LABORATORY
# EXERCISE S-1

# Skeletal Muscle Contraction

## Purpose of the Exercise

**To observe and record skeletal muscle contractions of an isolated frog gastrocnemius in response to electrical stimulation of varying strength and frequency.**

### MATERIALS NEEDED

Recording system (kymograph, Physiograph, etc.)
Stimulator and connecting wires
Live frog
Dissecting tray
Dissecting instruments
Probe for pithing
Heavy thread
Frog Ringer's solution
*Ph.I.L.S. 4.0: Lesson 8 Skeletal Muscle Function: Principles of Summation and Tetanus*

### SAFETY

- Wear disposable gloves and protective eyewear when handling the frogs.
- Dispose of gloves and frogs as instructed.
- Wash your hands before leaving the laboratory.

Chapter Opening Image: © Bryan Hainer/Getty Images

## Learning Outcomes

**After completing this exercise, you should be able to:**

O1 Determine the threshold level of electrical stimulation in frog muscle.
O2 Determine the intensity of stimulation needed for maximal muscle contraction.
O3 Record a single muscle twitch and identify its phases.
O4 Record the response of a muscle to increasing frequency of stimulation and identify the patterns of tetanic contraction and fatigue.
O5 Describe the relationship of calcium release from the sarcoplasmic reticulum to the mechanical events of muscle contraction and relaxation.

The O corresponds to the assessments A indicated in the Laboratory Assessment for this Exercise.

## Pre-Lab

**Carefully read the introductory material and examine the entire lab. Be familiar with a muscle twitch, threshold stimulus, summation, and tetanus from lecture or the textbook.**

**To study the characteristics** of certain physiological events such as muscle contractions, it often is necessary to directly record the events using either an analog (kymograph or physiograph) or digital recording device (BIOPAC, ADInstruments, IWORKs, etc.). These devices can provide accurate recordings of various physiological changes.

To observe the phenomenon of skeletal muscle contractions, muscles can be isolated from anesthetized frogs. These muscles can be attached to recording systems and activated by electrical stimuli of varying strength, duration, and frequency. Recordings obtained from such procedures can be used to demonstrate the basic characteristics of skeletal muscle contractions.

The principles of changing frequency of stimulation to investigate summation and tetanus in skeletal muscle can also be demonstrated with Ph.I.L.S. 4.0. The relationship of calcium levels in the sarcoplasm to the mechanical events of muscle contraction and relaxation is emphasized.

