Experiment AM-8: *Mytilus* Anterior Byssal Retractor Muscle

Equipment Required

PC or Mac Computer

IXTA, USB cable, IXTA power supply

FT-302 Force transducer

C-BNC-N2 Stimulating electrodes

Dissecting dish deep enough to hold seawater

Scalpel, dissecting scissors, and forceps

Insect pin bent into a hook shape

Modeling clay

Thread

Artificial seawater (See Appendix)

Acetylcholine (mixed in seawater at 10^{-4}M)

Serotonin (mixed in seawater at 10^{-5}M)

FT-302 and Stimulus Electrode Setup

1. Locate the following items: FT-302 force transducer and C-BNC-N2 stimulating electrodes.

Figure AM-8-S1: The FT-302 force transducer.

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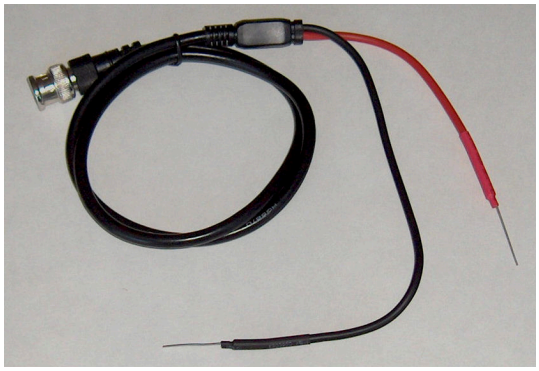

Figure AM-8-S2: The C-BNC-N2 stimulating electrodes.

2. Plug the DIN8 connector of the FT-302 into Channel A5.
3. Attach the C-BNC-N2 stimulating electrode cable to the S1 connection on the Low Voltage Stimulator on the front of the IXTA.

Figure AM-8-S3: The FT-302 force transducer and the C-BNC-N2 stimulating electrodes connected to the IXTA.

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Calibration of the FT-302 Force Transducer

1. Type **No Weight** in the Mark box. Click Record, and press the Enter key to attach the comment to the recording. Record for ten seconds with no weight hanging from the arm or hook of the transducer.
2. Type **5 grams** in the Mark box. Hang a 5 gram weight on the arm or hook of the transducer. Press the Enter key on the keyboard. Record for ten more seconds.
3. Click Stop to halt the recording.
4. Select Save As in the File menu, and name the file. Choose a destination on the computer in which to save the file. Click on the Save button to save the data file.

Unit Conversion

1. Scroll to the beginning of data when no weight was attached to the force transducer.
2. Use the Display Time icons on the LabScribe toolbar to adjust the display time of the Main window to show the complete calibration data on the Main window.
3. Click the Double Cursor icon. Place one cursor on the flat section of data collected when no weight was attached to the force transducer, and the second cursor on the flat section of data collected when the 5 gram weight was attached to the transducer.
4. To convert the voltages at the positions of the cursors to correct values, use the Simple Units Conversion dialogue window. Click V2-V1 on the force channel, then select Units, and select Simple.
    - Put a check mark in the box next to Apply units to all blocks. Notice that the voltages from the positions of the cursors are automatically entered into the value equations.
    - Enter “Zero” in the corresponding box to the right of the voltage recorded when no weight was attached to the transducer. Enter “5” in the box to the right of the corresponding voltage recorded when the 5 gram weight was hung on the hook of the transducer.
    - Enter the name of the units, grams, in the box below the weights. Click on the OK button in the lower right corner of the window to activate the units conversion.

In the 10 gram range, the FT-302 will deliver approximately 75 mV/gram at x1 gain and approximately one tenth of that in the 100 gram range. The FT-302 is now ready for use.

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Experiment AM-8: *Mytilus* Anterior Byssal Retractor Muscle

The Dissection

1. Place a mussel in the dissecting dish and cover it with cold seawater for 15 minutes.
2. Place a scalpel blade between the valves at the byssal gape on the ventral surface (the flatter edge) and cut the adductor muscles that keep the valves closed at the anterior and posterior end of the mussel. Try to cut these muscles close to the surface of the top valve. A flat blade screwdriver may help to pry the valves apart.
3. Pry open the mussel and cut any remaining muscles that are keeping the valves from opening.
4. Peel the mantle off of the surface of the top valve and remove the valve by twisting it off. It doesn't matter whether you remove the left or the right valve.
5. Put the mussel in the dissecting dish and observe its anatomy. Several soft structures surround the tougher muscles. The mantle spreads over the surface of both valves and secretes the material that builds the shell. Much of the rest of the soft structures are the ctenidia, the filamentous structures that move seawater though the mussel and filter out its food.
6. Remove the mantle, the ctenidia, and all other structures except for the adductor muscles, the muscular brown foot and the byssal muscles.

