

Labs #9 and #10: Vertebrate Skeletal Muscle

In this experiment, you will investigate the physiological properties of skeletal muscle from the isolated toad gastrocnemius. Concepts to understand include the single twitch, graded response and the relationship between muscle length and tension generated. You will also explore tetanus and muscle fatigue, and the effect of temperature on muscle contraction properties.

Background

The physiology of skeletal muscle was not fully understood until the early 20th century. Some of the earliest experiments on muscle physiology were performed between 1661 and 1665 by Jan Swammerdam, who demonstrated that an isolated toad muscle could be made to contract when the sciatic nerve was irritated with a metal object. Later, Luigi Galvani (1737–1798) demonstrated that toad muscle responded to electrical currents.

The basic unit of a muscle is the muscle cell, or fiber. Whole muscles are made up of bundles of these fibers. A single muscle fiber has a very regular structure, defined by myofibrils. Each myofibril consists of an arrangement of the contractile proteins actin and myosin, which are able to slide past each other in the presence of Ca^{2+} and ATP.

Skeletal muscle is similar to nerve tissue in that it responds to a stimulus in an all-or-none fashion. This response is called a twitch. Depending on the intensity and frequency of stimulation, greater numbers of fibers are activated. By increasing the number of active muscle fibers, the muscle is able to increase the force it generates. Muscles with large cross-sectional areas are able to generate larger forces than those with small cross-sectional areas.

The muscle fibers and their associated motor nerves are called a motor unit. Greater numbers of motor nerves associated with a muscle give finer control over that muscle. Motor nerves release the neurotransmitter acetylcholine from their synaptic bulbs onto the muscle. This junction between a nerve and a muscle is called the motor end plate. The release of acetylcholine at the motor end plate depolarizes the muscle tissue and in turn leads to the release of intracellular calcium from the sarcoplasmic reticulum, a variant of smooth endoplasmic reticulum. This release of intracellular calcium sets in motion the biochemical events that allow actin and myosin to move past each other and hydrolyze ATP for energy.

In this experiment, you will examine the basic principles of skeletal muscle physiology, including the all-or-none response, the effect of stimulus intensity and frequency on contraction force and the phenomenon of muscle fatigue. These experiments illustrate the collective understanding of muscle physiology gained from over 400 years of research.

During the laboratory session, you will be expected to generate hypotheses about muscle function that you will test during next week's lab (Lab #8). You will have access to toads and the equipment that you used during this week's lab. You might formulate hypotheses that concern force generation in different muscle groups (including other skeletal muscle, cardiac muscle and smooth muscle), or about how bathing the muscle in different sorts of solutions will affect contractile properties, for example. Before the end of class, each member of the group should generate one testable hypothesis and have it approved by the instructor. Each group member must generate a different hypothesis. During next week's lab, each group will test the hypotheses of all of the group members. The assignment will then be an individual lab report (see the handout from the crustacean heart lab) where each member of the class reports on only the one hypothesis that they developed.

Required Equipment

PowerLab
 Force transducer
 Thermocouple and T-pod
 Bipolar stimulator
 Ring Stand with micropositioner and clamps
 Toad Ringer's solutions at a range of temperatures.
 Petri dish and dissecting tools (including glass rod for blunt dissection)
 Strong nylon or silk thread, Small millimeter ruler
 Double-pithed toad

Procedures

Setting up the experiment

A. Setting up the software

1. Use the Toad Muscle Settings settings file. Connect the force transducer via the bridge pod to channel 1 (labeled Force) and the thermocouple via the T-pod to channel 2.
2. Select Bridge Pod from the Force channel pop-up menu. Zero the signal using the knob on the front of the Bridge Pod.

B. Toad Dissection

See the dissection guide on Page 7 for illustrations.

1. Obtain a double-pithed toad from your instructor.
2. Using a scalpel or sharp scissors, cut the skin of the toad around its abdomen.
3. Peel the skin down and off the legs of the toad.
4. Keep the tissue moist at all times with Ringer's solution.
5. From the dorsal side of the frog, use the glass probe to expose the sciatic nerve from between the thigh muscles down to the knee. The myelin sheath of vertebrate nerves makes them very white and shiny. **Be careful not to handle the sciatic nerve with metal instruments.**
6. Slip a 10" piece of strong thread under the Achilles tendon at the heel of the toad. Tie this thread securely to the tendon.
7. Carefully dissect the gastrocnemius muscle away from the tibio-fibula bone, but leave it attached to the knee. The gastrocnemius is the big calf muscle that extends the foot.
8. Remove the foot, thigh muscles, and the other muscles from the tibio-fibula, leaving the sciatic nerve and gastrocnemius attached at the knee.
9. (Optional) Using scissors, cut the tibio-fibula bone 8-10 mm below the knee.
10. Cut at the hip joint using a scalpel or scissors to separate the femur from the hip.
11. Keep the muscle in a Petri dish of cold Toad Ringer's until you are ready to mount it.

