Experiment AM-2: Skeletal Muscle, Summation and Tetanus

Equipment Required

PC Computer

IXTA data acquisition unit, USB cable, IXTA power supply

FT-302 Force transducer

A-BST-100 Stimulating electrodes

Ring stand and clamps

Femur clamp

Thread

15 cm Ruler

Amphibian Ringer's solution (See appendix)

FT-302 and Stimulus Electrode Setup

1. Locate the following items: FT-302 force transducer and the A-BST-100 bipolar stimulator cable.

Figure AM-2-S1: The FT-302 force transducer.

Figure AM-2-S2: The A-BST-100 bipolar stimulating electrode.

2. Plug the FT-302 into Channel A5 input.

Figure AM-2-S3: The FT-302 force transducer and the A-BST-100 bipolar stimulating electrode connected to the IXTA.

4. Connect the BNC connector of the A-BST-100 bipolar stimulator cable to the Stimulator 1 output on the IXTA.

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.

Note: *Moisten the exposed limbs of the frog with Ringer's solution every five minutes or so.*

4. Identify the Gastrocnemius muscle on the lower leg.
5. Use a glass hook to separate the Gastrocnemius muscle from the bone and other muscles of the lower leg.
6. Use scissors to free the Achilles tendon from the connective tissue around the heel of the foot. Double up a 24” piece of thread. Firmly tie the doubled thread around the Achilles tendon, leaving the ends of the thread long enough to attach the muscle to the transducer.

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

7. Cut the Achilles tendon as close to the bottom of the foot as possible, so the thread is still attached to the Gastrocnemius muscle.
8. Move the Gastrocnemius muscle away from the rest of the lower leg. Cut the tibia just below the knee to separate the rest of the lower leg from the preparation. Rinse the preparation with Ringer’s solution to moisten the tissue and rinse away any blood.
9. Dissect away the muscles of the upper leg and expose the femur. Use a stout pair of scissors to cut the femur close to the pelvis. Rinse the preparation with Ringer’s solution to moisten the tissue and rinse away any blood.

The Preparation

1. Use the femur clamp to mount the preparation on the ringstand.
2. Attach the thread on the Achilles tendon to the 100 g hook on the end of the force transducer.
3. Adjust the femur clamp and the force transducer so the thread from the Achilles tendon to the hook. There should be no slack in the thread, but do not stretch the muscle past its in situ length.
4. Position the stimulating electrodes so they lay against the muscle about midway between the knee and the tendon. The two electrodes should not touch one another.
5. When recording, use the “zeroing” knob at the top of the transducer to zero the baseline.

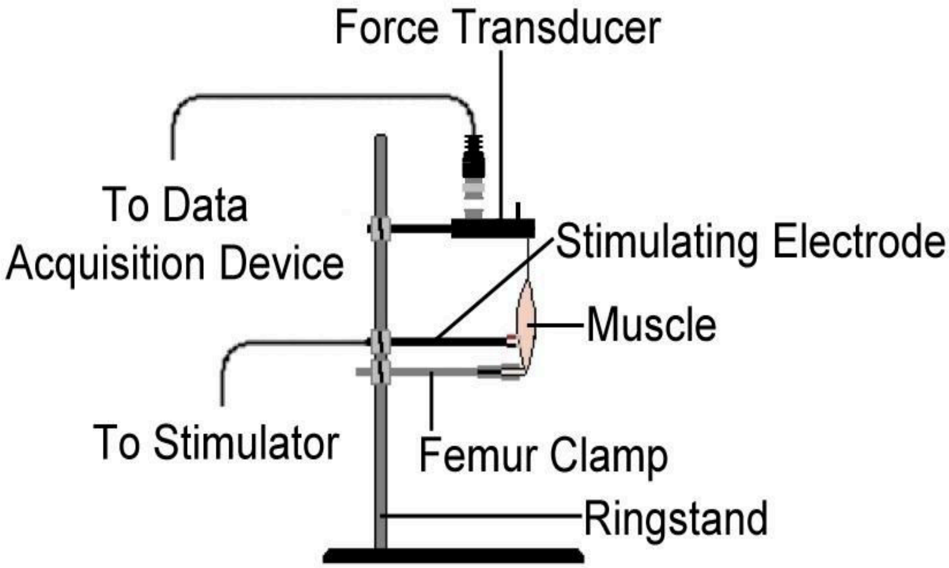

Figure AM-2-S4: The equipment used to record contractions from the frog Gastrocnemius muscle.