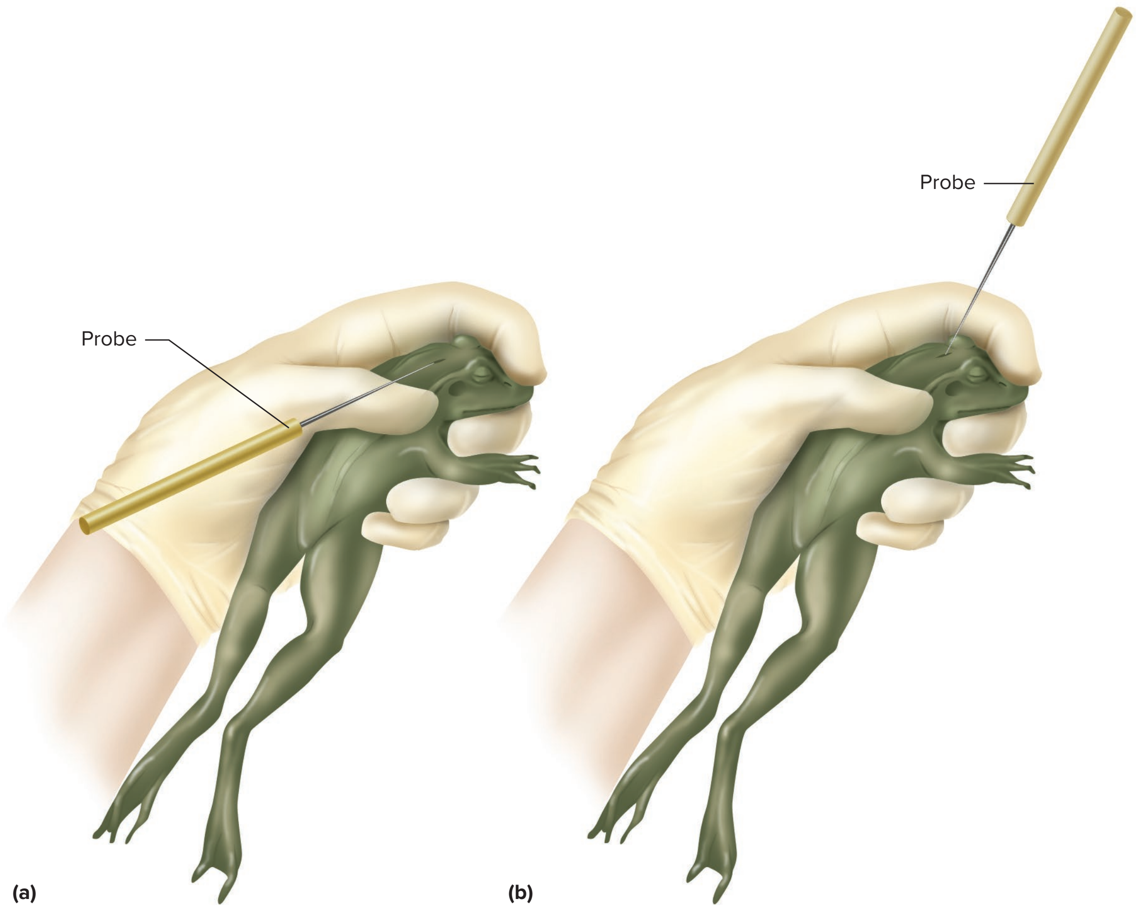
## PROCEDURE A: Recording System

1. Set up the recording system and stimulator that you have been provided to record the contractions of a frog muscle according to the directions provided by the laboratory instructor.
2. Obtain a live frog, and prepare one gastrocnemius as described in Procedure B.

## PROCEDURE B: Muscle Preparation

1. Prepare the live frog by pithing so that it will not have sensations or movements when its muscle is removed. To do this, follow these steps:
   - **a.** Hold the frog securely in one hand so that its limbs are extended downward.
   - **b.** Position the frog's head between your thumb and index finger (fig. S-1.1*a*).

**FIGURE S-1.1** Hold the frog's head between your thumb and index finger to pith (a) its brain and (b) its spinal cord.



c. Bend the frog's head forward at an angle of about 90° by pressing on its snout with your index finger.
d. Use a sharp probe to locate the foramen magnum between the occipital condyles in the midline between the frog's tympanic membranes.
e. Insert the probe through the skin and into the foramen magnum, and then quickly move the probe from side to side to separate the brain from the spinal cord.
f. Slide the probe forward into the braincase, and continue to move the probe from side to side to destroy the brain.
g. Remove the probe from the braincase, and insert it into the spinal cord through the same opening in the skin (fig. S-1.1*b*).
h. Slide the probe up and down the spinal cord to destroy it. If the frog has been pithed correctly, its legs will be extended and relaxed. Also, the eyes will not respond when touched with a probe.

## ALTERNATE PROCEDURE

An anesthetizing agent, tricaine methane sulfonate, can be used to prepare frogs for this lab. This procedure eliminates the need to pith frogs.

2. Isolate the frog's gastrocnemius muscle by proceeding as follows:
   a. Place the pithed frog in a dissecting tray.
   b. Use scissors to cut through the skin completely around the hindlimb in the thigh.
   c. Pull the skin downward and off the hindlimb.
   d. Locate the gastrocnemius muscle in the calf and the calcaneal tendon (Achilles tendon) at its distal end (fig. S-1.2*a*).
   e. Separate the calcaneal tendon from the underlying tissue, using forceps.
   f. Tie a thread firmly around the tendon (fig. S-1.2*b*).
   g. When the thread is secure, free the distal end of the tendon by cutting it with scissors.
   h. Attach the frog muscle to the recording system in the manner suggested by your laboratory instructor (fig. S-1.3).
   i. Insert the ends of the stimulator wires into the muscle so that one wire is located on either side of the belly of the muscle.