C. Mounting the muscle for recording

1. Securely mount the MLT500 force transducer in the micropositioner mounting bracket. Plug the MLT500 cable into the back of the ML301 Bridge Pod.
2. Tape a small metric ruler to the side of the micropositioner to use as a reference when adjusting height (Figure 1).

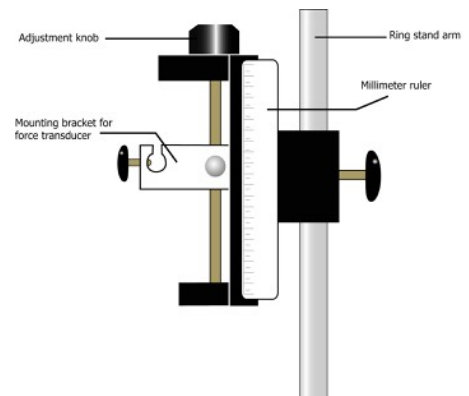


Figure 1. MLA40 Micropositioner mounted on ring stand

3. Connect the Bridge Pod to the Pod Port on Input 1 of the PowerLab.
4. Mount the bipolar stimulator handle to the ring stand using a clamp.
5. Clamp the femur of the toad to the ring stand using a femur clamp or strong metal clamp.
6. Attach the thread through the hole in the metal tab of the force transducer. Make sure that there is some slack in the thread.
7. Adjust the angle of the micropositioner so that the gastrocnemius is perpendicular to the table (Figure 2).
8. Raise the micropositioner using the adjustment knob so that the muscle is vertical but not under tension. The thread should not be loose, but have a slight amount of slack in it. Make sure that there is room to increase the height of the force transducer by at least 10 mm.
9. Rinse the muscle with Ringer's solution to keep it moist.
10. Position the bipolar stimulator so that both stimulus leads are touching the middle of the muscle. (You may also hold the stimulator manually with your hand if it is easier). As you proceed through the experiments, check that the connection between the stimulator and muscle is good and that the tissue remains fresh and moist (i.e., it's not getting 'cooked').
11. Attach the BNC (+/-) leads from the stimulator handle to the Output (+/-) connectors on the front of the PowerLab.

