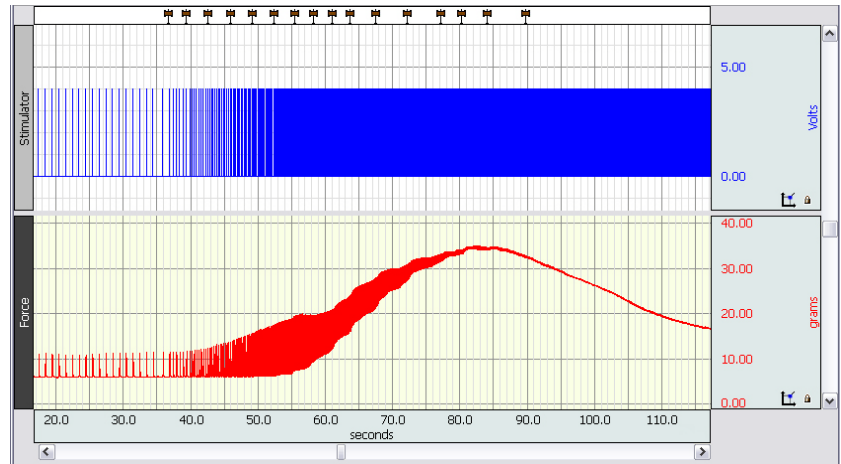
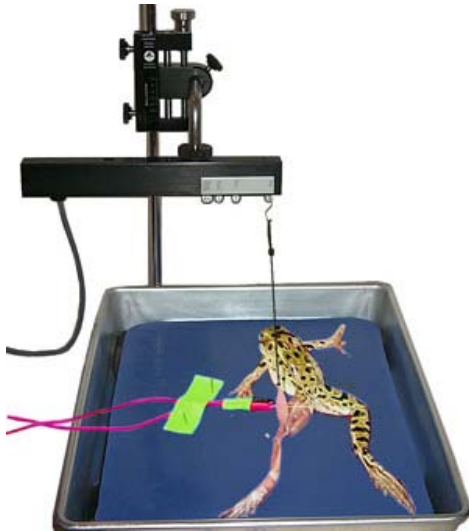


BSL PRO Lesson A02: Contractility of Skeletal Muscle Using Frog Gastrocnemius Muscle

Developed in conjunction with Department of Biology, University of Northern Iowa, Cedar Falls



This BSL PRO lesson explains how to isolate the gastrocnemius muscle of the frog and its somatic motor nerve (sciatic nerve) and describes the hardware and software setup necessary to record contractile responses resulting from an applied electrical stimulus.

Objectives:

1. Prepare a pithed frog for the study of muscle physiology.
2. Describe how muscle can contract when an electrical stimulus is applied.
3. Explain how motor nerves stimulate the contraction of skeletal muscles.
4. To record the twitch Threshold, maximal twitch response, summation, tetanus and fatigue for frog skeletal muscle and its motor nerve.
5. Examine the effects of the length of the sarcomere on the force of contraction.
6. Observe how the pH of the muscle changes as it becomes fatigued.

Equipment:

- Biopac Student Lab System:
 - MP36 or MP35 hardware
 - BSL 4.0.1 or greater software
- BSL PRO template file: "a02.gtl"
- Force Transducer (SS12LA includes S-hooks)
- Stimulator (one of the following):
 - **BSLSTMB/A**
 - **SS58L** MP35 Low-Voltage Stimulator
 - **OUT3** BNC adapter for MP36 built in Low-Voltage Stimulator
- Needle Electrodes (ELSTM2)
- Ring stand
- Tension adjuster (HDW100A or equivalent)
- 50 gram calibration weight
- Thread (non-stretch nylon or equivalent)
- Live frog
- 12 inch Ruler
- Examination/surgical gloves
- Dissection Pan
- Disposable syringes
- Dissection Kit: Scalpel, Forceps, Scissors, Dissection Pins, Tape
- Glass probes
- Acrylic Board (for dissection)
- BIOPAC abrasive pad (ELPAD) or fine sandpaper
- Amphibian Ringer's solution (3.0 mM KCl, 113 mM NaCl, 2.7 mM CaCl₂, 2.5 mM NaHCO₃, pH 7.2)
- Drug preparation: 0.1 ml of 0.5% Bromothymol Blue dissolved in 0.7% NaCl and adjusted to pH 7.0

Background:

The ability to move is a basic property of life permitting an animal to respond to its environment. Depending upon the structural complexity of the organism, the mechanisms for motion can appear very different. For example, amoeboid movement, ciliary and flagellar motion, and **muscular activity** all appear very diverse. However, upon close inspection, the basic molecular mechanisms are very similar involving almost identical chemicals but in different spatial arrangements. For metazoan, contractile structures are organized into individual or groups of cells known as **muscle fibers**. **Myocytes** can efficiently transform chemical energy into a mechanical force that is capable of work.

Muscle, perhaps more than any other physiological system, has been studied thoroughly. Of the three muscle types, **smooth, cardiac and skeletal**, the latter is best understood. We know more about skeletal muscles at all levels of organization than any other system. Through comparative anatomical studies for hundreds of years, the relationship of muscles to each other and other organs systems is well established. In more recent times, the physiology of skeletal muscle **contractility** has been studied from almost every conceivable physical and biochemical perspective. With the advent of electron microscopy, its detailed cytology and molecular organization has been clearly established. In addition it is an excellent experimental subject. In this laboratory, we will look at the contractile properties of the **gastrocnemius muscle** from the lower leg of the grass frog, *Rana pipiens*.

Muscle tissues are relatively easy to work with experimentally. They can be removed from an organism intact with little loss of function. Also, muscle tissue can be taken from an organism without removing other non-contractile tissue. Since muscular contractions are large in magnitude, they can be accurately measured with simple recording equipment. For **in vitro** contraction studies, there are two major preparations: 1) **isotonic** and 2) **isometric**. In the former, isotonic, the muscle can move exerting a constant force as the fiber changes length. In the latter, isometric, the length of the muscle is held constant as the force of contract varies. The physiology of contraction can be studied in muscles isolated from a pithed frog. The preparation can be stimulated directly by an electric shock or indirectly through activation of the appropriate motor nerve. In the gastrocnemius muscle experiments described here, we will examine properties of isotonic contractions.

Stimulation of Motor Nerve

In the vertebrates, skeletal muscle contractions are evoked **in vivo** by impulses in **somatic motor neurons**. The **soma** or cell body of motor or **efferent neurons** is located in the ventral gray horn of the spinal cord.

