Bio 397NP Neurobiology and Physiology Lab

# Muscle Physiology - The "catch" of a mussel's muscle

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## Background

Mussels are common marine and freshwater bivalves. *Mytilus edulis* is a common saltwater mussel known as a "blue mussel". It is edible and widely available in seafood markets. The adductor muscles and the byssal retractor muscles represent the main muscular systems in bivalve mollusks. The adductor muscles enable the animal to close its valves when necessary. The byssal retractor muscles control the position of the foot of the mussel, the direction that the mussel aligns itself on the substrate, and the tightness of the attachment of the mussel to the substrate. While many bivalves use their muscular foot to burrow into the sand, members of the genus *Mytilus* anchor themselves by using a collection of sticky threads.

A great deal of physiological and pharmacological work has been carried out on the anterior byssal retractor muscle (ABRM) of the *Mytilus edulis*, which has an interesting and unusual property. The ABRM is an example of a catch muscle, which is able to maintain a steady contraction for a long time period, with very little energy expenditure. This ability is important in anaerobic environments, because it allows the muscle to maintain tension for a prolonged time without using additional energy.

The byssal muscle consists of smooth muscle fibers. In invertebrate, smooth muscle contraction is initiated with calcium directly binding to myosin and then rapidly cycling cross bridges generating force. Smooth muscles do not depend on motor neurons to be stimulated. However, motor neurons reach smooth muscle and, depending on the neurotransmitters they release, can stimulate it or relax it. It is now well known that the ABRM is innervated by at least two efferent nerves. These are cholinergic (producing acetylcholine, abbreviated ACh) and serotonergic (producing serotonin, abbreviated 5-HT) nerves.

Excitation of one fiber versus the other generates a sustained tonic contraction characteristic of the 'catch' muscle. The mechanism by which catch is generated is not well understood. Most likely a catch protein known as twitchin, (similar to titin) becomes phosphorylated, allowing it to maintain a contraction state even at low concentrations of intracellular calcium.

**Objectives of Lab Cycle:**

- You will learn about mollusk biology, from identifying key parts of their anatomy to understanding the connection between muscle physiological properties and their functions.
- You will connect the principles of muscle contractions you have learned in lecture courses to the actual process of stimulating and recording muscle contractions in lab.
- You will learn to use transducers and stimulators to examine basic physiological systems.
- You will gain a more realistic view of scientific research by leading a real investigation
- You will pose a question, generate a hypothesis and make predictions, and begin to appreciate the difference between these three steps in formulating an experiment.
- You will work in groups to design an experiment, conduct the experiment, collect and analyze data, and present results.
- You will learn how to use inferential statistics to analyze data; drawing conclusions and placing them in a biological context.
- You will learn how to decide which data to present; how to use graphs, tables, and other visual displays effectively; and how to discuss those graphic supports in the accompanying prose.

**Lab Cycle Overview**

In this lab cycle you will work with your group mate to propose an experiment to test the regulatory mechanism of a 'catch' muscle contraction, and how this regulation generates transient phasic contraction, versus a sustained tonic contraction characteristic of the 'catch' contraction. The instructor will help you to refine your question and to ensure that the experimental procedures can be carried out using methods and materials supported by the laboratory. At the end of the lab cycle your group will present your results and each individual will write up a lab report in the form of a scientific manuscript (I. Background and Significance, II. Methodology, III. Results, IV. Discussion, V. References Cited).

**Week 1 (Lab 1)**
- You will learn about the anatomy of a mussel, how to dissect a mussel, how to isolate the ABRM, and the basic techniques to study muscle contractions using a force transducer.
- Your team will develop a hypothesis about the regulation of a 'catch' muscle, and an experiment to test it.

**Week 2 (Lab 2)** - You will perform the experiments you proposed.
**Week 3 (Lab 3)** - You will work on analysis, interpretation, and presentation of results.
*** Written Reports/Scientific Manuscripts will be due at the beginning of the next lab period.***

## Lab 1- Introduction to Muscle Physiology

Today you will stimulate the muscle using electrodes and measure the force of muscle contraction using the force transducer setup used in the "Physics of Physiology" lab. The muscle will be stimulated with a range of different electrical pulses. During the experiments, the amplitude, frequency and duration of these electrical pulses will be varied. Analysis of the muscle contraction will include measurements such as contraction amplitude and contraction duration. In the last part of the lab you will come up with a question/hypothesis about the system and make a prediction that will be tested next week.