Warning: The muscle preparation used in this experiment is functional for a limited period of time. If the muscle 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.

Calibration of the FT-302 Force Transducer

1. Type **No Weight** in the Mark box. Click Record, and click the mark button. 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. Click the mark button. 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 pressure 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.

Experiment AM-2: Skeletal Muscle, Summation and Tetanus

Warning: The muscle preparation used in this experiment is functional for a limited period of time. If the muscle 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.

Exercise 1: Stimulus-Response

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

Approximate Time: 45 minutes

Procedure

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

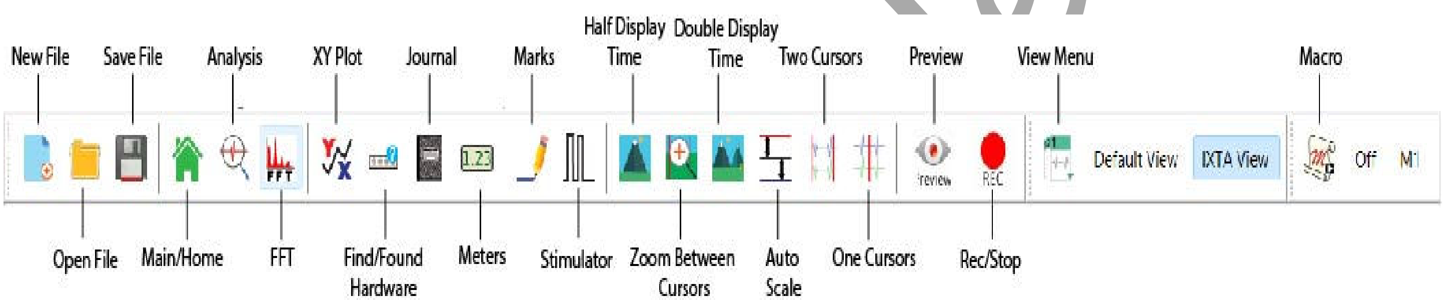

Figure AM-2-L61: The LabScribe toolbar.

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

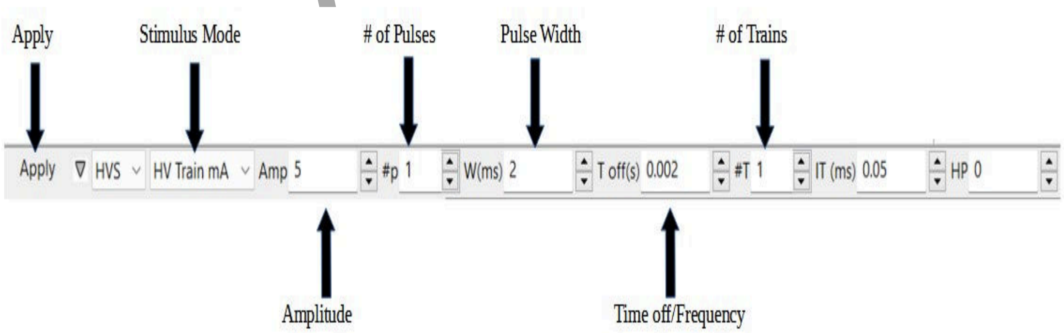

Figure AM-2-L2: The stimulator control panel

3. Click Record to stimulate the nerve with 0V. Type **0** in the Mark box and then click the mark button.
4. Click Stop to halt the recording.
5. Change the stimulus amplitude (Amp) to 0.250V. Click the Apply button to finalize the change in the stimulus amplitude.
6. Click Record to stimulate the nerve with 0.250V. Type **0.250V** in the Mark box and click the mark button. The recording should look like the image below.