Keep the frog muscle moist at all times by dripping frog Ringer's solution on it. When the muscle is not being used, cover it with some paper toweling that has been saturated with frog Ringer's solution.

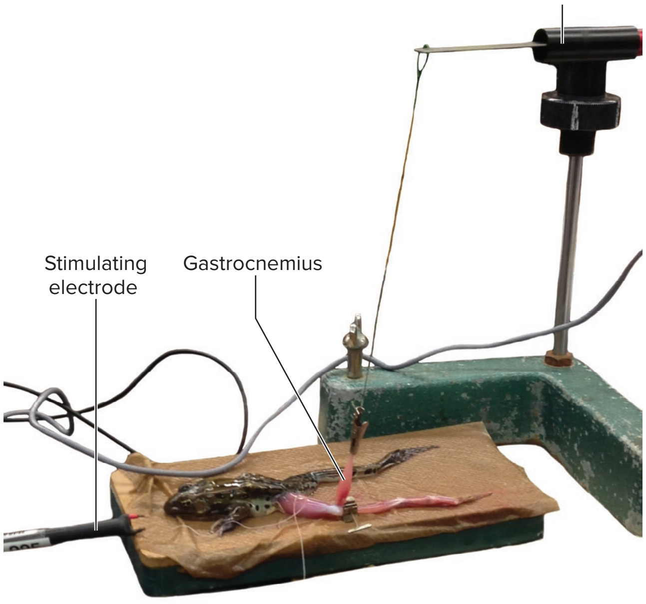
**FIGURE S-1.2** (a) Separate the calcaneal (Achilles) tendon from the underlying tissue. (b) Tie a thread around the tendon, and cut its distal attachments.

(a)



(b)

**FIGURE S-1.3** Typical set up for frog muscle recording. The gastrocnemius is attached to the force transducer that will send signals to the recording system.

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*Before you begin operating the recording system and stimulator, have the laboratory instructor inspect your setup.*

## PROCEDURE C: Threshold Stimulation

1. To determine the threshold stimulus or minimal strength of electrical stimulation (voltage) needed to elicit a contraction in the frog muscle, follow these steps:
   a. Set the stimulus duration to a minimum (about 0.1 milliseconds).
   b. Set the voltage to a minimum (about 0.1 volts).
   c. Set the stimulator so that it will administer single stimuli.
2. Administer a single stimulus to the muscle and watch to see if it responds. If no response is observed, increase the voltage to the next higher setting and repeat the procedure until the muscle responds by contracting.
3. After determining the threshold level of stimulation, continue to increase the voltage in increments of 1 or 2 volts until a maximal muscle contraction is obtained.
4. Complete Part A of Laboratory Assessment S-1.

## PROCEDURE D: Single Muscle Twitch

1. To record a single muscle twitch, set the voltage at the maximal muscle contraction as determined in Procedure C.
2. Set the recording speed at maximum, and while recording moving, administer a single electrical stimulus to the frog muscle.
3. Repeat this procedure to obtain several recordings of single muscle twitches.
4. Complete Part B of the laboratory assessment.
5. Refer to Laboratory Exercise 20 where you performed Ph.I.L.S. Exercise 4.0, Exercise 5

## PROCEDURE E: Sustained Contraction

1. To record a sustained muscle contraction, follow these steps:
   a. Set the stimulator for continuous stimulation.
   b. Set the voltage for maximal muscle contraction as determined in Procedure C.
   c. Set the frequency of stimulation at a minimum.
   d. Set the recording speed at about 0.05 cm/sec.
   e. With the recording, administer electrical stimulation and slowly increase the frequency of stimulation until the muscle sustains a contraction (tetanic contraction or tetanus).
   f. Continue to stimulate the muscle at the frequency that produces sustained contractions until the muscle fatigues and relaxes (this may take several minutes).
2. Every 15 seconds for the next several minutes, stimulate the muscle to see how long it takes to recover from the fatigue.
3. Complete Part C of the laboratory assessment.