### Equipment Set-up
- Make sure the FT-302 force transducer is in place, and all the connectors are plugged in.
- Choose the Mac OS X configuration (in Microsoft some of the settings are different).
- Log on using the username biouser and the password Biology!.
- Use the power switch to turn on the unit, confirm with a lit green power light.
- Log on to the computer, then turn on the power to the iWorx unit, and then load the software – in that exact order - for the components to work properly together.

### Start the software
- Click on the LabScribe shortcut on the computer's desktop to open the program.
- On the Main window, pull down the Settings menu and select Load Group.
- In Complete Settings locate and open IPLMv6Complete.iwxgrp file.
- Pull down the File menu and select Open; select CompleteSettings, open Animal Muscle folder, and then the ByssalMuscle-LS2 settings file. After a short time, LabScribe will appear on the computer screen as configured by the ByssalMuscle-LS2 settings.

**The Dissection**
1. Your TA will open the shells of the mussels for you. Put the mussel in the dissecting dish and observe its anatomy. Several soft structures surround the tougher muscles. The mantle spreads over the surface of both valves and secretes the material that builds the shell. Much of the rest of the soft structures are the ctenidia, the filamentous structures that move seawater though the mussel and filter out its food. Remove the mantle, the muscles, ctenidia, and all other structures except for the adductor muscle, the muscular brown foot and the byssal muscles (See Figure 1).
2. Moisten the exposed muscles with cold seawater every few minutes.

3. The anterior byssal retractor muscle is a white to yellow muscle that extends from its origin at the anterior tip of the shell to its insertion at the base of the foot.

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

**Figure 1**: Dissected *Mytilus*. The anterior byssal retractor muscle stretches from the apex of the valve to the base of the foot.

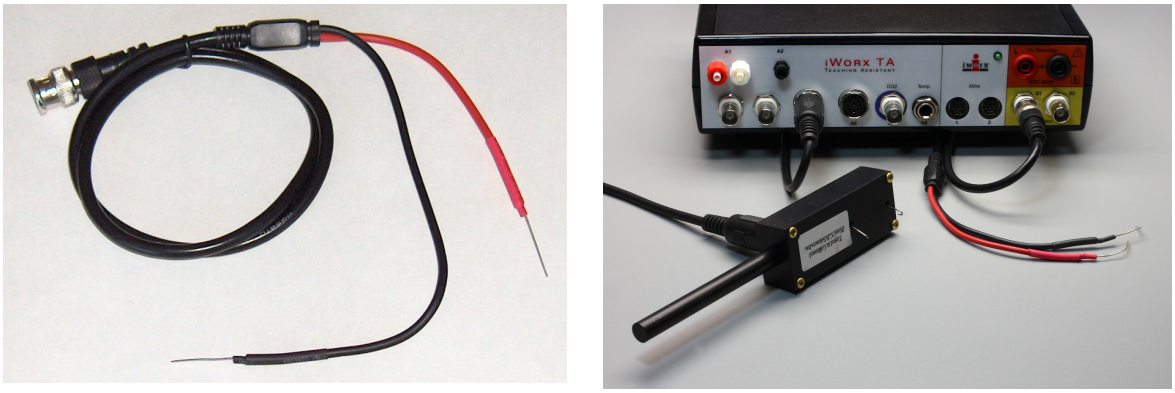


**Figure 2**: The C-BNC-N2 stimulating electrodes (left) and FT-302 force transducer (right) with the CBNC- N2 stimulating electrodes connected to the IXTA.

**Stimulus Electrode Setup**

1. Make sure the FT-302 force transducer and C-BNC-N2 stimulating electrodes are in place and connected with the IXTA unit (Figure 2).
2. Make sure the force transducer is correctly placed on the ring stand, and a piece of thread is tied under the head of the hook-shaped pin (use 0-10g).
3. Place the hook around the center of the anterior byssal retractor muscle.
4. Adjust the height of the transducer so that the byssal muscle is lifted slightly. What is the relationship between force and length in muscle?
5. Place one of the stimulating needle electrodes through the byssal muscle on either side of the insect pin hook. (Figure 3)

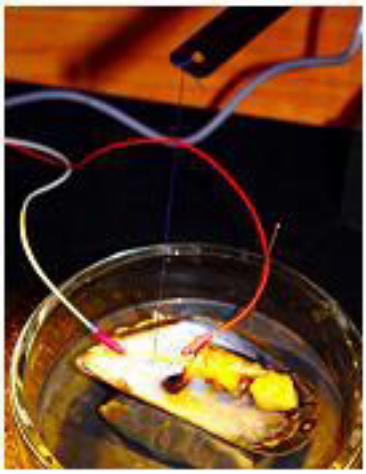

**Figure 3**. Electrodes in place to evoke and record contractions from the ABRM.

Note: The muscle preparation used in this experiment is functional for a limited period of time. If the muscle is bathed periodically in seawater (avoid the electrodes), it will work for about 2 hours.