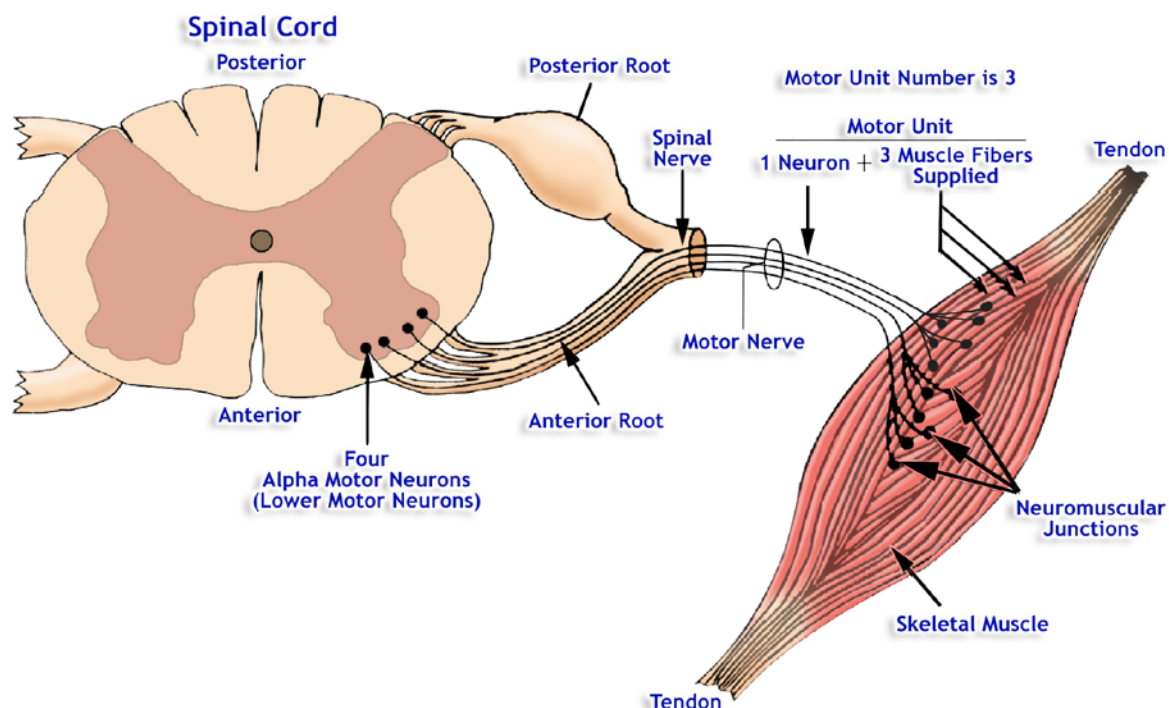


Figure 1

Normally, electrical activity in the somatomotor fibers originates in the spinal cord and travels over a single axon to a myocyte or to a group of myocytes known as a **motor unit**. The motor neurons are activated either by other neurons in the brain or spinal cord, or by local **sensory neurons**. Impulses in efferent neurons may also be stimulated by damaging fibers peripherally. This damage produces an injury current, which stimulates action potentials. This is why a **muscle twitch** occurs when a nerve is pinched. Once the axon of motor neurons leaves the spinal cord, it joins a **nerve** along with hundreds of other motor and sensory neurons. Each efferent somatic neuron transmits impulses from the spinal cord directly to a specific group of myocytes evoking contraction. The form of the contraction expressed by skeletal myocytes, or motor unit depends upon the pattern of motor neuron stimulation. For example, the gastrocnemius is innervated by hundreds of motor neurons in the **sciatic nerve**. Each motor neuron has a **threshold voltage** for activation. If a neuron is stimulated with a single, small **supra-threshold voltage**, a single **all-or-none contraction** occurs in all myocytes of a motor unit. This is a **twitch**. When more neurons are activated by increasing stimulus strength, additional motor units contract increasing muscular strength, and the twitch is larger. When all efferent neurons are activated for a muscle, a maximum-sized twitch occurs indicating all motor units have been **recruited**. **Supra-maximal voltages** can recruit no additional motor units and do not increase muscular strength. This relationship between neuron stimulation, motor unit activation, and muscle contraction is known as **excitation-contraction coupling**. Because increasing stimulus amplitude recruits more motor units, this pattern or kind of response is known as **spatial or motor-unit summation**.

In addition to recruitment of motor units by increasing stimulus amplitude (i.e. voltage,) variation in contraction can be achieved by **temporal summation**. Action potentials in the motor nerve fibers elicit the release of a chemical neurotransmitter called **acetylcholine (ACh)** from the axon endings. This transmitter combines with **nicotinic receptors** to produce action potentials on the membrane of the skeletal myocyte. An electrical impulse in the muscle cell, in turn, causes Ca^{++} to be released from the **sarcoplasmic reticulum (SR)**. At the molecular level, for contraction, the release of Ca^{++} into the cytosol permits actin and myosin to interact in the presence of ATP. Actin-myosin interactions and contractions are relaxed when Ca^{++} is re-absorbed into the SR by a Ca^{++} pump.

The **rate** at which myocytes are stimulated influences how long Ca^{++} lingers in the cytoplasm. If a myocyte receives a single, sub-maximum stimulus, Ca^{++} is released and quickly re-absorbed. If the myocyte receives a second impulse before relaxation is fully affected, the amount of cytoplasmic Ca^{++} in the second contraction is greater than the first. Consequently, the strength (i.e. force in grams) of the 2nd contraction is greater (Figure 2).

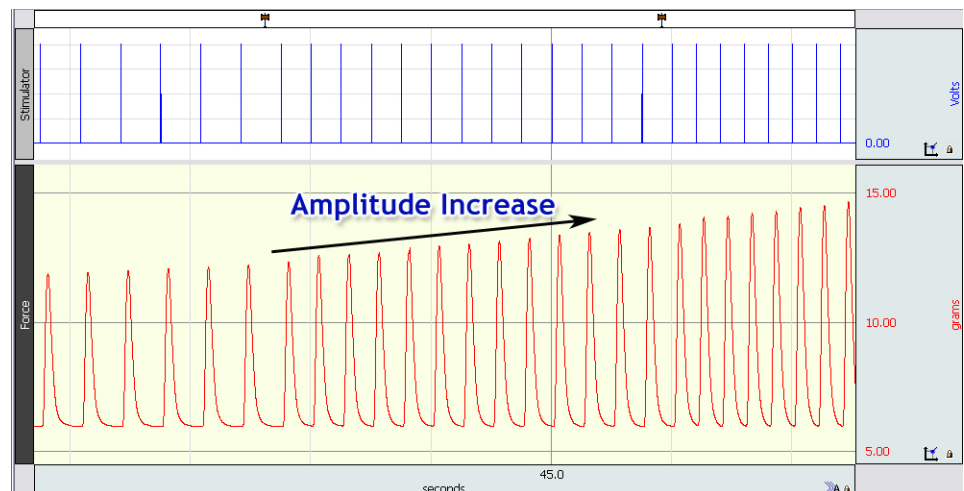


Figure 2

If no other impulse appears, all Ca^{++} is returned to the SR. If a train or volley of sub-maximal impulses is used to stimulate a myocyte, the strength of the contraction is proportion to the frequency of stimulation. At the maximum rate, the myocyte remains in a persistent, sustained contraction known as **tetanus**. If the rate of stimulation (pulses/second) is increased above the **tetanizing frequency**, no greater increase in strength occurs. At frequencies of stimulation between initial temporal response and tetany, contractions express partial relaxations. From its appearance, this response is called **treppe** or **stair-step**. In temporal summation, by varying the stimulating frequency we manipulate cytoplasmic Ca^{++} and molecular interactions within motor units to control the strength of contraction.

For the frog gastrocnemius, there are two modes or ways to stimulate the muscle with electrodes. The way most resembling a physiological state is to apply an exogenous electrical signal indirectly via the sciatic nerve.

Alternatively, the electrodes can be inserted directly into the muscle which applies the stimulus directly to the myocytes.

The difference between the two is that directly stimulating the muscle will only recruit fibers in the proximity of the electrodes, whereas the fibers recruited via the sciatic nerve will be more structurally uniform throughout the muscle. Either of these stimulating techniques can be used to perform this exercise. In part, which one you use will depend upon the quality of your dissection and care of the exposed tissue. If you are able to stimulate motor neurons through the sciatic nerve, your threshold voltage will be an order of magnitude lower than those required to stimulate the muscle tissue directly. For example, threshold voltage via sciatic nerve stimulations is often around 0.1 to 0.3 volts (100-300 mV) while direct muscular stimulation is usually between 0.5 and 1.1 volts (500 to 1100 mV). If you are able to activate muscular contraction via the sciatic nerve, DO NOT use excessively large supra-maximal stimuli. These large voltages will destroy the neurons.

In striated myocytes, contractile proteins are assembled in linear, parallel arrays stabilized by **z-disks**, **titan** and **nebulin** in each **sarcomere**. Sarcomeres are arranged in a series form myofibrils, hundreds of which fill the **sarcoplasm** of the muscle cell. When Ca^{++} is abundant in the sarcoplasm, actin and myosin bind forming the **rigor complex**. In the presence of ATP, the two molecules cycle continuously between the bound and unbound states. As a consequence, filamentous actin and myosin macromolecules slide past each other in opposite directions producing the force of contraction. When Ca^{++} is removed, *in lieu* of the ATP concentration, the **actinomyosin** complex is blocked and the rigor complex relaxes. The force generated during contraction of a sarcomere is related to its shape. If a myocyte is extirpated from tendons and elastic components of the fascia or **aponeuroses** contract, thick and thin filaments overlap maximally in each sarcomere. When the myocyte is stimulated, no force is generated. On the other hand, if the fiber is stretched to its limits just before tearing, actin and myosin filaments do not overlap. Again no force can be generated by the fiber with stimulation. At intermediate lengths, the thick and thin filaments overlap to various degrees depending upon degree of stretch. Thus, muscle strength (in grams) is related to length (in mm) in a very precise way. A plot of gastrocnemius muscle **force versus length** will illustrate an optimum length of the muscle for generating a force. This is usually a dome-shaped curve with maximum force generated at an intermediate length. *In situ*, skeletal muscles are usually stretched to intermediate length by ligaments anchoring them to bone so they can generate maximum force if it is desired.