### LEARNING EXTENSION ACTIVITY

To demonstrate the staircase effect (treppe), obtain a fresh frog gastrocnemius muscle and attach it to the recording system as before. Set the paper control for slow speed, and set the stimulator voltage to produce a maximal muscle contraction. Stimulate the muscle once each second for several seconds. How do you explain the differences in the lengths of successive muscle contractions?

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LABORATORY
# ASSESSMENT S-1

Name ______________________

Date ______________________

Section ______________________

The A corresponds to the indicated Learning Outcome(s) O found at the beginning of the Laboratory Exercise.

# Skeletal Muscle Contraction

## PART A: Threshold Stimulation Assessments

**Complete the following:**

1. What recording system was used for this laboratory exercise? ______________________
2. What was the threshold voltage for stimulation of the frog gastrocnemius muscle? A1 ______________________
3. What voltage produced maximal contraction of this muscle? A2 ______________________

### CRITICAL THINKING ASSESSMENT

Do you think other frog muscles would respond in an identical way to these voltages of stimulation?

______________________

Why or why not?

______________________

______________________

______________________

## PART B: Single Muscle Twitch Assessments

**Complete the following:**

1. Fasten a recording of two single muscle twitches in the following space. A3

2. On a muscle twitch recording, label the *latent period, contraction phase,* and *relaxation phase,* and indicate the time it took for each of these phases to occur. A3

3. What differences, if any, do you note in the two myograms of a single muscle twitch? How do you explain these differences?

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## PART C: Sustained Contraction Assessments

**Complete the following:**

1. Fasten a recording demonstrating wave summation and sustained contraction in the following space. A4

2. On the recording, indicate when the muscle twitches began to combine (summate), and label the period of tetanic contraction and the period of fatigue. A4

3. At what frequency of stimulation did tetanic contraction occur? A4 ______________________________

4. How long did it take for the tetanic muscle to fatigue? A4 ______________________________

5. Is the length of muscle contraction at the beginning of tetanic contraction the same as or different from the length of the single muscle contractions before tetanic contraction occurred? ____________ How do you explain this? A4

______________________________________________________________________

______________________________________________________________________

6. How long did it take for the fatigued muscle to become responsive again? A4 ______________________________

# NOTES

# LESSON 8 Skeletal Muscle Function: *Principles of Summation and Tetanus*

The purpose of this exercise is to further explore the mechanics of skeletal muscle contraction, please complete the simulation of this exercise: Ph.I.L.S. 4.0: Lesson 8 Skeletal Muscle Function: *Principles of Summation and Tetanus* (Fig. S-1.4).

A skeletal muscle twitch results from the release of neurotransmitter from a somatic motor neuron. The neurotransmitter causes depolarization of the motor endplate and generates an action potential along the skeletal muscle membrane (sarcolemma). The depolarization travels down the transverse tubules to the dihydropyridine receptor, which stimulates the release of calcium ions from the sarcoplasmic reticulum (SR). The calcium ions bind to troponin C and initiate cross bridge cycling. When the stimulus is terminated, the calcium is quickly pumped back into the SR and the skeletal muscle will relax. In this exercise, you will deliver electrical stimuli to mimic the firing of motor neurons.

*State your hypothesis regarding the effect of increased frequency of stimulation on the force produced by a muscle.*

