrode on the muscle. Divide the muscle conduction distance (in mm) that was measured in Step 10 by the conduction velocity (in mm/msec) of the nerve that was calculated in Step 9.

For example, $40 \text{ mm} / (50 \text{ mm/msec}) = 0.8 \text{ msec}$

Record the conduction time of the nerve in the Journal, and on the data table.

12. Measure the latency of the muscle. Place one cursor on the onset of the stimulus artifact displayed on the Nerve CAP channel. Place the other cursor on the onset of the muscle

compound action potential displayed on the Muscle CAP channel. The value for T2-T1 function on either the Nerve or Muscle CAP Channel is the latency of the muscle. Record the latency of the muscle in the Journal, and on the data table.

13. Calculate synaptic delay. Subtract the nerve conduction time (stimulus electrode-NMJ) found in Step 11 from the latency of the muscle found in Step 12. Record the synaptic delay in the Journal, and on the data table.
14. Select Save in the File menu.
15. Click on the Main Window icon to return to that window.

Questions: Synaptic Delay

1. Is there a more accurate way to make this measurement?
2. What additional factors would be involved if the time between the nerve action potential and muscle contraction were used instead of the time between the nerve and muscle action potentials?

Table AN-3-L3: Times, Velocities, and Distances Needed to Determine Synaptic Delay

Parameter	Value
Nerve Latency (msec)	
Nerve Conduction Distance (mm)	
Nerve Conduction Velocity (mm/msec)	
Muscle Conduction Distance (mm)	
Nerve Conduction Time, Stimulus to NMJ (msec)	
Muscle Latency (msec)	
Synaptic Delay (msec)	

Exercise 3: Facilitation of the Muscle Response

1. Use the Sweep Selection bar at the bottom of the Main window to display the first sweep recorded in Exercise 3. Click on the tab on the selection bar for that sweep and the sweep will appear on the Main window. According to the design of the exercise, the five stimulus pulses delivered to the nerve in this sweep were 20 msec apart.
2. Transfer the sweep to the Analysis window by clicking on the Analysis window icon in the toolbar or selecting Analysis from the Windows menu.

3. Maximize the height of the traces on the Nerve and Muscle CAP channels by clicking on the arrow to the left of the title of each channel to open the channel menu. Select Scale from the menu and AutoScale from the Scale submenu.
4. Measure the amplitude of the first muscle compound action potential in the series of five. Place one cursor on the baseline of the recording on the Muscle CAP channel in the section before the first stimulus was delivered. Place the other cursor on the peak of the first muscle CAP. The value for the V2-V1 function on the Muscle CAP channel is the amplitude of this muscle compound action potential.
5. Record the amplitude of the first muscle compound action potential in the Journal using one of the techniques described in the data analysis section of Exercise 2, and on [Table AN-3-L4](#).
6. Repeat Steps 4 and 5 on each of the other muscle compound action potentials in this sweep.
7. To display another sweep made with a different interval between stimulus pulses, click on the tab for that sweep in the Sweep Selection bar at the bottom of the Analysis window. The sweep that is selected will appear on the Analysis window.

Table AN-3-L4: Amplitudes of Multiple Muscle Compound Action Potentials with Different Stimulus Frequencies.

Interval Between Stimulus Pulses (msec)	Amplitude (mV) of Muscle Compound Action Potential in Series				
	1st	2nd	3rd	4th	5th
20					
15					
10					
5					
2					

8. To take measurements from the second sweep displayed on the Analysis window, select its name from the Sweep menu in the upper left margin of the data display window ([Figure AN-3-L5](#)). Repeat Steps 4 and 5 to measure and record the amplitudes of the five muscle compound action potentials in the sweep.

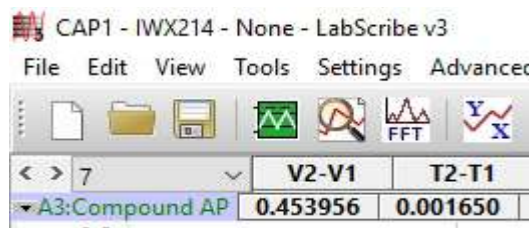


Figure AN-3-L5: The upper left corner of the Analysis window showing the Sweep menu used to select the sweep to be measured.