Numerous factors influence the force of muscle contraction. One interesting factor is **pH**. A change in hydrogen ion concentration (**pH = $-\log [\text{H}^+]$**) will alter the strength of muscle contraction. When muscles experience acidosis, the increase in protons produces a decline in force by at least four different mechanisms. If intact myocytes are washed with low pH solutions, membrane proteins including ion channels undergo small conformational changes. This will alter the ability of K^+ and Na^+ channels to function efficiently disrupting membrane voltages (V_m). Using **glycerated muscle** fibers to remove the **sarcolemma**, the intracellular effects of pH are revealed. Enzymes in the cytoplasm function optimally when pH = 7.2-7.6 for most vertebrates. If **acidosis** (or **alkalosis**) occurs in the **sarcoplasm**, enzyme efficiency is diminished. An increased proton concentration will block or inhibit both 1,) the **myosin-ATPase** which is required for relaxation of the rigor complex and 2,) the **Ca^{++} -ATPase** on the sarcoplasmic reticulum which keeps cytoplasmic Ca^{++} at a low concentration. Also, the decline in pH will influence the shape of a Ca^{++} -binding molecule known as **troponin**. When pH changes away from 7.2-7.6, the shape of troponin is altered. Then Ca^{++} can no longer permit actin and myosin to form a rigor complex. Consequently, muscle strength is decreased. During vigorous activity in some types of myocytes, twitch muscle contractions are supported anaerobically by the synthesis of lactic acid to produce ATP. As lactate metabolism proceeds, protons (H^+) evolve lowering pH of the cytoplasm. In this case, the decline in muscle strength is due primarily to the effects of protons on macromolecules and enzymes rather than any direct effect of lactate anions in the cell.

Fatigue is a physiology state commonly observed in biological preparations. It is a decline or decrease in the ability to respond when repeatedly presented the same stimulus. In muscle preparations, fatigue can occur for several reasons. First, impulse frequency in motor neurons can decrease. Recall that the action potentials in neurons and myocytes are all-or-none signals, reoccurring with the same amplitude (or height). Since the frequency of impulses regulates amount of neurotransmitter released, a decline in neuronal impulse frequency results in fewer impulses and less neurotransmitter to the myocyte. Second, the neuron and myocyte communicate via a synapse at the **neuromuscular junction** (or endplate).

If the pre-synaptic membrane does not release vesicle containing neurotransmitter, the ability to produce a post-synaptic impulse in the myocyte will decrease. And third, the myocyte can become less responsive with prolonged stimulation. This occurs by either of two ways, decrease in Ca^{++} availability or decrease in ATP (or other energetic molecules). Since it is abundant in external solutions and stored in the sarcoplasmic reticulum, Ca^{++} depletion occurs infrequently and is not usually related to fatigue. However, a sudden influx Ca^{++} without an impulse produces a spontaneous twitch known as a “**spasm**” or “**cramp**.” Most often fatigue results from a decline in the availability of biological energy. When a myocyte is stimulated rapidly to tetany, ATP is consumed rapidly sustaining the process. Since the myocyte manufactures ATP at a finite rate, energy supply cannot match demand and consumption. Consequently, when stimulation produces tetany, fatigue results from a decline in available ATP. If tetany is interrupted for a short period (few seconds), energy production will restore maximum muscular performance. You can observe this in your gastrocnemius preparation.

In the following experiment, the frog sciatic and gastrocnemius will be exposed to study basic physiological properties of muscle contraction. Initially you will determine **Threshold** and examine the components of muscular contraction: **latent, contraction and relaxation phases** or periods. Also, you will study **motor unit summation and temporal summation** by independently changing stimulus amplitude and frequency while observing the effect on muscle responses. Within the vertebrate body, fine motor control is a product of the simultaneous **integration** of temporal and spatial summation

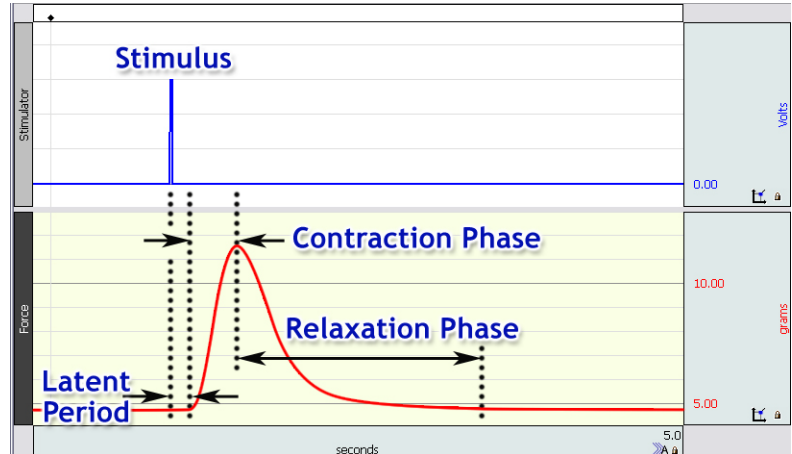


Figure 3

We will also determine a strength-length relationship for the gastrocnemius muscle and examine the effect of pH on its ability to produce a force.

Clinical Significance

Some types of muscle degeneration are secondary to nerve damage or to dysfunctions at the neuromuscular junctions. **Muscle degeneration** follows damage to the motor nerve pathway because proper neuromuscular activity and resulting muscle tone seem to be required for the health of the muscle. In the disease **myasthenia gravis** (myasthenia means that a muscle fatigues too easily), antibodies are produced against the muscle membrane receptors for acetylcholine, which is the neurotransmitter of somatic motor neurons. This **autoimmune disease** thus prevents the muscle from being properly stimulated by somatic motor neurons.

Instructor Note:

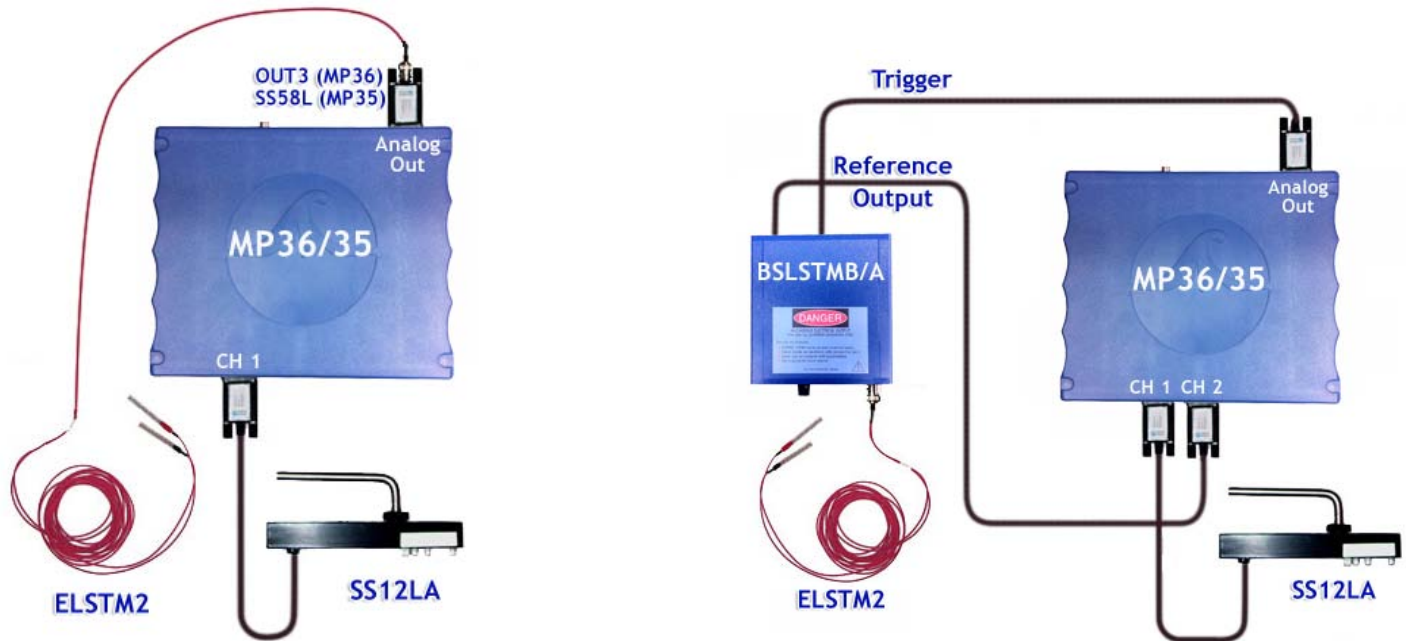
There are four available recording modes for this lesson; Threshold, Summation (includes Tetanus and Fatigue), Length-Tension and Muscle pH. If your lab does not have the time or the setup for the Length-Tension or Muscle pH recordings, they can be skipped by clicking “**Next**.” Because the lesson has the option of applying the stimulus to the sciatic nerve or directly to the muscle, you may find it useful to have the students record the lesson both ways and then compare the results.