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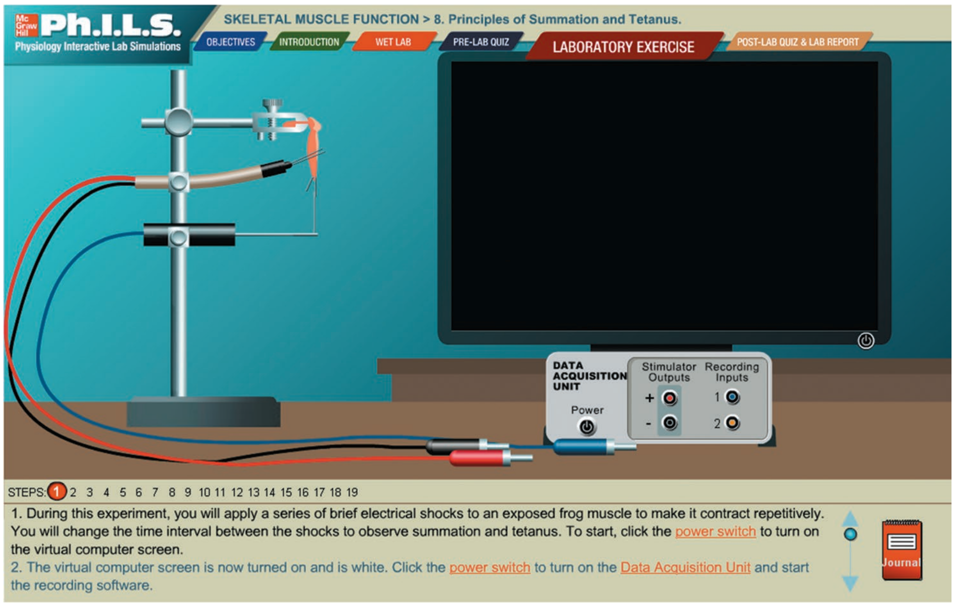
First you will adjust the voltage to initiate individual twitches of the frog muscle (for specific directions, refer to the P.H.I.L.S. lesson 8). Once individual twitches are being recorded, you will increase the frequency of the stimulation. An increase in frequency of the electrical stimulation mimics the functioning of the nervous sys-

**FIGURE S-1.4** Opening screen for the laboratory exercise on Skeletal Muscle Function: *Principles of Summation and Tetanus*.



tem. Action potentials are all or none and unchanging, so to stimulate the muscle to a greater or lesser degree, the nervous system changes the frequency, or time between, action potentials. As the frequency of action potentials increases (stimuli are closer together), the muscle does not fully relax and calcium concentrations build in the sarcoplasm. With greater concentrations of calcium a stronger contraction is produced. This phenomenon is known as wave summation, in that the waves of stimuli are added together to cause an increase in muscle tension. As you continue to increase the frequency, muscle will generate greater tension with shorter and less complete rest periods. This type of contraction is known as incomplete tetanus. If the stimuli are increased to the point that the muscle does not relax and there no rest period, the recording will be a flat line. This steady muscular contraction is complete tetanus (Table S1.1).

Record the frequencies where you first saw wave summation, incomplete, and complete tetanus. Complete the supplemental questions at the end of this exercise.

Wave summation: ______________________

Incomplete tetanus: ______________________

Complete tetanus: ______________________

**Table S-1.1** Observing Muscle Response

| Muscle Response | Observation on Computer Screen |
|---|---|
| Wave summation | Trace moves off the baseline (lower gray line) |
| Incomplete tetanus | Trace becomes higher than in a single twitch (above upper gray line) |
| Complete tetanus | Trace is higher than a single twitch (above upper gray line) and becomes smooth |

## Ph.I.L.S. Lesson 8, Skeletal Muscle Function: *Principles of Summation and Tetanus* Assessments

**1.** At what frequency did muscle twitches begin to combine (summate)? ______________________ Demonstrate incomplete tetanus? ______________________ Demonstrate complete tetanus? ______________________

**2.** Calcium is released from the sarcoplasmic reticulum through voltage-gated calcium channels to initiate muscle contraction. When the muscle is no longer stimulated, calcium pumps move the calcium from the sarcoplasm back into the sarcoplasmic reticulum. Complete the following: A5

**a.** During muscle *contraction,* is the net movement of calcium into or out of the sarcoplasmic reticulum?

______________________________________________

**b.** During muscle *relaxation,* is the net movement of calcium into or out of the sarcoplasmic reticulum?

______________________________________________

**c.** During incomplete tetanus, the amount of tension produced by the muscle is greater than during a single twitch. What does this tell you about the relative amount of calcium in the muscle sarcoplasm during incomplete tetanus?

______________________________________________

**d.** Describe the net movement of calcium (into or out of the sarcoplasmic reticulum) during complete tetanus.

______________________________________________

**e.** Explain why the rate of muscle relaxation would be slower after tetanus than after a single twitch.

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### CRITICAL THINKING ASSESSMENT

The toxins released from the bacterium that causes tetanus bind permanently to the acetylcholine receptors of the skeletal muscle, resulting in continuous stimulation of the muscle. Explain the result to the skeletal muscle of the body.

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