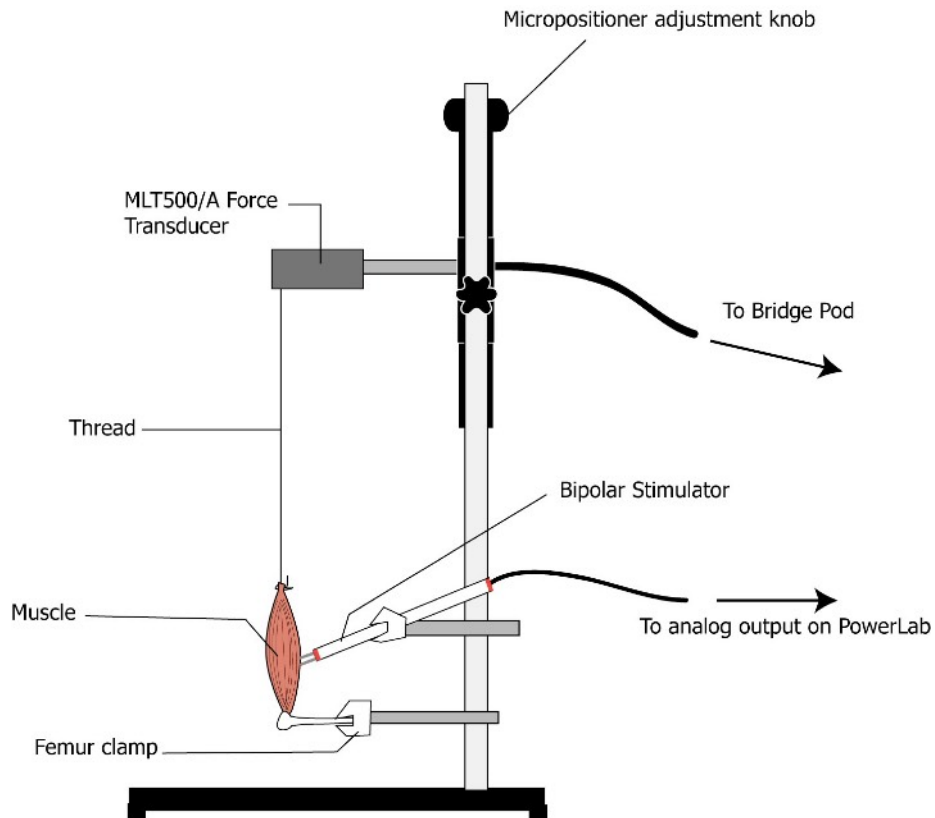


Figure 2. Set-up of the mounted gastrocnemius muscle.

Part 1. The graded response

In this experiment, you will give the muscle a series of stimuli of increasing amplitude to examine the effects of stimulus amplitude on contraction force.

1. Make sure that the muscle is moist and that both leads from the stimulator are touching it.
2. In Chart, select **Macro** from the menu bar and choose **Recruitment**. The recording will proceed automatically, with the PowerLab stimulating the muscle with increasing strength.
3. **Before proceeding to Part 2, complete Tables 1 and 2 (see Data Tables section). Follow the instructions in the Analysis section for Part 1 to obtain the minimum voltage for maximum contraction force.**
4. **As always, after each experiment, add a comment to each block of data to identify what was done (treatment) and any important description (the stimulus frequency, stimuli interval, etc.). Your data will not be interpretable without your notes.**

Part 2. Effect of load on contraction force

In this experiment, you will increase the load on the muscle by raising the micropositioner (increasing the tension on the muscle).

5. Make sure the muscle is moist and the stimulator handle is positioned correctly.
6. Note the position of the micropositioner using the ruler. This is your reference position.
7. Use the supramaximal stimulus voltage that you calculated in Table 2 for this experiment. Select **Macro** from the Chart menu bar and select **Stimulus Amplitude**. This will open the **Stimulator** dialog box. Change stimulator mode to pulse. Input your value in volts for the supramaximal stimulus voltage that you calculated in the the Output field (Amplitude in V). **Do not adjust the other settings.** When you are done, close the Stimulator dialog box.
8. Set the **Macro** to **Muscle tension**.
9. After each recording, you will be prompted to raise the micropositioner by one millimeter. Before you do so, wait at least 10 seconds to allow the muscle to recover. To raise the micropositioner, turn the adjustment knob located on the top of it. Do not try to reposition the entire unit on the ring stand. During the experiment, you will increase the height of the micropositioner a total of ten millimeters.
10. At the end of the recording, return the micropositioner to its original position, releasing the tension on the muscle.

Part 3. Effect of pulse frequency on contraction force

In this experiment, you will stimulate the muscle with twin pulses at different pulse intervals and observe their effect on muscle contractions.

11. Make sure that the muscle is moist and in good condition, and the stimulator handle is positioned correctly.
12. Set the **Macro** to **Muscle Summation**. The PowerLab will stimulate the muscle with twin pulses 400ms, 200 ms, 100ms, 50 ms and 20 ms apart. Each recording will appear in a separate block.

Part 4. Tetanus

In this part of the experiment, you will examine the muscle's response to a continuous stimulus at different frequencies.

13. Make sure the muscle is moist, in good condition, and the stimulator handle is positioned correctly.
14. Set the **Macro** to **Tetanus**. The PowerLab will stimulate the muscle for one second with continuous pulses at intervals of 400 ms, 200 ms, 100 ms, 50 ms and 20 ms.

Part 5. Muscle fatigue

In this experiment, you will stimulate the muscle constantly for a long duration to examine the effects of muscle fatigue.

15. Make sure the muscle is moist, in good condition, and the stimulator handle is positioned correctly.
16. Set the **Macro** to **Fatigue**. The PowerLab will stimulate the muscle continuously with a stimulus interval of 20 ms for 30 seconds. The recording duration is 45 seconds.

Part 6. Effect of Temperature

1. In this experiment, you will bathe the muscle in Ringer's of different temperatures to examine the effect of temperature on striated muscle contractile properties.