Setup and Calibration:

General Cable Connections

It is assumed that the MP36/35 and BSLSTMB/A (if used) are connected to their power sources, but are turned OFF. It is also assumed that the MP36/35 is connected to the host computer and that the BSL PRO software has been installed and is known to work with the MP unit.

The connections will depend on the type of stimulator used as shown in Figure 4. In all cases, the Force transducer (SS12LA) is plugged into CH 1.



If using OUT3 (MP36) or SS58L (MP35)

If using BSLSTMB/A

Figure 4

1. Place the tension adjuster (BIOPAC HDW100A or equivalent) on the ring stand, and attach the BIOPAC SS12LA Force transducer such that the hook holes are pointing down. The SS12LA should be roughly set so that it is level both horizontally and vertically.
2. Set the tension adjuster such that it is approximately $\frac{1}{4}$ the distance from the lowest setting. This will allow the majority of the range to be used for adding tension (raising the adjuster).

Note: Do not firmly tighten any of the thumb-screws at this stage.



Figure 5

3. Determine the optimal force range for this experiment (0 to 100, 0 to 200, or 0 to 500 grams).

If you have a small or medium frog, there is a good chance that the default 0 to 100 gram range will be optimal. However, if large frogs are used, the range may need to be increased.

4. Place the S-hook into the hole on the SS12LA that is labeled with the desired range.
5. Click on the **Preferences** button and select **Force transducer range**. Make sure the range selection matches range on the force transducer (“S-hook”).

Turn ON the hardware

6. After all cables and leads are connected, the MP36/35 and BSLSTMB/A (if used) may be turned ON. Turning ON the hardware after the connections are made minimizes the chance of instrumentation errors caused by Electrostatic Discharge (ESD) during plug-in.
7. Establish the BSLSTMB/A stimulator settings (if used).
 - Turn the **Level** knob counter-clockwise until 0 is displayed on digital readout.
 - Set **Range** to “10 Volts” position using the key switch.
 - Place the **Reference** switch in the down position – “**Fixed (15ms)**.” Note that this setting only applies to the Reference out (to MP unit) and not the stimulator output pulse (to nerve or muscle). It is used to make sure the stimulator level will be accurately recorded even with sample rates as low as 200 samples/second.



Figure 6

8. Lightly abrade the needle electrodes to assure they will make good electrical contact.
- Note:** The BIOPAC ELSTM2 needle electrodes are often shipped with a clear protective Teflon coating. This coating must be removed prior to first use.

Software Setup

9. Launch the BSL PRO software and open the template file “a02.gtl” in one of two ways:
 - a) From Startup dialog, click the **PRO Lessons tab** and double-click on the lesson title in the list. If you do not see the lesson title, try the next opening method.
 - b) From Startup dialog, choose the **Create/Record a new experiment** option, click **Open Graph template from disk** and then click OK. Navigate to the file, select it and click **Open**.
10. Enter in a unique name and click OK. A folder with that name will be created and at the end of the lesson the data file will be automatically stored in this folder. Figure 7 shows the basic controls.

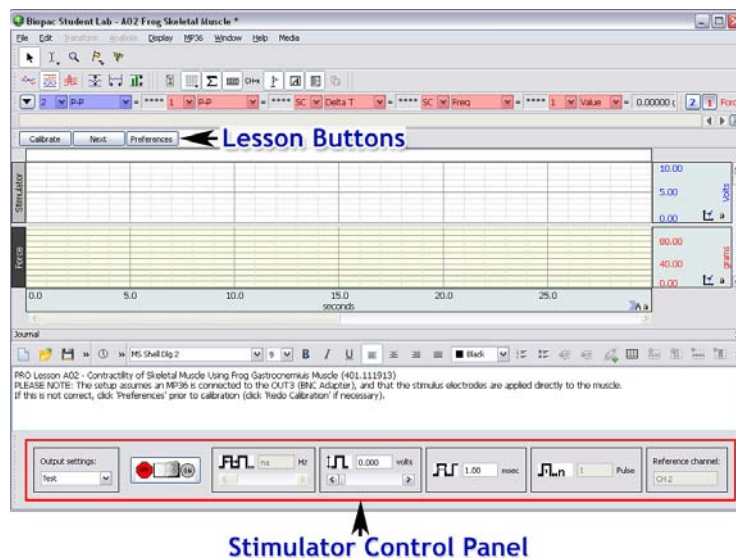


Figure 7

Note: If you do not see the Lesson Buttons upon launching the graph template, go to “**Display > Preferences > Graph**” and change the Display Style from “**Tabbed**” to “**Windowed**.”

11. Check the assigned **stimulator type** noted in the journal and make sure it matches the one you are using. If it does not, click the Preferences button and choose the “Stimulator hardware” option. Select the proper stimulator type and click OK.
12. Verify that the stimulus **electrode placement** noted in the journal is correct. If not, click the **Preferences** button and choose “**Stimulus electrode placement.**” Select either Sciatic Nerve or Muscle. The only difference between the two is that the stimulus levels required to produce contractions are approximately 10 times more when stimulating the muscle directly.

Calibration:

Note: If the last calibration was performed using same force transducer, calibration can be skipped by clicking “**Next.**”

13. Click “**Calibrate**” and follow the instructions in the pop-up dialogs. The first calibration is performed with only the S-hook attached to the force transducer (Figure 8). The second calibration is performed with a 50 gram weight attached, but after it has stopped swinging (Figure 9).

Note: Do not touch the bronze weight with exposed fingers, hands or frog Ringer’s solution.