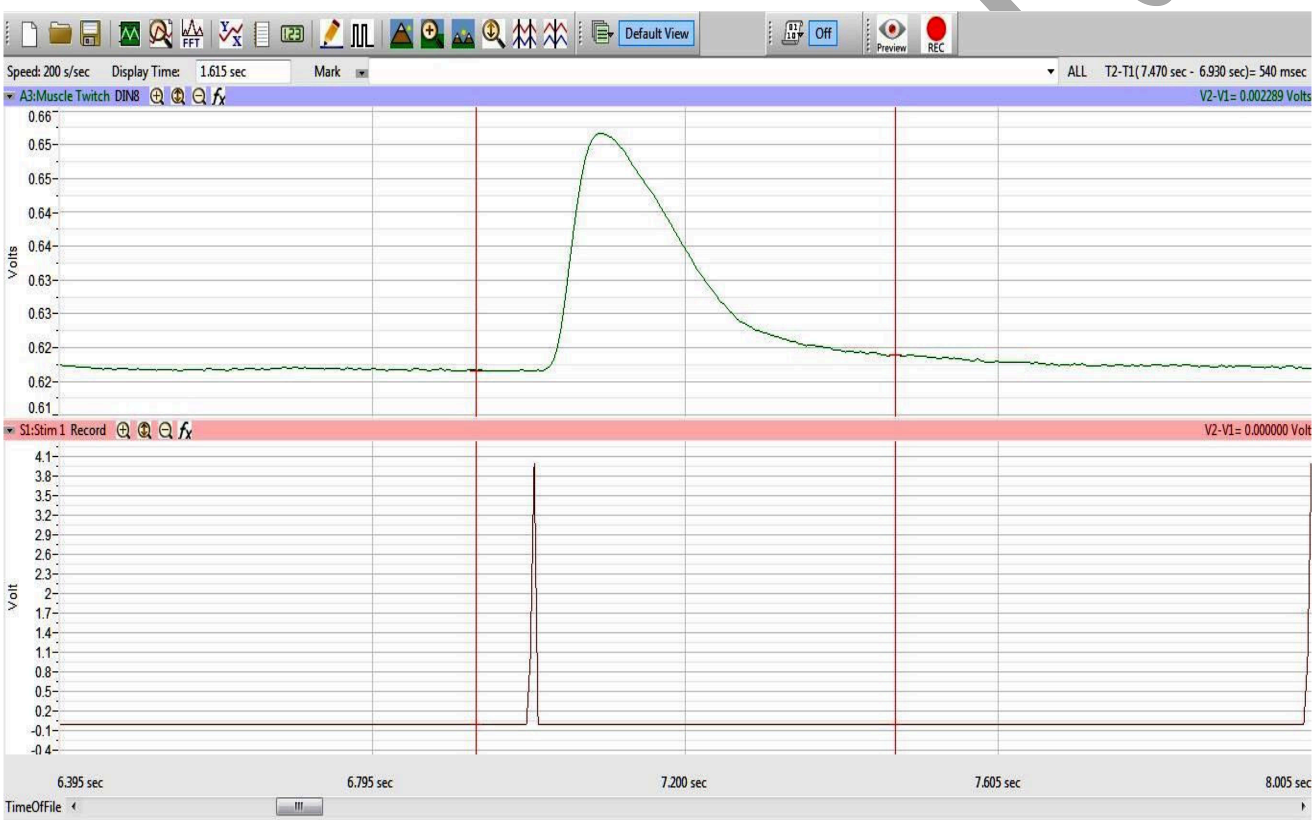

Figure AM-2-L3: A recording of a muscle twitch (upper trace) and the stimulus pulse (lower trace). The cursors were placed on the baseline (left) and the peak of the twitch (right).

7. Increase the stimulus amplitude in increments of 0.250 V. Record and mark the muscle response. Increase the stimulus amplitude until the muscle twitch reaches a maximum amplitude or the stimulus amplitude is 5V.
8. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.
9. Moisten the muscle with frog Ringer's solution.