Procedures

30. In Chart choose **Setup: Stimulator**.
31. Input the following settings: Stimulator Mode: Pulse, Output: fixed number of pulses = 1, Start: manually, Range: 200 Hz, Frequency: 50Hz, Pulse Durations, 1.00 ms.
32. Input the supramaximal stimulus value in the output voltage field.
33. Perfuse the muscle with the cold toad Ringer's for 8-10 minutes.
34. Click Start to begin recording.
35. Click the Stimulate button in the Stimulator Panel.
36. Record the muscle response and then click Stop to end the recording.
37. Repeat steps 33-36 using the toad Ringer's solution that is 10°C warmer until you have reached a final Ringer's temperature of 40°C. **Be careful not to cook the muscle by going over 40°C.**

Analysis

Part 1. The graded response

Examine your recording from Part 1. You should see up to 20 contractions in the Force channel. The Stimulus Marker channel indicates the time of the first pulse. Each subsequent pulse occurs 500 milliseconds after the last.

1. Place the **marker** on the baseline of the waveform in the Force channel.
2. Using the mouse, place the **waveform cursor** at the top of the **last** contraction peak.
3. Record the peak height for each of the peaks and record your data in Table 1, starting at the last point and working your way backward to the beginning of the trace. Fill out Table 1 starting at the bottom. **Note:** the PowerLab will have stimulated the muscle 20 times, but not all of the stimuli may have elicited a twitch. If you have fewer than 20 contractions, enter a zero in your data table for those stimulus intensities without a twitch.
4. From your data, determine the minimum voltage required to elicit a maximal contraction. This is the maximum excitation voltage. Determine your value for a supramaximal stimulus by multiplying this voltage by 1.5. Record these values in Table 2, below.

Part 2. The effect of load on contraction force

5. Return the **marker** to its resting position by clicking in the lower left corner of the Chart document.
6. Using the **waveform cursor**, measure the baseline value. This is the preload force.
7. Next, determine the raw twitch force with the **waveform cursor** by placing the cursor on the maximum value in the block. Enter your results in Table 3, below.
8. Repeat steps 2 and 3 for the other ten data blocks.
9. Calculate the net twitch force by subtracting the preload (baseline) value from the raw twitch force value.

Part 3. The effect of stimulus frequency on contraction force

10. For each of the 5 blocks of data, determine the maximum contraction force of the first and second peaks using the marker and waveform cursor and record your results in Table 4, below.

Part 4. Tetanus

11. Determine the maximum contraction force for each stimulation frequency and record your results in Table 5, below. There are five blocks of data.

Part 5. Muscle Fatigue

12. Place the marker on the waveform baseline in the Force channel immediately prior to stimulation.
13. Use the waveform cursor to determine maximum contraction force. Record this value and the time of maximal stimulation in Table 6.
14. Determine the contraction force at peak amplitude around $t = 1, 5, 10, 15, 20, 25$, and 30 seconds after stimulation. Record your results in Table 6, below.