Figure AM-8-L1: Dissected Mytilus. The anterior byssal retractor muscle stretches from the apex of the valve to the base of the foot.

7. Moisten the exposed muscles with cold seawater every few minutes.
8. The anterior byssal retractor muscle is a white to yellow muscle that extends from its origin at the anterior tip of the shell to its insertion at the base of the foot.

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Note: *Isolate as much of the ABRM as possible, since it will be used to attach the muscle to the transducer.*

9. Secure the mussel securely to the dissecting dish. Placing modeling clay beneath and around the mussel may provide sufficient anchorage. The mussel may be fastened more securely by placing a rubber band across the mussel and fastening it to the wax or Sylgard substrate with insect pins.

The Preparation

1. Place the force transducer on a manipulator stand or a small ring stand.
2. Tie a piece of thread under the head of the hook-shaped pin.
3. Place the hook around the center of the anterior byssal retractor muscle.

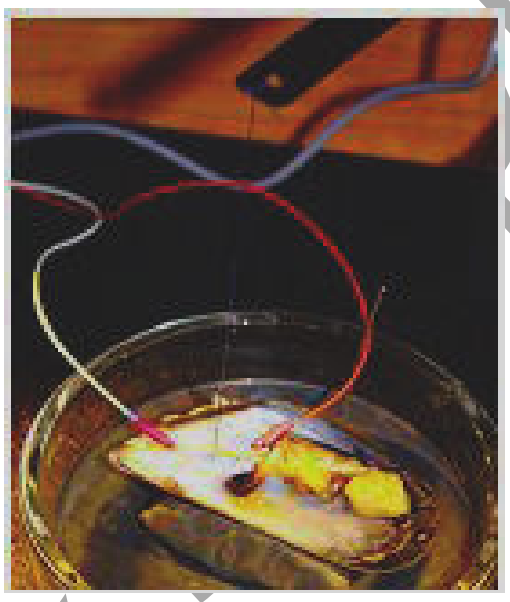

Figure AM-8-L2: The equipment used to evoke and record contractions from the anterior byssal retractor muscle.

4. Attach the thread to the hole on the end of the blade of the force transducer.
5. Adjust the height of the transducer so that the byssal muscle is lifted slightly.
6. Place one of the stimulating needle electrodes through the byssal muscle on either side of the insect pin hook.
7. The muscle preparation used in this experiment is functional for a limited period of time. If the muscle is bathed periodically in seawater, it will work for about two hours. To conserve time, complete all the exercises in the experiment before analyzing the data.

Worx Muscle Lab

Exercise 1: Stimulus-Response

Aim: To determine the relationship between the strength of the stimulus and the response of the muscle.

Approximate Time: 15 minutes

Procedure

1. Click the Stimulator Preferences icon on the LabScribe toolbar to open the stimulator control panel on the Main window.

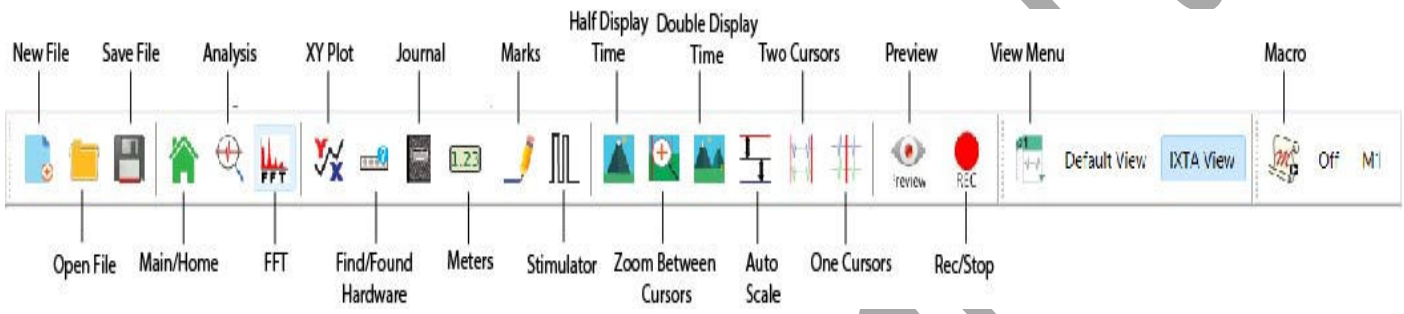

Figure AM-8-L3: The LabScribe toolbar.

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 0.000 V; the number of pulses (#pulses) to 1; and the pulse width (W) to 10ms. 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
   - 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.