Exercise 2: Summation and Tetanus

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

Approximate Time: 45 minutes

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 voltage that causes a maximal muscle response; the number of pulses (#pulses) to 15; and, the frequency (F) to 0.5 Hz. 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.
2. Click the Apply button to finalize the change in any stimulus parameter.
3. Click Record to stimulate the nerve at 0.5 Hz. Type **0.5 Hz** in the Mark box and click the mark button.
4. Click Stop to halt the recording.
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. Click Record to stimulate the nerve at 1 Hz. Type **1 Hz** in the Mark box and click the mark button to attach a comment to the recording.
7. Click Stop to halt the recording.
8. Repeat Steps 4 through 6 for each of the following frequencies: 2, 3, 4, 5, 10, 20, and 30 Hz. A muscle response to a stimulus frequency of 5 Hz is displayed below.
9. Select Save in the File menu.

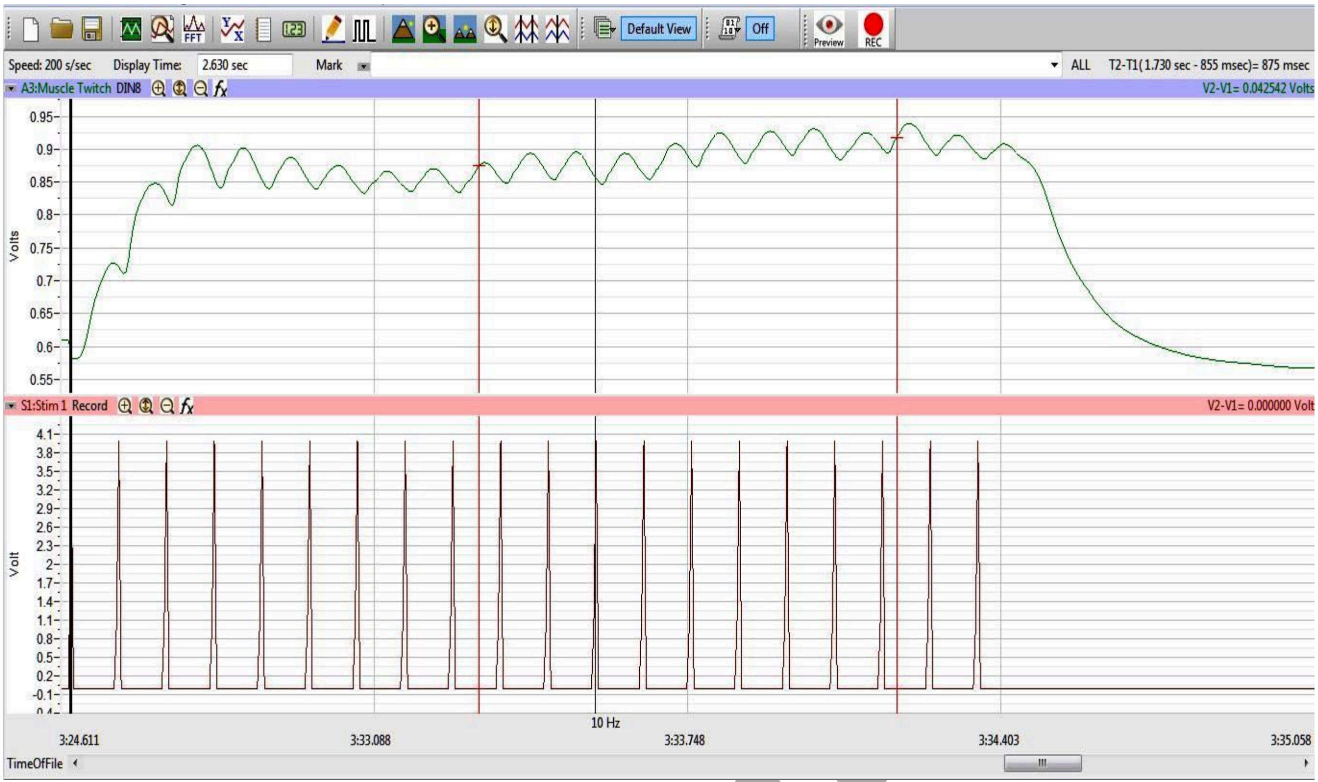

Figure AM-2-L4: A recording of mechanical summation at a stimulus frequency of 5 Hz. The muscle does not have time to return to resting tension between contractions.