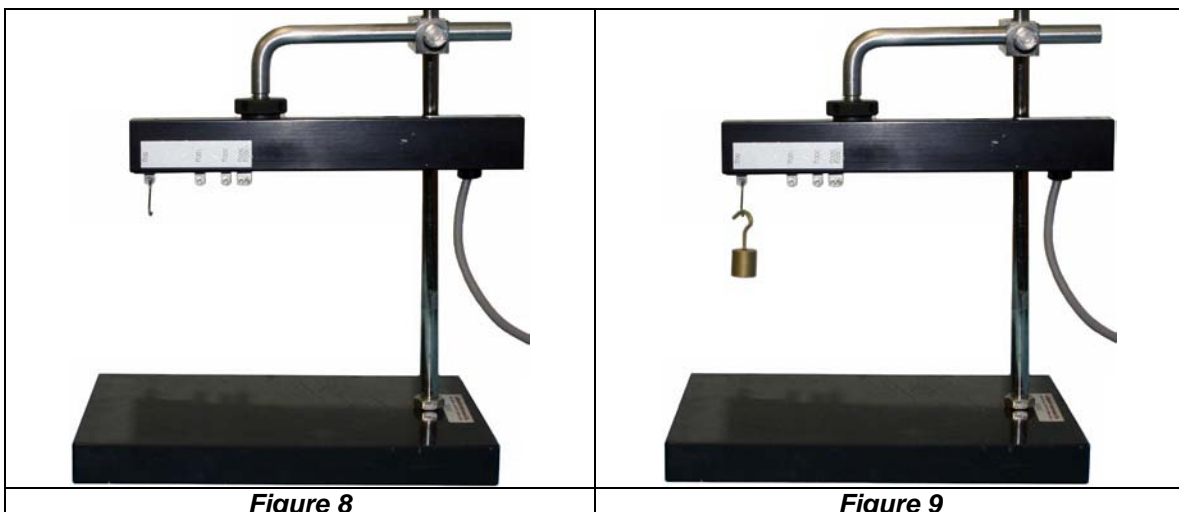


Figure 8

Figure 9

Frog Preparation:

1. Pith the frog (if necessary, see [BSL PRO Lesson A01 – Frog Preparation](#) for details).

Notes:

- You should leave the frog intact for all segments of your experiment. Leaving the leg, muscle, and nerve attached to the frog will improve circulation and preserve the muscle and nerve.
 - Apply amphibian Ringer's solution to the frog and muscle in five-minute intervals. Ringer's solution contains inorganic salts in concentrations similar to those found in extracellular fluid and will prolong the life of your preparation. The size and condition of the frog can influence the recording result.
2. Cut the skin around the leg close to the hip. Be sure to cut around the full circumference of the leg.
 3. Carefully remove the skin from the leg with a pair of forceps. Grasp the top edge of the cut skin and pull downward. The skin will come off inside out.

Note: Do not touch the bare surface of the muscle with the outer surface of the skin.



Figure 10

Isolate the Gastrocnemius Muscle:

4. Insert a probe between the gastrocnemius and the other muscles of the leg. Carefully slide the probe back and forth to free the muscle from the surrounding tissue.

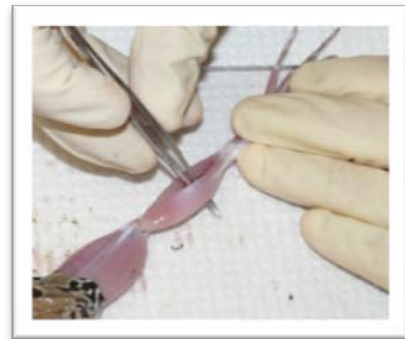


Figure 11

Isolate the Sciatic Nerve:

Skip this step if you will only be stimulating the muscle directly.

5. Use a blunt dissection to free up the nerve, being very careful not to damage the nerve.

Note: If possible, use a glass probe as metal will conduct any static charge from your body which can damage the nerve.

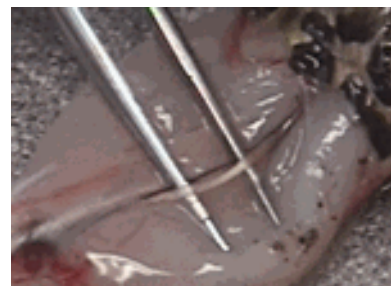


Figure 12

6. Cut a length of thread about 30 cm long and fold it double. Tie the double thread around the Achilles tendon and knot it very securely.
7. Insert a scissor blade into the opening created by the probe. Cut down through the attachment of the Achilles tendon to the heel. Be sure to leave a large segment of the tendon attached to the gastrocnemius below the knot.



Figure 13

8. Pin the knee in place, being very careful not to penetrate or damage the sciatic nerve. To stabilize knee position, pin the muscles of the anterior thigh and the anterior leg.

9. Position the force transducer

- a) Adjust the tension adjuster/force transducer assembly such that the S-hook is over the knee.
- b) Slide the tension adjuster/force transducer assembly down the ring stand to a point where you can loosely hang the loop off the S-hook.
- c) Slide the tension adjuster/force transducer assembly up the ring stand to a point where the line slack is removed but the muscle is not stretched.
- d) Adjust the assembly such that the thread line falls vertically.

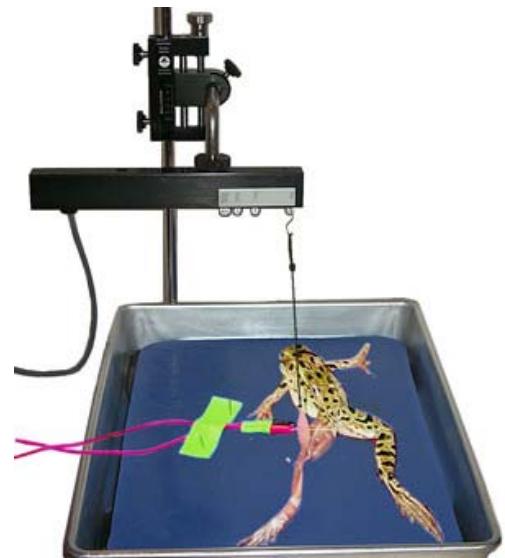


Figure 14

- e) For a true reflection of the muscle's contractile force, the muscle must not be pulled at an angle.
- f) Tighten all thumb-screws to secure the positioning of the assembly.

10. Position the stimulus electrodes

- If stimulating the sciatic nerve:
 - a. Remove the covers from the electrode tips.
- DO NOT discard the covers and always put them back on after the experiment.
 - b. Use tape to connect the two electrode leads, making sure the needle tips do not touch.
 - c. Insert the electrode needles underneath the nerve, being very careful not to penetrate or damage the sciatic nerve.
 - d. Take care in placing the electrodes to ensure that the surrounding muscle is not stimulated by needle contact as that will distort the result.
- If directly stimulating the muscle:
 - a. Using the Red needle electrode, pierce the muscle near the knee.
 - b. Place the Black needle electrode through the muscle above the Achilles tendon.

Hints for minimizing measurement error:

- The SS12LA Force transducer must be level on the horizontal and vertical planes.
- Set up the Tension Adjuster and Force Transducer in positions that will minimize movement when tension is applied. In other words, try to keep the point of S-hook attachment as close as possible to the Ring Stand support.
- Position the Tension Adjuster such that the Adjustment knob is easily accessible so you will not bump cables or the tray during adjustment.
- Position the Stimulator and Force transducer cables such that they cannot be easily pulled or bumped. If necessary, use tape to adhere cables to the tray or ring stand to relieve strain or pull.
- Set up the dissection tray such that it is very stable and will not wobble or rock.
- Make sure the end of the frog muscle is firmly pinned to the dissection tray, so that it will not rise up when tension is applied.
- Position the entire setup on a solid workbench, so that it will not wobble if the table vibrates.
- Use a thread that will not stretch under tension (such as non-stretch nylon).
- Use a knot that will not allow the line to lengthen or come undone under tension (such as the Bowline knot).
- **REMEMBER** to periodically wet the muscle with Ringer's solution.

If you will be performing the muscle pH experiment:

Now is the time to inject the Bromothymol Blue solution to determine the muscle pH while the muscle is in a resting state.

1. Inject two muscles with 0.1 ml of 0.5% Bromothymol Blue dissolved in 0.7% NaCl and adjusted to pH 7.0.
 - a. Inject into the gastrocnemius muscle.
 - b. Inject into an unused muscle such as the hamstring or quadriceps to be used as a control.
2. Wait a few minutes then note the color of the gastrocnemius muscle at the site of injection and determine the corresponding pH. Record this on your data sheet or in the graph journal.

Note: Bromothymol Blue is a pH indicator that is yellow at pH 6.0, green at pH 7.0, and blue at pH 7.6.

Adjust Tension mode

This mode is used to adjust the line tension and/or test the setup prior to recording. Data displayed in this mode will not be saved.

1. Click on the **Adjust Tension** button.
2. Use the tension adjuster knob (HDW100A) to make the line taught and then continue until approximately 5 grams of tension is displayed. **DO NOT OVER-TIGHTEN!**
3. Let the setup sit for a minute, then re-check the tension to make sure nothing has slipped or stretched.
4. Click **Stop**.

Stimulus Level Control:

The adjustment for stimulus level will be different depending on the type of stimulator you are using.