**Exercise 1: Stimulus-Response**
Aim: To determine the relationship between the strength of the stimulus and the response of the muscle.

In this exercise, you will stimulate the muscle at increased amplitudes, and examine the effects of changing the stimulus voltage with which the muscle is stimulated.

What effect do you think the increasing stimulus voltages might have on the force of contraction of the muscle?

State this as a hypothesis and record your hypothesis in the last page of this manual (question #1 of post-lab assignment).

**Procedure of testing your hypothesis:**

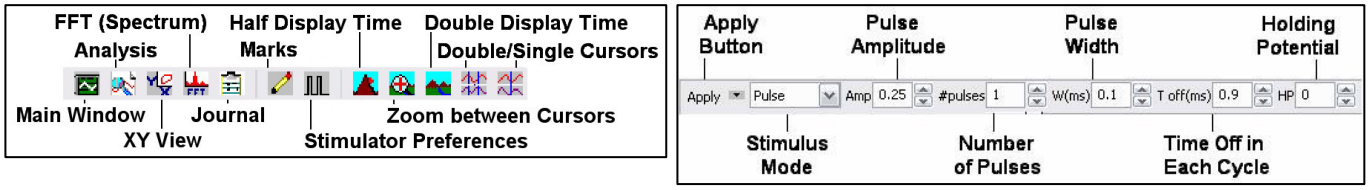


Figure 4: The LabScribe toolbar (left), and stimulator control panel (right)

1. Click the Stimulator Preferences icon on the LabScribe toolbar to open the stimulator control panel on the Main window (Figure 4).
2. Check the values for the stimulus parameters that are listed in the stimulator control panel on the Main window: the pulse amplitude (Amp) should be set to 0.000 V; the number of pulses (#pulses) to 1; and the pulse width (W) to 10ms. The value for a stimulus parameter can be changed by either of two methods:
   -Click on the arrow buttons to the right of the window that displays the value of the parameter to increase or decrease the value.
   -Type the value of the parameter in the window next to the label of the parameter.
   IMPORTANT: Click the Apply button to finalize the change in any stimulus parameter.
3. Type 0.000V in the Mark box to the right of the Mark button.
4. Click Record to stimulate the muscle. Press the Enter key to attach the comment to the recording.
5. Click Stop to halt the recording.
6. Change the stimulus amplitude (Amp) to 0.250V using one of the techniques described in Step 5. Click the Apply button to finalize the change in the stimulus amplitude.
7. Type 0.250V in the Mark box.
8. Click Record to stimulate the muscle. Press the Enter key to attach the comment to the recording.
9. Increase the stimulus amplitude in increments of 0.250 Volt. Record and mark the muscle responses. Increase the stimulus amplitude until the muscle twitch reaches a maximum amplitude or the stimulus amplitude is 5V. The recording should look like Figure 5.
10. Select Save As in the File menu, type a name for the file. Choose a destination on the computer in which to save the file, like your lab group folder). Designate the file type as *.iwxdata. Click on the Save button to save the data file.
11. Moisten the muscle with seawater.

Figure 5: A recording of a muscle twitch (upper trace) and the stimulus pulse (lower trace).

**Exercise 2: Summation and Tetanus**

Aim: To measure the amplitude of contraction produced in a muscle that is stimulated with a long current pulse, and repeated pulses delivered at progressively higher frequencies

In this exercise, you will keep the stimulus amplitude constant at the value that produced a maximal contraction in the preceding experiment, and examine the effects of changing the frequency with which the muscle is stimulated. With increasing frequency of stimulation, the muscle may not relax completely before the next stimulus arrives. As a result, a new contraction begins in a muscle fiber that is already partly contracted.

What effect do you think this might have on the force of contraction of the muscle?

State this as a hypothesis and record your hypothesis in the last page of this manual (question #2 of post-lab assignment).

**Procedure of testing your hypothesis:**

1. Check the values for the stimulus parameters that are listed in the stimulator control panel on the Main window: the pulse amplitude (Amp) should be set to the voltage that caused a maximal muscle response in Exercise 1; the number of pulses (#pulses) to 25; and, the frequency (F) to 0.5 Hz.
2. Click the Apply button to finalize the change in any stimulus parameter.
3. Type 0.5 Hz in the Mark box to the right of the Mark button.
4. Click Record to stimulate the muscle. Press the Enter key to attach the comment to the recording.
5. Click Stop to halt the recording. A muscle response to a stimulus frequency of 0.5Hz is displayed in Figure 6.