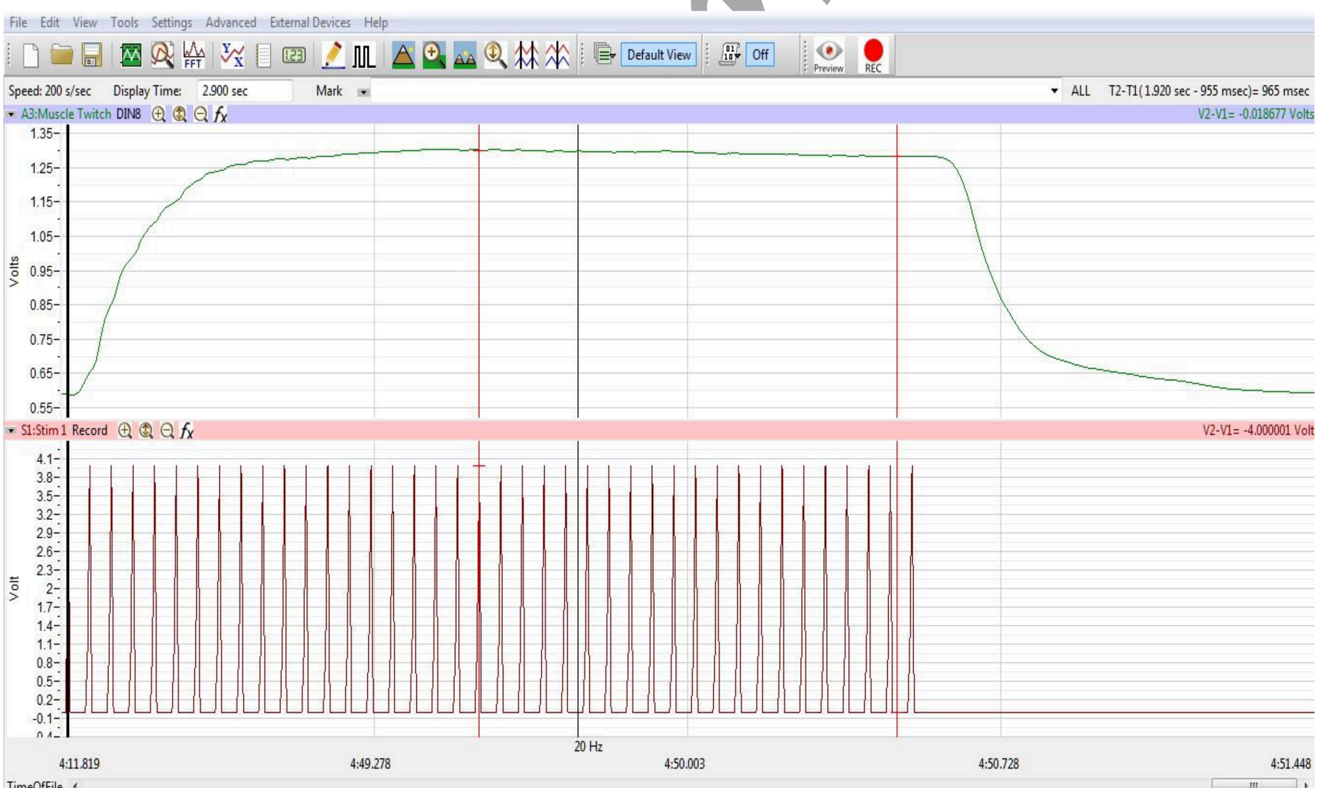

Figure AM-2-L5: Recording from a muscle stimulated at a frequency of 20Hz demonstrating tetanus.

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. 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.