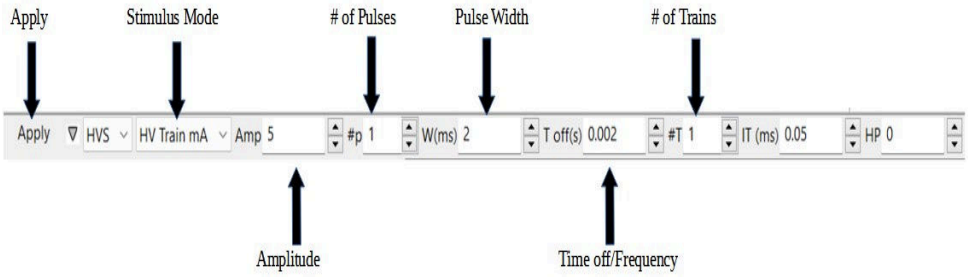

Figure AM-8-L4: The stimulator control panel

3. Type 0.000V in the Mark box.
4. Click **Record** to stimulate the muscle. Click the mark button to attach the comment to the recording.

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5. Click **Stop** to halt the recording.
6. Change the stimulus amplitude (Amp) to 0.250V using one of the techniques described in Step 2. Click the **Apply** button to finalize the change in the stimulus amplitude.
7. Type 0.250V in the Mark box.
8. Click **Record** to stimulate the muscle. Click the mark button to attach the comment to the recording.

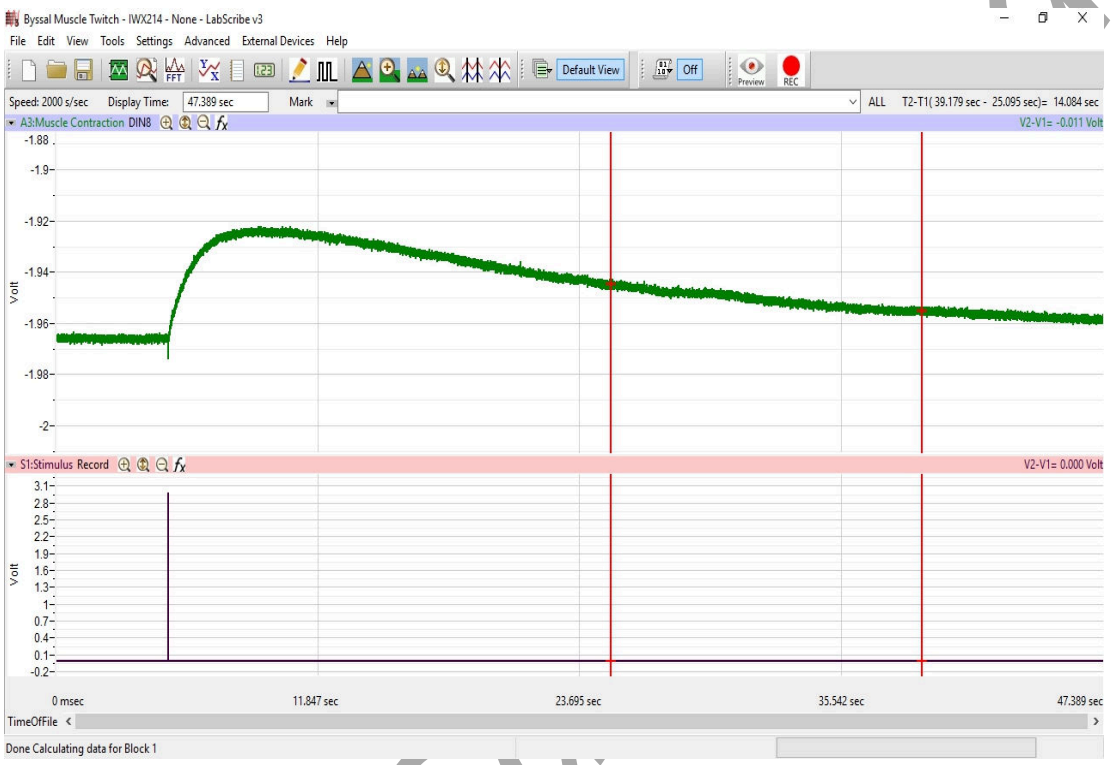

Figure AM-8-L5: A recording of a muscle twitch (upper trace) and the stimulus pulse (lower trace).

9. Increase the stimulus amplitude in increments of 0.250 Volt. Record and mark the muscle responses. Increase the stimulus amplitude until the muscle twitch reaches a maximum amplitude or the stimulus amplitude is 5V. The recording should look like the data above.
10. Select **Save As** in the File menu, type a name for the file. Click on the **Save** button to save the data file.
11. Moisten the muscle with seawater.