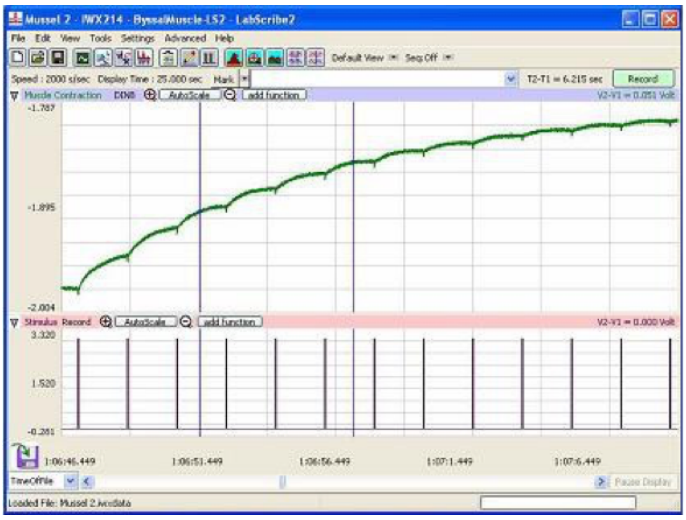

Figure 6: A recording of mechanical summation at a stimulus frequency of 0.5 Hz. The muscle does not have time to return to resting tension between contractions.

6. Change the stimulus frequency (F) to 1 Hz. Click the Apply button to finalize the change in the stimulus amplitude.
7. Type 1 Hz in the Mark box.
8. Click Record to stimulate the muscle at 1 Hz. Press the Enter key to attach the comment to the recording.
9. Click Stop to halt the recording.
10. Repeat Steps 6 through 9 for each of the following frequencies: 2, 5, 10, 20, and 30 Hz. A muscle response to a stimulus frequency of 20 Hz is displayed in Figure 7.
11. Finally, stimulate the muscle with a five second pulse. Change the number of pulses to 1 and the pulse width to 5 seconds. Click the Apply button to finalize the change in pulse number and width.
12. Type 5-second pulse in the Mark box.
13. Click Record to stimulate the muscle. Press the Enter key to attach the comment to the recording.
14. Once the muscle has completely relaxed, click Stop to halt the recording
15. Select Save in the File menu.

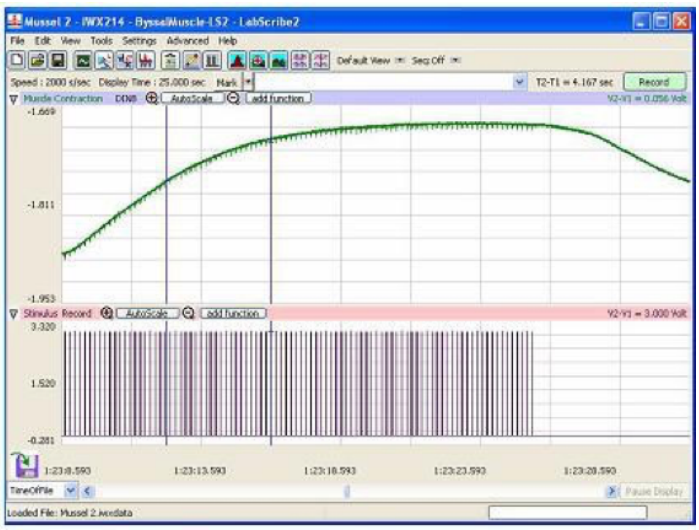


Figure AM-8-L7: Recording from a muscle stimulated at a frequency of 20Hz demonstrating tetanus.