- If using an external stimulator (BSLSTMB/A), use the knob on the stimulator and adjust the level according to its digital readout.
- If using a Low Voltage stimulator (OUT3 or SS58L), use the level control that is in the stimulator control panel.



Level control when using OUT3 or SS58L

Figure 15

Testing the setup:

1. Click **Adjust Tension**.

Note: If stimulating the sciatic nerve, increase in 0.1 Volt increments, and if stimulating the muscle directly, increase in 1 Volt increments.

2. Set the stimulus level to 0.1/1 Volts.
3. Click on the **OFF/ON** button in the stimulator control panel to stimulate with one pulse.
4. Continue increasing the level and stimulating (OFF/ON button) in 0.1/1 Volt increments until a Force response is seen on the display.
5. If no response is detected:
 - Make sure there is approximately 5 grams of tension on the force transducer.
 - Make sure the needle electrodes are abraded to remove any coating or corrosion (see Setup Step 7).
 - If necessary, slightly move the needle electrodes to a new position and repeat the test.
6. Click **Next** to proceed to the recordings

Recording Modes

One data file containing several recordings will be saved for this lesson. Each successive recording will be appended onto the previous one. This lesson is broken down into a sequential series of recording modes, each of which can contain multiple recordings. The recording mode will be shown in the far left lesson button.

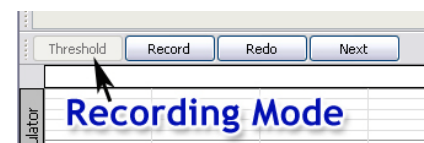


Figure 16

Notes:

- Check with your instructor to determine which recording modes will be used. To skip a recording mode click **Next**.
- It may be necessary to re-adjust the thread tension if the amplitude on CH 1 (Force) falls to zero when no stimulus is applied. If, after beginning a recording, you notice a signal problem, carefully adjust the tension then click **Stop**, **Redo**, and then **Record**.
- It is assumed that the user has a basic knowledge of the software, which includes data selection, zooming in and out, auto-scaling, taking measurements, and placing event markers. Review the tutorial if necessary (see options in the Help menu).
- Always review the recording steps prior to clicking **Record**.

1. Threshold

In this mode, each time the **Record** button is clicked, a recording will begin, followed by one stimulus pulse. The recording will stop automatically after enough time has occurred to capture a response.

Notes:

- If stimulating the sciatic nerve, increase in 0.1 Volt increments and if stimulating the muscle directly, increase in 1 Volt increments.
- If using the OUT3 or SS58L stimulators, you can increase the level by clicking the right scroll bar arrow of the Level control.

1. Set the **stimulus level** to 0 Volts.
2. Click **Record**. This is your baseline recording.
3. Increase the stimulus level to 0.1/1 Volt and click **Record**.
4. Continue increasing the level by 0.1/1 Volts and then **Record** until a Force response is detected.
 - This is the **Threshold response** and should be noted in the journal.

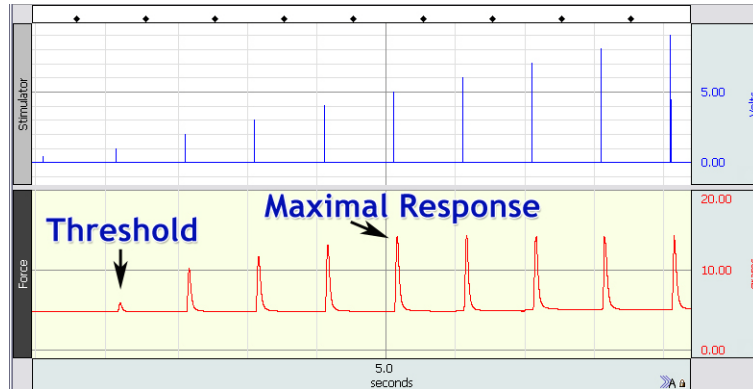


Figure 17

5. Continue increasing the level by 0.1/1 Volts while clicking **Record**, until the point where further increases in stimulator voltage produce no further increase in the response amplitude.
 - This is the **Maximal response** and should be noted in the journal.

Note: To make comparisons easier, you can view all of the recordings by clicking on the **Autoscale Horizontal** toolbar button



6. Click **Next** to proceed.

2. Summation

This mode is used to record Summation, Tetanus and Fatigue. When **Record** is clicked, a recording will begin, followed by a continuous series of stimulus pulses at a defined frequency. The recording will continue until **Stop** is clicked. You will adjust the frequency during the recording.

1. Check the line tension:
 - a) Click **Record** and examine the output of the Force transducer (CH 1). Adjust the tension if needed.

Note: You can turn on textual values by clicking within the vertical scale region of CH 1 (Force) and selecting “**Show textual value display**” from the pop-up dialog.
 - b) Click **Stop**.
 - c) Click **Redo** and then click **Yes** to redo the recording.
2. Set the **stimulus level** to a value that is between that required for Threshold and maximal response. The stimulus level will remain throughout this recording mode.
3. Click **Record**.
4. Click on the **pulse frequency** right scroll arrow (in stimulator control panel) to increase the stimulator pulse rate by 1 Hz.



Figure 18

5. Continue to increase the pulse frequency by **1 Hz** approximately every 2 seconds, until the Force response shows that a point of complete tetanus has been reached.
6. Let the recording continue, without changing the pulse frequency, until after the point of fatigue has been reached (see example in Figure 19).
7. Click **Stop**.
8. Click **Next** to proceed.

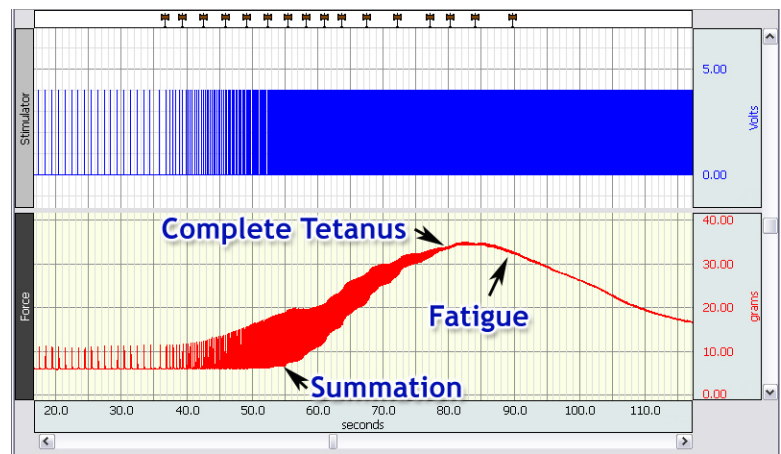


Figure 19

3. Length-Tension

In this recording mode, each time the **Record** button is clicked, a recording will begin, followed by one stimulus pulse. The recording will stop automatically. For this recording it is very important that the knee is pinned down securely so it will not lift when the muscle is stretched.

Note: If you have just finished the Summation recording, wet the muscle with Ringer's solution and let the muscle rest for at least 5 minutes before recording.

1. **Reposition the Force transducer:**
 - a) Loosen the thumb screws on the tension adjuster (i.e. HDW100A) and slide it down on the Ring Stand until there is slack in the thread.
 - b) Adjust the tension knob such that the position indicator reads 0 mm.
 - c) Carefully slide the tension adjuster up on the ring stand until the thread is just taut but the muscle is not stretched and then tighten the thumb screws.
2. Set the **stimulus level** to a value that is between that required for Threshold and maximal response (same as Summation mode).
3. Click **Record**.

Notes:

- If there was a visible contraction but no response recorded, then the tension adjuster must be moved up on the ring stand following Step 1.
 - If there was no contraction, check the needle electrodes and if still in place, turn up the stimulus voltage slightly.
 - If any changes were made, click **Redo** and then **Record** again. Repeat until a response is recorded.
4. Type "- 0 mm" into the append marker label region that is above the lesson button region. This text will be added onto the existing label (i.e. "Length-Tension 1 - 0 mm").
 5. Use the tension knob to raise the force transducer by 1 mm.