Exercise 2: Summation and Tetanus

Aim: To measure the amplitude of contraction produced in a muscle that is stimulated with a long current pulse, and repeated pulses delivered at progressively higher frequencies.

Approximate Time: 15 minutes

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Procedure

1. 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 the voltage that caused a maximal muscle response in Exercise 1; the number of pulses (#pulses) to 25; and, the frequency (F) to 0.5 Hz. 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 the change in any stimulus parameter
2. Type 0.5 Hz in the Mark box.
3. Click **Record** to stimulate the muscle. Click the mark button to attach the comment to the recording.
4. Click **Stop** to halt the recording. A muscle response to a stimulus frequency of 0.5Hz is displayed below.

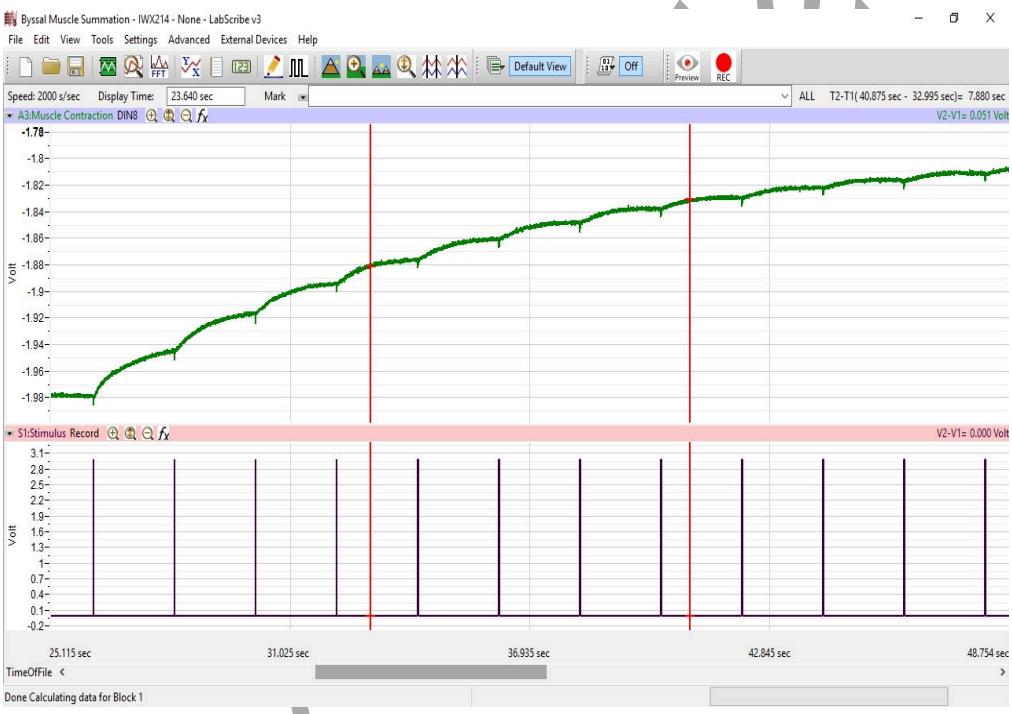

Figure AM-8-L6: A recording of mechanical summation at a stimulus frequency of 0.5 Hz. The muscle does not have time to return to resting tension between contractions.

5. Change the stimulus frequency (F) to 1 Hz using one of the techniques described in Step 1. Click the **Apply** button to finalize the change in the stimulus amplitude.
6. Type 1 Hz in the Mark box.
7. Click **Record** to stimulate the muscle at 1 Hz. Click the mark button to attach the comment to the recording.
8. Click **Stop** to halt the recording.

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9. Repeat Steps 5 through 8 for each of the following frequencies: 2, 5, 10, 20, and 30 Hz. A muscle response to a stimulus frequency of 20 Hz is shown below.
10. Finally, stimulate the muscle with a five second pulse. Change the number of pulses to 1 and the pulse width to 5 seconds. Click the **Apply** button to finalize the change in pulse number and width.
11. Type 5-second pulse in the Mark box.
12. Click **Record** to stimulate the muscle. Click the mark button.
13. Once the muscle has completely relaxed, click **Stop** to halt the recording
14. Select Save in the File menu.