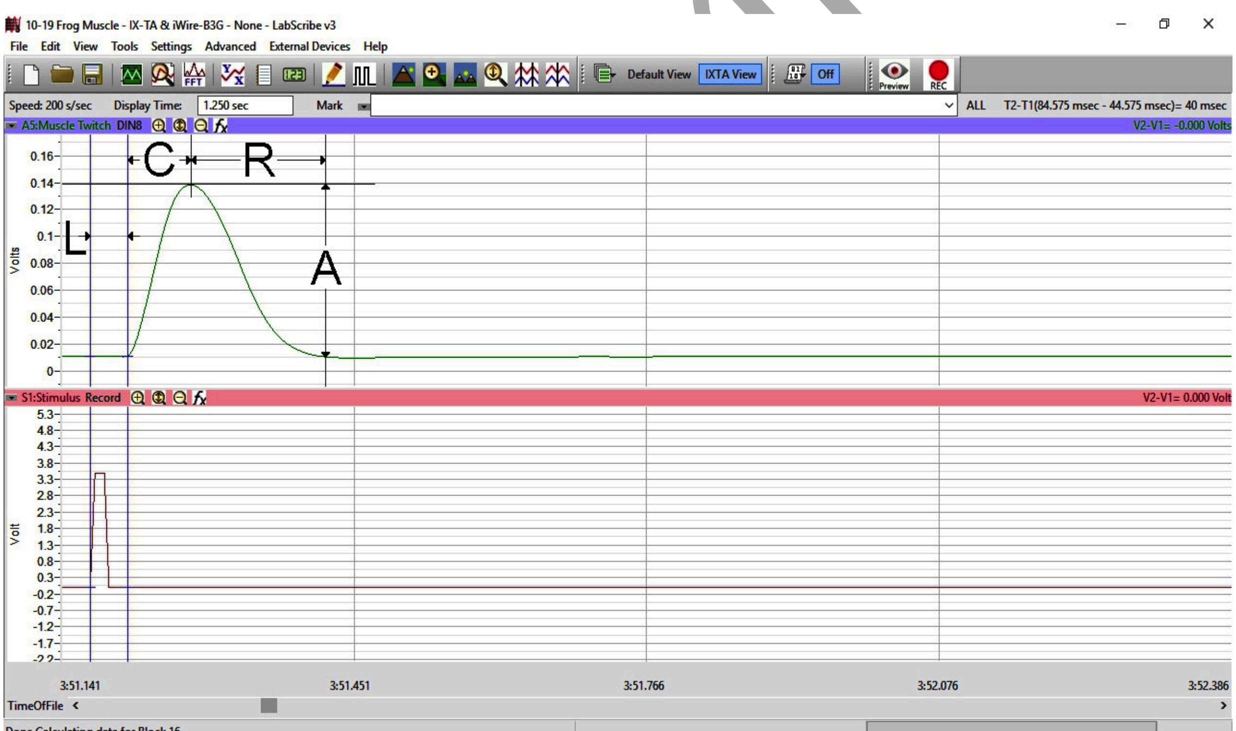

Figure AM-2-L6: A single muscle twitch and stimulus pulse displayed in the Analysis window. The labels indicate: latency (L); contraction time (C); relaxation tine (R); and twitch amplitude (A).

6. Once the cursors are placed in the correct positions for determining the amplitudes and times for each muscle twitch, the values of the parameters in the Function Table can be recorded in the on-line notebook of LabScribe by typing their names and values directly into the Journal, or on a separate data table.
7. 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 Twitch 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 Twitch Channel pull-down menu.
8. On the Muscle Twitch Channel, use the mouse to click on and drag the cursors to specific points on the recording to measure the following parameters:
    - Muscle Twitch Amplitude, which 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, which 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 is the contraction time of the twitch.
    - Relaxation Time, which 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 is the relaxation time of the twitch.
    - Latency, which 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 is the latency of the muscle response
9. Record the values in the Journal using the one of the techniques described in Steps 7 or 8, and on Table 1.
10. Repeat Steps 2 through 9 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 on the table.
11. Select Save in the File menu.
12. Graph the muscle twitch amplitude and the contraction time as a function of the stimulus amplitude.

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-2-L1: Amplitude and Times of Muscle Twitches Generated by Stimulus Pulses of Different Amplitudes.

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

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.