6. Click **Record**.
7. Add "-1 mm" onto the append marker label.
8. Continue to raise the force transducer (increase muscle length) by 1 mm, record and then add the length value onto the event marker label until the muscle fails to contract or the string slips off the muscle. When completed, the data should look similar to that shown in Figure 20.
9. Click **Next** to proceed.

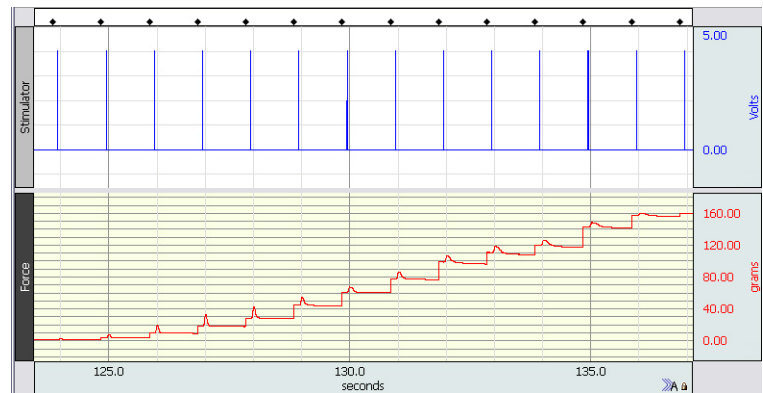


Figure 20

4. Muscle pH

The Bromothymol Blue solution should have been injected into two muscles prior to the recordings. If you have just completed the Summation recording, go directly to Step 5. If you did not perform the Summation recording, or did not inject the Bromothymol Blue solution during setup, you should inject it now (see last section of "Frog Preparation") and then perform the following:

1. Set the **stimulus level** to that which produced the maximal response in Threshold recordings.
2. Set the **stimulus frequency** to 20 Hz.
3. Click **Record**.
4. Continue to record until the muscle is fatigued and then click **Stop**.
5. Note the color of the muscle at the site of injection and determine the corresponding pH. Record this on your data sheet or in the graph journal.

Note: Bromothymol Blue is a pH indicator that is yellow at pH 6.0, green at pH 7.0, and blue at pH 7.6.

Done

Click **Done** to end the lesson and automatically save the data.

From the **Done** dialog, you may choose to analyze the data immediately or copy it to a different location (i.e. thumb drive) for analysis on another computer.

Data Analysis:

Use the software measurement tools to find the values for the tables and answer the questions for the recordings you performed.

Notes:

- Duration of the contraction phase is the time interval (Delta T) from the onset of contraction to the peak of contraction.
- Duration of relaxation phase is the time interval from the peak of the contraction to the return to baseline.
- The twitch duration is the sum of the duration of contraction phase and duration of relaxation phase.
- See [Appendix 1](#) for basic information on how to use the software tools.

Measurement examples:

To find the **latent period**, zoom in on data that includes the stimulus and the contraction. Select an area that starts at the beginning of the stimulus pulse and ends at start of the contraction. The “SC Delta T” measurement displays the duration in seconds.

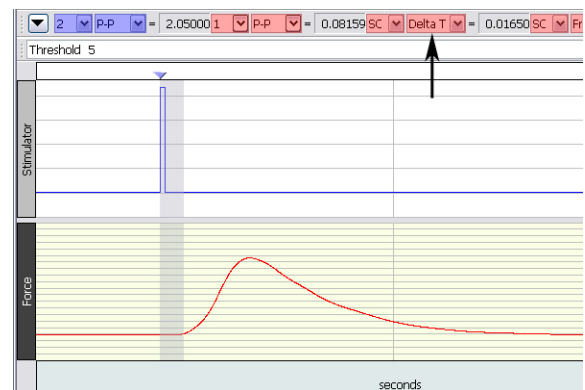


Figure 21

To find the **amplitude of contraction**, zoom in on the contraction and then select an area that encompasses the peak and baseline. For this lesson the bottom channel is CH 1 (Red) so the measurement to use is “1 P-P.”

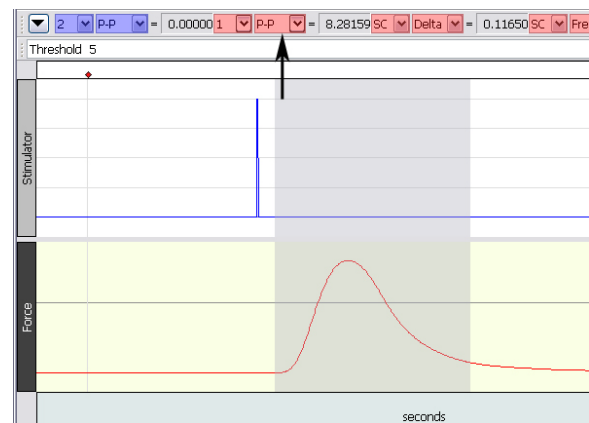


Figure 22

To find the duration of the **contraction phase**, zoom in on the contraction and select an area from the start of the contraction to the peak of the contraction. The “SC Delta T” measurement displays the duration in seconds.

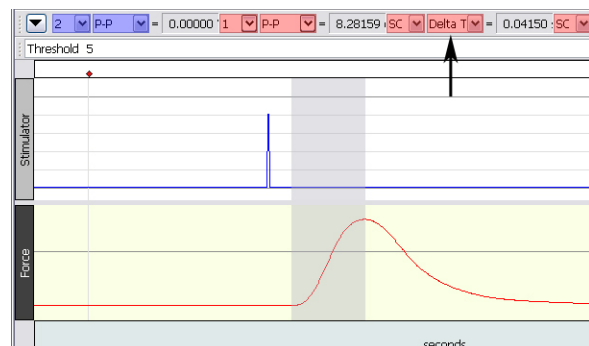


Figure 23

To find the duration of the **relaxation phase**, zoom in on the contraction and select an area from the peak of the contraction to the end of the contraction. The “SC Delta T” measurement will display the duration in seconds.

Note: The relaxation phase returns to baseline more slowly than the contraction phase. If it is difficult to see the point where the signal returns to baseline, zoom out more (compress the data) then re-select the area.



Figure 24

Did you stimulate the sciatic nerve or directly stimulate the muscle? _____

Threshold

| Condition | Stimulus Level (Volts) | Amplitude of Contraction (grams) | Latent Period (msec) | Duration of Contraction Phase (msec) | Duration of Relaxation Phase (msec) |
|--------------------|------------------------|----------------------------------|----------------------|--------------------------------------|-------------------------------------|
| | 2 p-p | 1 p-p | SC Delta T | SC Delta T | SC Delta T |
| Threshold Response | | | | | |
| Maximal Response | | | | | |

Table 1

Calculate the Stimulus level between Threshold and Maximal (used for Summation): _____ Volts

Calculate the twitch duration for the Threshold response: _____ msec

Calculate the twitch duration for the maximal response: _____ msec

Questions:

- When stimulating the sciatic nerve, what happens to the number of fibers producing action potentials as you increased the voltage?
- When you directly stimulate the muscle:
 - Why does the force increase as the voltage is increased?
 - Would this be considered motor unit recruitment?

3. After reaching the maximal response, why doesn't the force of contraction increase as the stimulus amplitude increases?

4. Does the Latent Period, Duration of Contraction, or Duration of Relaxation change with stimulus strength?

Summation, Tetanus and Fatigue

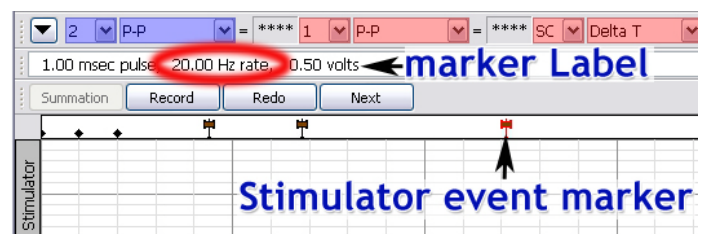
| | Normal Twitches | Summation | Tetanus |
|-----------------|-----------------|-----------|---------|
| Frequency (Hz): | | | |

Table 2

Duration from onset of tetanus to onset of fatigue: _____ seconds

Notes:

- Summation begins when the frequency of stimulation increases to a point where the muscle cannot fully relax between stimuli.
- Each time the stimulus frequency was changed, a stimulus event marker was automatically inserted. The marker label displays the frequency used. To determine the frequencies:
 - a. Click on the Autoscale Horizontal toolbar button to view all of the data.
 - b. Zoom in on only the Summation, Tetanus and Fatigue recording.
 - c. Find the stimulus event marker that is nearest to the data of interest.
 - d. Click on the event marker (activate it) and read the frequency from the event marker label.