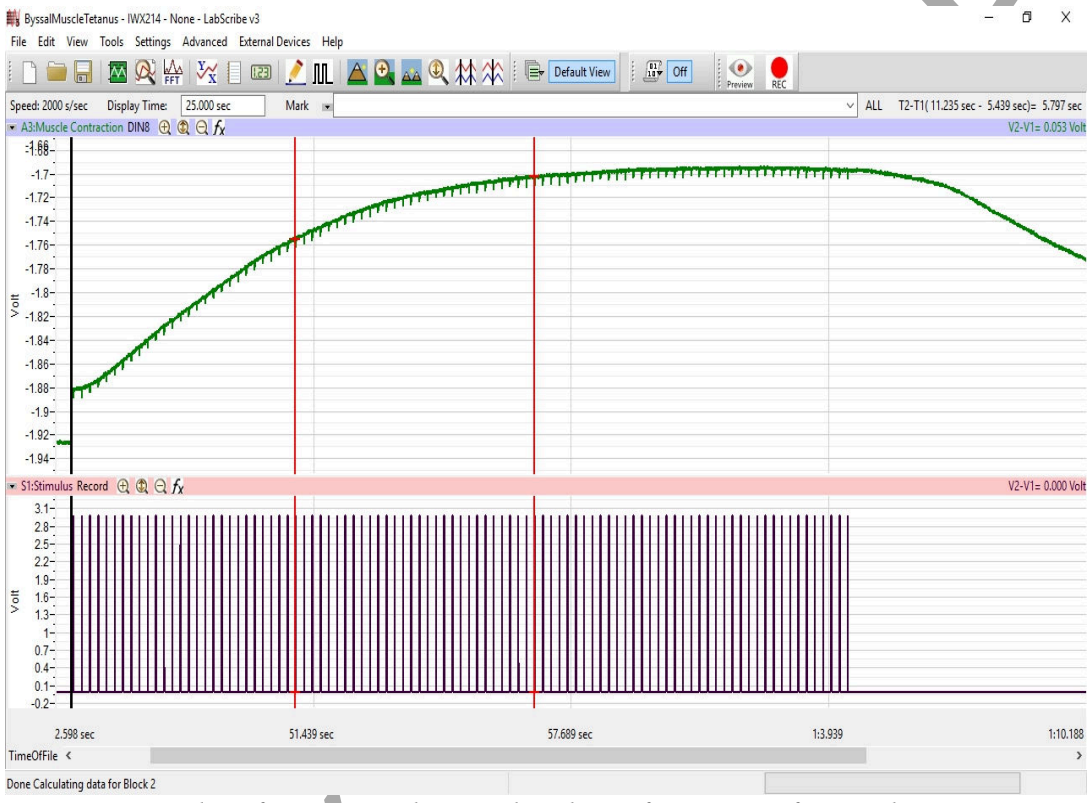

Figure AM-8-L7: Recording from a muscle stimulated at a frequency of 20Hz demonstrating tetanus.

Exercise 3: Effects of Neurotransmitters on Muscle Contraction.

Aim: To observe the effects of acetylcholine and serotonin, the neurotransmitters that effect contraction and relaxation of the anterior byssal retractor muscle.

Approximate Time: 30 minutes

1. Pour enough seawater into the dissecting dish to cover the preparation and re-adjust the tension on the force transducer thread if necessary.
2. Type Acetylcholine in the Mark box.
3. Click **Record** and click the mark button to attach a comment to the recording.

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4. Remove the seawater with a syringe and replace it with the seawater solution of 10^{-4} M acetylcholine.
5. Once the maximal contraction from the acetylcholine has been reached, type Seawater in the Mark box, replace the acetylcholine solution with seawater, and click the mark button to attach the comment to the recording.
6. Allow the muscle to relax completely.
7. Click **Stop** to halt the recording.
8. Use a syringe to remove the seawater from the dissecting dish.
9. Type 5-second pulse and serotonin addition in the Mark box, and set the stimulator to deliver a single 5-second pulse that will produce a maximal contraction as you did in Step 10 of Exercise 2.
10. Click **Record** to stimulate the muscle with a 5-second pulse. Click the mark button to attach the comment to the recording.
11. As soon as possible after the muscle has reached its peak contraction from the 5-second pulse, pour enough of the 10^{-5}M serotonin solution into the dish to cover the muscle.
12. Once the muscle has relaxed, remove the serotonin solution with a syringe.
13. Click **Stop** to halt the recording.
14. Type 5-second pulse after serotonin in the Mark box.
15. Set the stimulator to deliver a single 5-second pulse that should produce a maximal contraction.
16. Click **Record** to deliver the 5-second pulse. Click the mark button to attach the comment to the recording.
17. Once the muscle has completely relaxed, click **Stop** to halt the recording.
18. Wait one minute, type 2nd pulse after serotonin in the Mark box.
19. Click **Record** to deliver the five-second pulse. Click the mark button to attach the comment to the recording
20. After the muscle has completely relaxed, click **Stop** to halt the recording.
21. Rinse the prep several times with seawater.
22. Wait one minute, type 1st pulse after seawater washes in the Mark box.
23. Click **Record** to stimulate the muscle with another 5-second pulse. Click the mark button to attach the comment to the recording.
24. After the muscle has relaxed completely, click **Stop** to halt the recording.
25. Repeat steps 22 to 24 every five minutes for the next twenty minutes, changing the mark with each pulse to reflect the order. Keep the muscle moist by dripping seawater on it between stimulations.
26. After the muscle has relaxed for the final time, click **Stop** to halt the recording.
27. Select Save in the File menu.