9. Measure and record the amplitudes of the muscle compound action potentials in the three other sweeps in Exercise 3. Follow the directions explained earlier in this data analysis section to display, select, measure, and record data.
10. Select Save in the File menu.
11. Click on the Main Window icon to return to that window.

Questions: Facilitation of the Muscle Response

1. How does decreasing the interval between successive stimulus pulses affect the amplitude of the compound muscle action potential?
2. What practical implication does this phenomenon have for muscle function?

Exercise 4: Effects of Eserine

1. Use the Sweep Selection bar at the bottom of the Main window to display the first sweep recorded in Exercise 4. Click on the tab on the selection bar for that sweep and the sweep will appear on the Main window. According to the design of the exercise, the nerve-muscle preparation was bathed with normal Ringer's solution and the stimulus pulse delivered to the nerve in this sweep had an amplitude of 0.100 V.
2. Transfer the sweep to the Analysis window by clicking on the Analysis window icon in the toolbar or selecting Analysis from the Windows menu.
3. Maximize the height of the traces on the Nerve and Muscle CAP channels by clicking on the arrow to the left of the title of each channel to open the channel menu. Select Scale from the menu and AutoScale from the Scale submenu.
4. Measure the amplitude of the muscle compound action potential. Place one cursor on the baseline of the recording on the Muscle CAP channel in the section before the stimulus was delivered. Place the other cursor on the peak of the muscle CAP. The value for the V2-V1 function on the Muscle CAP channel is the amplitude of this muscle compound action potential.
5. Record the amplitude of the muscle compound action potential in the Journal using the one of the techniques described in data analysis section of Exercise 2, and on [Table AN-3-L5](#).
6. Measure the duration of the muscle compound action potential (CAP). Place one cursor on the beginning of the muscle CAP displayed on the Muscle CAP channel. Place the other cursor at

the end of the muscle CAP. The value for the T2-T1 function on the Muscle CAP channel is the duration of the muscle compound action potential in this sweep.

7. Record the duration of the muscle compound action potential in the Journal using the one of the techniques described in data analysis section of Exercise 2, and on the data table.
8. To display another sweep made with a different stimulus amplitude, click on the tab for that sweep in the Sweep Selection bar at the bottom of the Analysis window. The sweep that is selected will appear on the Analysis window.
9. To take measurements from the second 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 4 through 7 to measure and record the amplitude and duration of the muscle compound action potential in the sweep.
10. Measure and record the amplitudes and durations of the muscle compound action potentials in the other sweeps made with different stimulus amplitudes in both treatments (Ringer's solution and Ringer's solution with eserine). Use the same techniques explained earlier to display, select, measure, and record the data.
11. Select Save in the File menu.
12. Click on the Main Window icon to return to that window.

Table AN-3-L5: Amplitudes and Durations of Muscle Compound Action Potentials Before and After Treatment with Eserine.

	Normal Ringer's		Ringer's with Eserine	
Stimulus Amplitude (V)	Muscle CAP Amp (V)	Muscle CAP Period (msec)	Muscle CAP Amp (V)	Muscle CAP Period (msec)
0.100				
0.200				
0.300				
0.400				
0.500				

Questions: Effects of Eserine

1. Does eserine have any effect on the excitability or threshold of the muscle?
2. What is the mechanism of action of eserine?

Exercise 5: Effects of Other Agents

1. Use the same techniques used in the analysis of Exercise 4 to take measurements from the data in Exercise 5.
2. Use the same techniques used in Exercises 2, 3 and 4 to record the measurements from this exercise in the Journal, and on [Table AN-3-L6](#).
3. Select Save in the File menu.

Questions: Effects of Other Agents

1. Does the agent that you tested have any effect on the excitability or threshold of the muscle? Those of the nerve?
2. What is the mechanism of action of your agent?

Table AN-3-L6: Amplitudes and Durations of Muscle Compound Action Potentials Before and After Treatment with Other Agents.

	Normal Ringer's		Ringer's with _____	
Stimulus Amplitude (V)	Muscle CAP Amp (V)	Muscle CAP Period (msec)	Muscle CAP Amp (V)	Muscle CAP Period (msec)
0.100				
0.200				
0.300				
0.400				
0.500				