Questions:

1. Based on the durations of the muscle contractions from the Threshold data, what is the lowest stimulation frequency that should have caused summation?

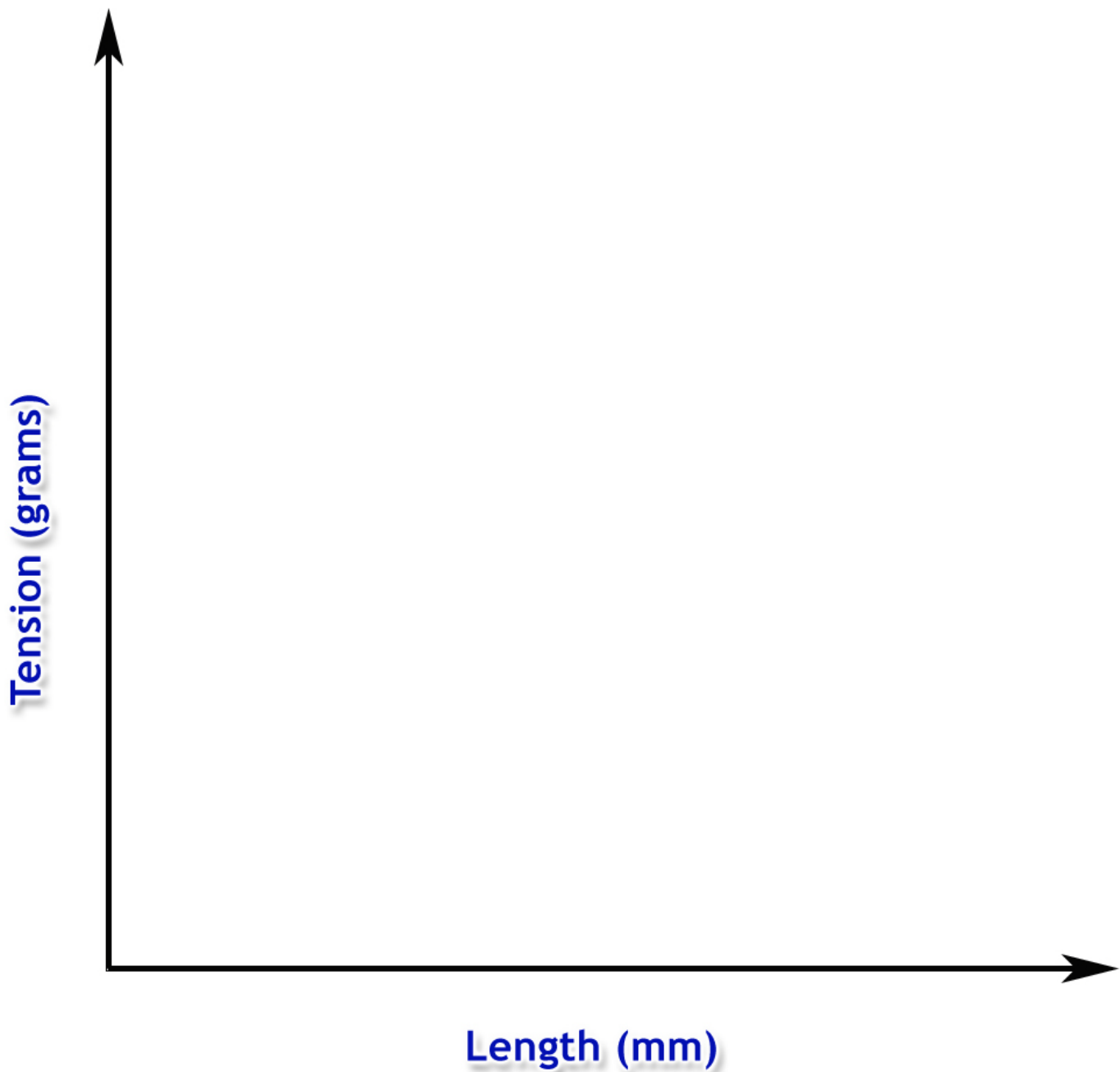
Note: Frequency (Hz) = 1000/ Pulse Interval (msec)

2. Is your calculated frequency the same as the frequency measured in your data?

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[illegible]**Table 4**

Graph the relationship between Length (amount of stretch) and Tension (amplitude of contraction). Use Excel or manually plot in the area below. If using Excel, make sure to label axes and plot "Length" on the x-axis. After the graph is complete, label the region of the muscle stretch that corresponds with the maximal force production as the optimal length of the muscle.

**Graph 1****Questions:**

1. Use your graph to answer the following question: Was there a direct linear relationship between the amount of stretch and the amplitude of the contraction? _____ If not, describe the relationship.
2. Why does this relationship between the amount of stretch and the amplitude of the contraction occur?

Muscle pH

Note: Bromothymol Blue is a pH indicator that is yellow at pH 6.0, green at pH 7.0, and blue at pH 7.6.

What muscle did you use for the control? _____

Resting state (prior to recordings):

Color of gastrocnemius muscle around injection site: _____

Corresponding pH: _____

Fatigued state:

Color of gastrocnemius muscle around injection site: _____

Corresponding pH: _____

Color of muscle used for control around injection site: _____

Corresponding pH: _____



Questions:

1. Why does muscle fatigue occur?

Appendix 1:


Autoscaling data:

Graph data can be autoscaled vertically and horizontally for enhanced viewing.


- Autoscale vertically by using the  toolbar button or “Display > Autoscale Waveforms” to optimize the vertical display and allow closer examination of the waveform.
- Autoscale horizontally by using the  toolbar button or “Display > Autoscale Horizontal” to display the entire horizontal time scale in a single graph window.

Zooming in and out of data:

Use the Zoom tool to magnify portions of the waveform for a closer look.

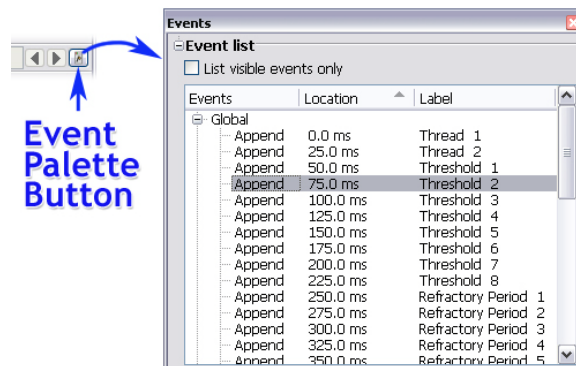
- Zoom in by selecting the  toolbar icon and click/drag over the area of interest.
- To zoom back, use Ctrl – (minus) or “Display > Zoom Back.”

Using the Event Palette to quickly locate Event Markers in the graph:


An easy way to locate event markers in the graph is by using the Event Palette, accessible by clicking the  toolbar icon or via “Display > Show > Event Palette.”

Zoom in on any section of data, simply highlight an event in the Event list, and the portion of data corresponding to that event will be displayed in the graph. Other features in the Event Palette include summarizing, renaming, removing or editing existing events.

To navigate quickly through consecutive event markers, use the arrows to the left of the Event Palette toolbar icon.



Measurements used in BSL PRO Lesson A02:

Graph data measurements are taken by using the I-beam tool  to select an area of interest. The following basic measurements are used in this experiment:

- P-P (Peak-to Peak) Shows the difference between the maximum amplitude value and the minimum amplitude value in the selected area.
- Value Shows the data value at the cursor position, or if an area is selected, the data value at the end of the selection.
- Delta-T Shows the horizontal axis time value in the selected area.

For a full explanation of the features described above, see the BSL PRO Tutorial or BSL PRO Software Guide.