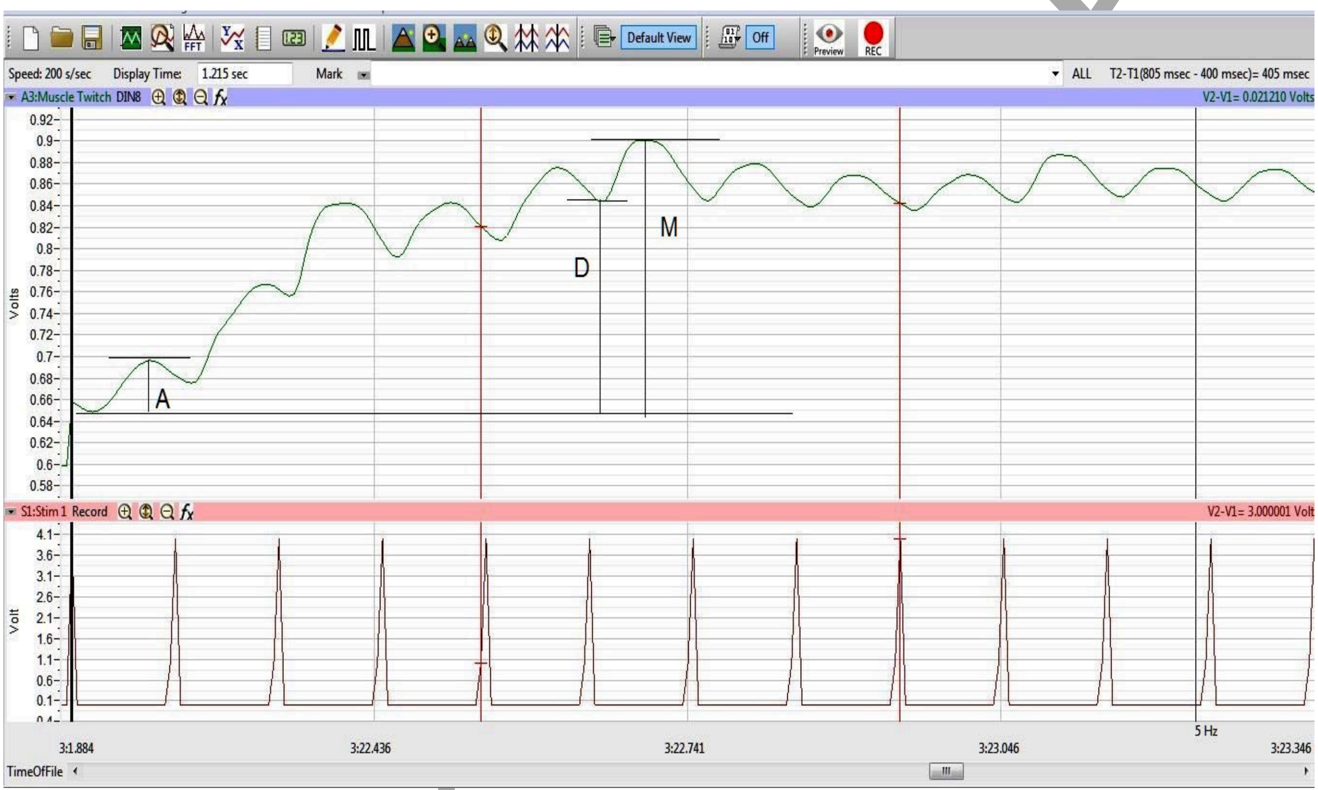

Figure AM-2-L7: A recording of mechanical summation at a stimulus frequency of 5 Hz displayed in the Analysis window. The labels indicate: the amplitude of the first muscle twitch (A); the maximum amplitude during summation (M); and the change in passive tension (D).

5. The mathematical functions, V2-V1 and T2-T1 should show in either Main or Analysis windows.
6. Maximize the height of the trace on the Muscle Twitch Channel by selecting AutoScale All.
7. On the Muscle Twitch 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 Twitch 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 Twitch Channel is the amplitude due to mechanical summation.
- 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 Muscle Twitch 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 the table.
10. Select Save in the File menu.
11. Determine the frequencies at which the following first appear:
    - Mechanical summation.
    - Incomplete tetanus
    - Complete tetanus

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 requires high stimulus frequencies. What does this tell you about calcium re-uptake by the sarcoplasmic reticulum?
3. Why is the rate of muscle relaxation much slower after tetanus than after a single twitch?

Table AM-2-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
0.5			0	No
1				
2				
3				
4				
5				
10				
20				
30				