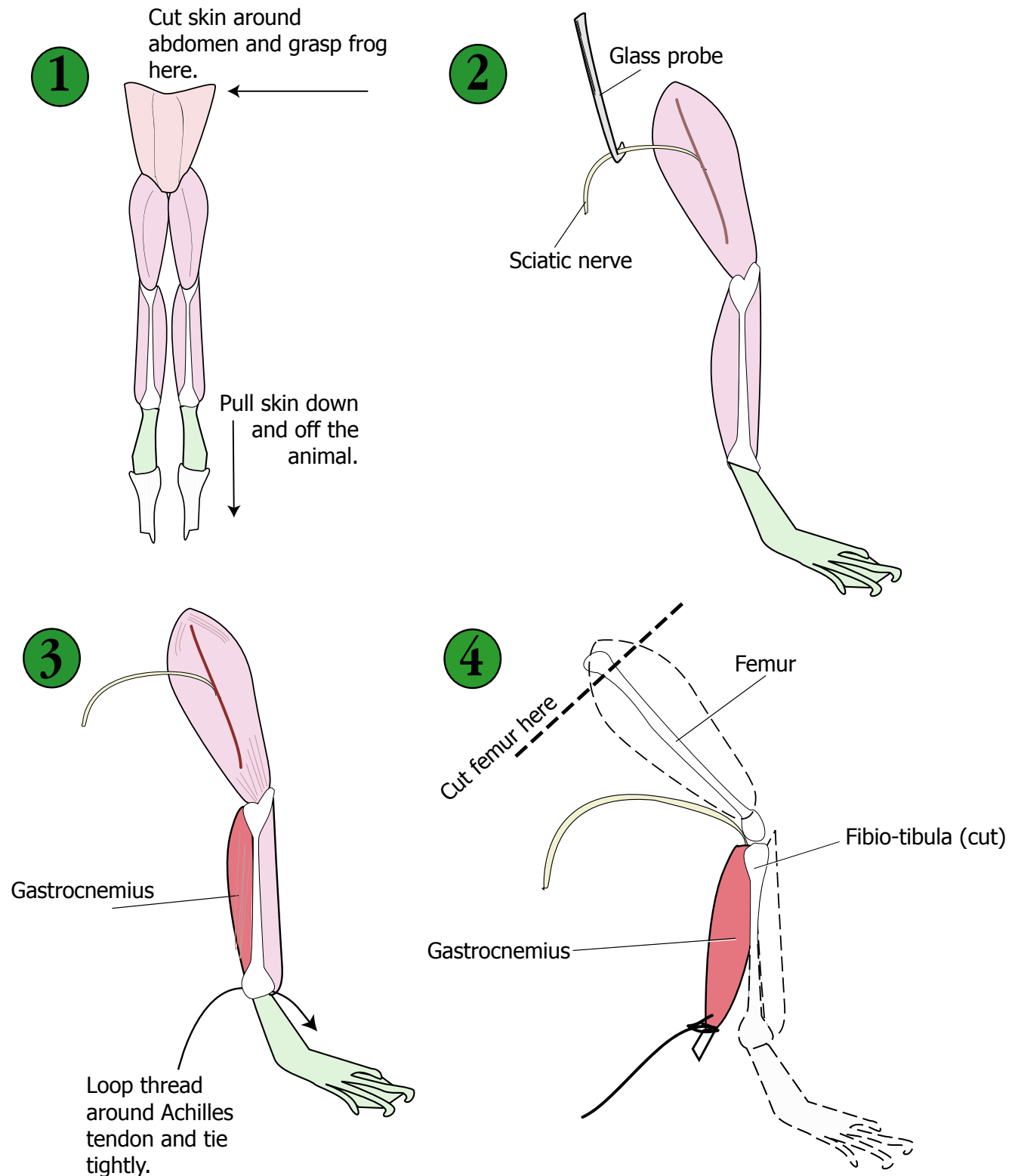
Part 6. Temperature

1. Record the contraction force at each temperature in Table 7, and calculate the Q_{10} for the temperature range between 0 and 20°C and 20 and 40°C .

Questions to consider in your report --

- In light of vertebrate muscle contraction being initiated by action potentials which are "all or none", why do you observe a graded response to increasing stimulus intensity?
- What effect does stretching the muscle have on contraction strength? Is the effect linear? What preload force resulted in the highest contraction force? Can you explain this in terms of what you know about muscle contraction?
- What happens to the muscle at the highest preload forces?
- What happens to the force of contraction when the muscle is stimulated repeatedly in quick succession? Explain the mechanism behind this phenomenon. What happens if the stimuli are spaced far apart in time? Why?
- Define tetanus. At which stimulus interval did you observe tetanus?
- Why does muscle fatigue occur? What is happening physiologically? How long was your preparation able to maintain a maximal contraction? Would your results have changed if you were measuring from smooth muscle tissue? Why?
- What was the Q_{10} for muscle contractile properties? What is the mechanism by which temperature influences muscle contractile properties?

Frog Muscle Dissection Guide



Data Tables

Suggestions to help you organize your data and analysis.

Table 1. Effect of stimulus intensity on contraction force

Stimulus Amplitude (V)	Contraction Force (N)
0.05	
0.10	
0.15	
0.20	
0.25	
0.30	
0.35	
0.40	
0.45	
0.50	
0.55	
0.60	
0.65	
0.70	
0.75	
0.80	
0.85	
0.90	
.950	
1.00	

Table 2. The supramaximal stimulus voltage.

Minimum voltage required for maximal contraction (V)	Supramaximal stimulus voltage (V)

Table 3. The effect of preload on muscle contraction force

Preload (N)	Raw twitch force (N)	Net twitch force (N) (Raw twitch force – preload)

Table 4. The effect of stimulus frequency on contraction force

Stimulus interval (ms)	Force of first peak (N)	Force of second peak (N)
400		
200		
100		
50		
20		

Table 5. Tetanus

Stimulus interval (ms)	Maximum force (N)
400	
200	
100	
50	
20	

Table 6. Muscle fatigue

Time since stimulation (sec)	Contraction force (N)
1	
5	
10	
15	
20	
25	
30	

Maximum force (N)= _____
Time of max force= _____ sec post-stimulation

Table 7. Temperature

Temperature (°C)	Contraction force (N)
0	
10	
20	
30	
40	
$Q_{10} =$	