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Data Analysis

Exercise 1: Stimulus-Response

1. Scroll through the data from Exercise 1 and find the first muscle twitch to be generated by a stimulus pulse. Click the AutoScale button to maximize the size of the muscle twitch on the window. Note the stimulus voltage used to generate this twitch.

Note: *At stimulus voltages that are below the threshold of the muscle, the amplitude of the muscle twitch is zero.*

2. Use the Display Time icons to adjust the Display Time of the Main window to show the stimulus pulse used to generate the twitch and the complete twitch on the Main window. The stimulus pulse and the twitch can be selected by:
   - Placing a cursor before the stimulus pulse, and a cursor after the muscle has completely relaxed; and
   - Clicking the Zoom between Cursors button on the LabScribe toolbar to expand the display of the stimulus pulse and the twitch to the width of the Main window.
3. Data can be collected from the Main window or the Analysis window. If you choose to use the Analysis window, click on the Analysis window icon in the toolbar.
4. Values for V2-V1 and T2-T1 on each channel are seen in the table across the top margin of each channel, or to the right of each graph.
5. Maximize the height of the trace on the Muscle Contraction Channel by clicking the AutoScale All button on the toolbar.

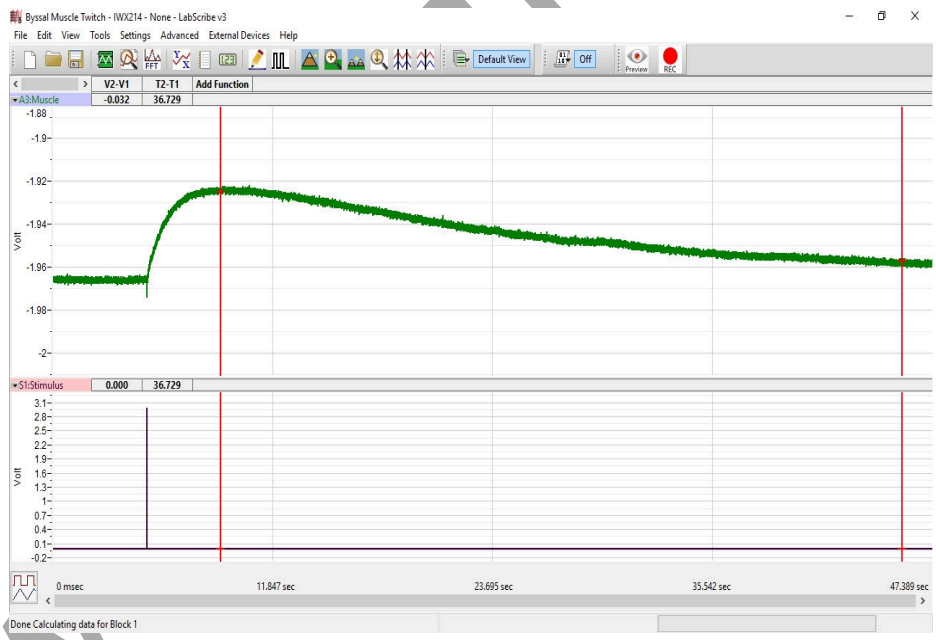

Figure AM-8-L8: A single muscle twitch and stimulus pulse displayed in the Analysis window. The cursors are located at the peak of the contraction and the point at which the muscle has returned to its baseline tension. The time between them represents the relaxation duration.

