Experiment AM-1: Skeletal Muscle, Weight and Work

Equipment Required
PC Computer
IXTA, USB cable, IXTA power supply
DT-475 Displacement transducer
A-BST-100 Stimulating electrodes
Ring stand and clamps
Femur clamp
Thread
6” Ruler
Set of weights and pan
Amphibian Ringer’s solution (See appendix)

DT-475 and Stimulus Electrode Setup
1. Locate the following items: DT-475 displacement transducer and the A-BST-100 bipolar stimulator cable.

Figure AM-1-S1: The DT-475 displacement transducer.

Figure AM-1-S2: The A-BST-100 bipolar stimulating electrode.
2. Plug the DIN8 connector of the DT-475 into Channel A5.
3. Attach the BNC connector of the A-BST-100 bipolar stimulator cable to the stimulator output.

Figure AM-1-S3: The DT-475 displacement transducer and the A-BST-100 bipolar stimulating electrode connected to 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 displacement 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 upper eyelet on the rod of the displacement transducer.

3. Use a paper clip to attach the weight pan to the lower eyelet on the rod.

4. Adjust the femur clamp and the displacement transducer so the thread from the Achilles tendon to the transducer rod is vertical.

5. To prevent the weight in the pan from stretching the muscle, the knob on the upper end of the rod should be resting on the bushing in the top of the transducer case.

6. 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.

7. Place two nickels (10 g) in the weight pan.
Figure AM-1-S4: The arrangement of the equipment used to evoke and 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.
Experiment AM-1: Skeletal Muscle, Weight and Work

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Exercise 1: Maximum Contraction
Aim: To make sure all fibers contract when the muscle is stimulated.
Approximate Time: 45 minutes

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

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 4.0; the number of pulses (#pulses) to 1; and, the pulse width (W) to 10ms. To change a stimulus parameter type the value of the parameter in the window next to the label of the parameter. Click the Apply button to finalize the changes.

3. Click Record to stimulate the nerve with 4.0mV. Type 4.0 in the Mark box and then click the Mark button.
4. Click Stop to halt the recording.

5. Change the stimulus amplitude (Amp) to 4.5mV using one of the techniques described in Step 2. Click the Apply button to finalize the change.

6. Click Record to stimulate the nerve with 4.5V. Type 4.5 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, 5, and 6 using a stimulus amplitude (Amp) of 5.0mV.

9. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.

10. Moisten the muscle with frog Ringer's solution.

**Exercise 2: After-loaded Weight and Contractile Strength**

Aim: To measure the strength of contraction while the muscle is lifting after-loaded weights.

Approximate Time: 45 minutes

**Procedure**

1. Make sure the thread connecting the Achilles tendon to the eye of the rod is vertical, and the knob on the upper end of the sliding rod of the transducer is resting on the top of the transducer case.

2. Check the values for the stimulus parameters that are listed in the stimulator control panel on the Main window. All stimulus parameters, except the pulse amplitude (Amp), should be set to the same values as the ones used in Exercise 1. Set the pulse amplitude (Amp) to a voltage that causes a maximal muscle response. To change a stimulus parameter, 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.

3. Type 10g in the Mark box. With the two nickels in the weight pan, click the Record button to record a single twitch. Click the mark button. Click Stop to halt the recording.

4. Add another 10g, or two more nickels, to bring the weight in the pan to a total of 20g. Type 20g in the Mark box. Click the Record button to record a single twitch. Click the mark button. Click Stop.

5. Increase the weight in the pan in 10g increments. Record the muscle response for each weight. Mark each recording to indicate the total weight lifted by the muscle. Continue to increase the weight until the muscle response is very small.

6. Select Save in the File menu.

7. Moisten the muscle with frog Ringer's solution.
Exercise 3: Pre-loaded Weight and Contractile Strength

Aim: To measure the strength of contraction while the muscle is lifting pre-loaded weights.

Procedure

1. Lower the displacement transducer. The knob on the lower end of the sliding rod should just be touching the bushing on the bottom of the transducer case. In this configuration, the weight in the pan stretches the muscle before it is stimulated. Make sure the thread connecting the Achilles tendon to the eye of the rod is vertical.

2. Repeat Exercise 2, applying the weights to the pan in 10g increments. Stop adding weight and recording responses when the muscle response is zero, or when the upper knob on the transducer rod contacts the bushing on the top of the transducer case.

3. Select Save in the File menu.

Data Analysis

Exercise 1: Maximum Contraction

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.

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 Twitch Channel by clicking AutoScale All on the toolbar.

6. Once the cursors are placed in the correct positions for determining the amplitudes and times for each muscle twitch, the values 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 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 Table 1.

11. Select Save in the File menu.
Figure AM-1-L3: A single muscle twitch and stimulus pulse displayed in the Main window. The labels indicate: latency (L); contraction time (C); relaxation time (R); and twitch amplitude (A).

Table AM-1-L1: Amplitudes and Times of Maximal Muscle Contractions.

<table>
<thead>
<tr>
<th>Stimulus Amplitude (V)</th>
<th>Muscle Twitch</th>
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<tbody>
<tr>
<td></td>
<td>Amplitude (mV)</td>
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Exercise 2: After-loaded Weight and Contractile Strength.

1. Scroll through the data from Exercise 2 and find the muscle twitch generated while the muscle lifted the lightest weight.

2. Use the same techniques to measure the data from this exercise as used to measure the data from Exercise 1.
3. Record the values for the amplitudes and times from the twitches in this exercise in the Journal and on Table 2 using the same techniques used in the analysis of the data from Exercise 1.

Table AM-1-L2: Amplitudes and Times of Contractions from an Afterloaded Muscle.

<table>
<thead>
<tr>
<th>Weight (g)</th>
<th>Muscle Twitch</th>
<th>Amplitude (mV)</th>
<th>Contract Time (msec)</th>
<th>Relax Time (msec)</th>
<th>Latency (msec)</th>
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4. Calculate the work performed and the rate of contraction for each twitch:
   • Work equals weight multiplied by the amplitude of muscle response.
   • Rate of contraction equals this amplitude divided by contraction time (C).

5. Repeat the measurements for all twitches.

6. Present your data in tables and graphs that relate the amplitude of the muscle response, the work performed, and the speed of contraction to weight.

Questions
1. Why did the amount of work initially increase with increased weight?
2. Why did the amount of work decrease when heavier weights were used?
3. Did any of the other parameters measured differ with weight? Why?
Exercise 3: Pre-loaded Weight and Contractile Strength.

1. Scroll through the data from Exercise 3 and find the muscle twitch generated while the muscle was holding the lightest weight.

2. Use the same techniques to measure the data from this exercise as used to measure the data from Exercise 1.

3. Record the values for the amplitudes and times from the twitches in this exercise in the Journal and on Table 3 using the same techniques used in the analysis of the data from Exercise 1.

Table AM-1-L3: Amplitudes and Times of Contractions from a Preloaded Muscle.

<table>
<thead>
<tr>
<th>Weight (g)</th>
<th>Muscle Twitch</th>
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<td>Amplitude (mV)</td>
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Question

How do the muscle response parameters of a pre-loaded muscle compare to those of an after-loaded muscle? Explain what is happening physiologically.