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6. The functions in the channel pull-down menus of the Analysis window can also be used to enter the names and values of the parameters from the recording to the Journal. To use these functions:
    - Place the cursors at the locations used to measure the amplitude and times of each muscle twitch.
    - Transfer the names of the mathematical functions used to determine the amplitude and times to the Journal using the Add Title to Journal function in the Muscle Contraction Channel pull-down menu.
    - Transfer the values for the amplitude and times to the Journal using the Add Ch. Data to Journal function in the Muscle Contraction Channel pull-down menu.
7. On the Muscle Contraction Channel, use the mouse to click on and drag the cursors to specific points on the recording to measure the following parameters:
    - **Muscle Contraction Amplitude** is the difference between the baseline tension of the muscle and the tension at the peak of the twitch. To measure this parameter, place one cursor at the beginning of the twitch, and the second cursor on the peak of the twitch. The value for the V2-V1 function on the Muscle Twitch Channel is the muscle twitch amplitude.
    - **Contraction Time** is the time between the beginning and the peak of the twitch. To measure this parameter, keep the cursors in the same positions used to measure the muscle twitch amplitude. The value for the T2-T1 function on the Muscle Twitch Channel is the contraction time of the twitch.
    - **Relaxation Time** is the time between the peak of the twitch and the return of the muscle tension to the baseline level. To measure this parameter, keep the cursor on the peak of the twitch and place the other cursor at the end of the twitch. The value for the T2-T1 function on the Muscle Contraction Channel is the relaxation time of the twitch.
    - **Latency** is the time it takes the muscle to start responding to a stimulus. Place one cursor at the beginning of the stimulus pulse, and the other cursor at the beginning of the muscle twitch. The value for the T2-T1 function on the Muscle Contraction Channel is the latency of the muscle response
8. Record the values in the Journal using the one of the techniques described in Step 6, and in Table 1.
9. Repeat Steps 2 through 7 to find the muscle twitch amplitude, contraction time, relaxation time, and latency of the other muscle twitches recorded in this exercise. Record the values in the Journal and in Table 1.
10. Select Save in the File menu.
11. Graph the muscle twitch amplitude and the contraction time as a function of the stimulus amplitude.

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Questions

1. How does direct electrical stimulation produce contractions of the muscle?
2. Why doesn't the muscle respond to low stimulus voltages?
3. Why does the amplitude of the muscle response increase with increasing stimulus voltages?
4. At high stimulus voltages, the muscle response reaches a maximum amplitude. Why doesn't the muscle response continue to increase with increasing stimulus voltages?

Table AM-8-L1: Amplitudes and Times of Muscle Twitches Generated by Stimulus Pulses of Different Amplitudes.

	Muscle Twitch			
Stimulus Amplitude (V)	Amplitude (mV)	Contract Time (sec)	Relax Time (sec)	Latency (msec)
0.000	0	0	0	0
0.250				
0.500				
0.750				
1.000				
1.250				
1.500				
1.750				
2.000				
2.250				
2.500				
2.750				
3.000				

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3.250				
3.500				
3.750				
4.000				

Exercise 2: Summation and Tetanus

1. Scroll to the beginning of the data recorded for Exercise 2. Click the AutoScale button to maximize the size of the muscle twitches on the window.
2. Scroll through the data from Exercise 2 and find the first series of muscle twitches in which the muscle does not have sufficient time to fully relax to the baseline tension level between twitches. This phenomenon is known as mechanical summation
3. Use the Display Time icons to adjust the Display Time of the Main window to show all the twitches in the series on the Main window. The twitches can also be selected by using the Zoom between Cursors function explained in Step 1 of the data analysis section for Exercise 1.
4. Data can be collected from the Main window or the Analysis window. If you choose to use the Analysis window, click on the Analysis window icon in the toolbar.
5. Values for V2-V1 and T2-T1 on each channel are seen in the table across the top margin of each channel, or to the right of each graph.
6. Maximize the height of the trace on the Muscle Twitch Channel by clicking the AutoScale All button on the toolbar.
7. On the Muscle Contraction Channel, use the mouse to click on and drag the cursors to specific points on the recording to measure the following parameters:
    - **Amplitude of First Muscle Twitch**, which is the difference between the baseline tension of the muscle and the tension at the peak of the first twitch in the series. To measure this parameter, place one cursor at the beginning of the first twitch, and the second cursor on the peak of the twitch. The value for the V2-V1 function on the Muscle Contraction Channel is the amplitude of the first muscle twitch in the series.
    - **Maximum Amplitude in Summation/Tetanus**, which is the difference between the baseline tension of the muscle and the tension at the peak of the tallest twitch in the series. To measure this parameter, place one cursor at the beginning of the first twitch, and the second cursor on the peak of the tallest twitch in the series. The value for the V2-V1 function on the Muscle Contraction Channel is the amplitude due to mechanical summation.

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- **Change in Passive Tension**, which is the difference between the baseline tension of the muscle and the tension at the highest relaxation point between the twitches in the series. To measure this parameter, place one cursor at the beginning of the first twitch, and the second cursor on the highest relaxation point between any pair twitches in the series. The value for the V2-V1 function on the MuscleContraction Channel is the increase in the passive tension in the muscle during the series of twitches.

8. Record the values in the Journal using the one of the techniques described in Steps 7 or 8 in the data analysis section for Exercise 1, and on Table 2.
9. Repeat Steps 2 through 9 to find the amplitude of the first twitch, maximum amplitude in summation, and change in passive tension for each of the other series of twitches recorded in this exercise. Record the values in the Journal and on Table 2.
10. Select Save in the File menu.
11. Determine the frequencies at which the following first appear:
    - Mechanical summation.
    - Incomplete tetanus
    - Complete tetanus
12. Find the continuous current pulse on the trace. Determine the maximum amplitude of the contraction and record the value in the Journal and in Table 2.
13. Place one cursor at the end of the continuous current pulse, and the other at the point when the muscle returns to its original baseline. Record the T2-T1 value in the Journal and in Table 2.

Questions

1. If contraction amplitude is dependent upon the increases in concentration and persistence of intracellular calcium, why are the contraction amplitudes of single twitches the same?
2. Tetanus occurs at a much lower stimulus frequency in this muscle than it does in vertebrate skeletal muscle. How might this be advantageous to a mussel, given the function of the anterior byssal retractor muscle?
3. Why is the rate of muscle relaxation much slower after tetanus than after a single twitch?
4. After the 5-second current pulse, how long does it take for the muscle to return to its baseline tension? How does this compare to the time it takes for the muscle to return to baseline levels after a series of short pulses?

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Table AM-8-L2: Strength of Muscle Contractions during Mechanical Summation and Tetanus

Stimulus Frequency (Hz)	Amplitude 1st Twitch (V)	Maximum Amplitude (V)	Change in Passive Tension (V)	Sum/Tetanus	T2-T1 (sec)
0.5			0	No	
1					
2					
3					
4					
5					
10					
20					
30					

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Exercise 3: Effects of Neurotransmitters on Muscle Contraction.

1. Scroll through the trace to the introduction of acetylcholine. Click the AutoScale button to maximize the size of the muscle contraction that resulted from the introduction of acetylcholine. Find the maximum contraction.
2. If necessary, use the Display Time icons to adjust the Display Time of the Main window to show the entire contraction on the Main window.
3. Data can be collected from the Main window or the Analysis window. If you choose to use the Analysis window, click on the Analysis window icon in the toolbar.
4. Values for V2-V1 and T2-T1 on each channel are seen in the table across the top margin of each channel, or to the right of each graph.

5. On the Muscle Contraction Channel, use the mouse to click on and drag the cursors to specific points on the recording to measure the Maximum Contraction Amplitude, which is the difference between the baseline tension of the muscle and the tension at the peak of the contraction. To measure this parameter, place one cursor at the beginning of the contracting, and the second cursor on the peak. The value for the V2-V1 function on the Muscle Contraction Channel is the maximal amplitude of the contraction.
6. Record this value in the Journal and in Table 3.
7. Determine the duration of the relaxation after the peak contraction that resulted from the addition of acetylcholine by positioning the cursors at the peak of the contraction and at the point at which the muscle returned to its baseline tension. Record T1-T2 in the Journal and in Table 3.
8. Find the contraction in which serotonin was added at the beginning of the relaxation. Position the cursors at the peak of the contraction and the point at which the muscle returns to its baseline tension. Enter the T1-T2 value in the Journal and in Table 3. Determine the amplitude of this contraction by using the procedure you previously used in Step 2. Enter this value in the Journal and in the table.
9. Determine the contraction amplitude and the relaxation duration for the muscle contractions resulting from the 5-second pulses after the removal of the serotonin solution and after the seawater washes. Enter these values in the Journal and in the table.

Questions

1. Was the maximum contraction with the addition of acetylcholine similar to the maximum contraction produced by electrical stimulation? Why might they differ?
2. Compared to previous contractions, did the rate of relaxation change immediately after the addition of serotonin?
3. Did the contraction produced by the first 5-second current pulse after the serotonin solution was removed last as long as that produced by the 5-second current pulse in Experiment 2? Did the amplitude of contraction change? Discuss the effect of serotonin on the contraction and relaxation of the ABRM.
4. Did the rate of relaxation and the amplitude of the contractions return to normal after the serotonin was removed? Describe the time course of recovery and explain why it may be so slow.

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Table AM-8-L3: Effects of Acetylcholine and Serotonin

Condition	Amplitude of Contraction (mV)	Duration of Relaxation (sec)
Addition of acetylcholine		
5-second pulse just before the addition of serotonin		
1st stimulating pulse after the removal of serotonin		
2nd stimulating pulse after the removal of serotonin		
1st stimulating pulse after seawater washed		
2nd pulse after seawater washes		
3rd pulse after seawater washes		
4th pulse after seawater washes		
5th pulse after seawater washes		

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