**Data Analysis**

**Exercise 1: Stimulus-Response**

1. Scroll through the data from Exercise 1 and find the first muscle twitch to be generated by a stimulus pulse. Click the AutoScale button to maximize the size of the muscle twitch on the window. Note the stimulus voltage used to generate this twitch.
   Note: At stimulus voltages that are below the threshold of the muscle, the amplitude of the muscle twitch is zero.
2. Use the Display Time icons to adjust the Display Time of the Main window to show the stimulus pulse used to generate the twitch and the complete twitch on the Main window. The stimulus pulse and the twitch can be selected by:
   - -Placing a cursor before the stimulus pulse, and a cursor after the muscle has completely relaxed;
   - -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. Click on the Analysis window icon in the toolbar or select Analysis from the Windows menu to transfer the data displayed in the Main window to the Analysis window.
4. Look at the Function Table that is above the uppermost channel displayed in the Analysis window. The mathematical functions, V2-V1 and T2-T1 should appear in this table. Values for V2-V1 and T2-T1 on each channel are seen in the table across the top margin of each channel.
5. Maximize the height of the trace on the Muscle Contraction Channel by clicking on the arrow to the left of the channel's title to open the channel menu. Select Scale from the menu and AutoScale from the Scale submenu to increase the height of the data on that channel.
6. 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.
7. 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 Contraction Channel pulldown menu.
8. Transfer the values for the amplitude and times to the Journal using the Add Ch. Data to Journal function in the Muscle Contraction Channel pull-down menu.
9. On the Muscle Contraction Channel, use the mouse to click on and drag the cursors to specific points on the recording to measure the following parameters:
   • Muscle Contraction Amplitude 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 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 function on the Muscle Twitch Channel is the contraction time of the twitch.
• Relaxation Time 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 function on the Muscle Contraction Channel is the relaxation time of the twitch.
• Latency 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 function on the Muscle Contraction Channel is the latency of the muscle response.

10. Record the values in the Journal.
11. Select Save in the File menu.

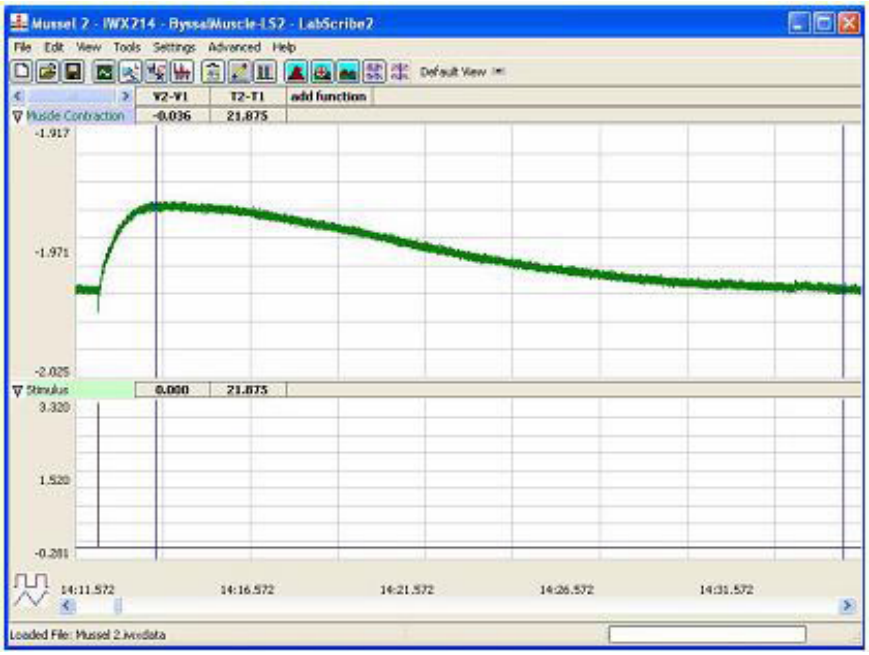


Figure AM-8-L8: A single muscle twitch and stimulus pulse displayed in the Analysis window. The cursors are located at the peak of the contraction and the point at which the muscle has returned to its baseline tension. The time between them represents the relaxation duration.

**Exercise 2: Summation and Tetanus**

1. Scroll to the beginning of the data recorded for Exercise 2. Click the AutoScale button to maximize the size of the muscle twitches on the window.
2. Scroll through the data from Exercise 2 and find the first series of muscle twitches in which the muscle does not have sufficient time to fully relax to the baseline tension level between twitches. This phenomenon is known as mechanical summation.
3. Use the Display Time icons to adjust the Display Time of the Main window to show all the twitches in the series on the Main window. The twitches can also be selected by using the Zoom between Cursors function explained in the data analysis section for Exercise 1.
4. Click on the Analysis window icon in the toolbar or select Analysis from the Windows menu to transfer the data displayed in the Main window to the Analysis window.
5. The mathematical functions, V2-V1 and T2-T1 should appear in the Function Table above the uppermost channel in the Analysis window. The values for these parameters are seen in the table across the top of each channel.
6. Maximize the height of the trace on the Muscle Twitch Channel by selecting AutoScale from the Scale submenu on the channel menu.
7. On the Muscle Contraction Channel, use the mouse to click on and drag the cursors to specific points on the recording to measure the following parameters:
   - Amplitude of First Muscle Twitch, which is the difference between the baseline tension of the muscle

and the tension at the peak of the first twitch in the series. To measure this parameter, place one cursor at the beginning of the first twitch, and the second cursor on the peak of the twitch. The value for the V2-V1 function on the Muscle Contraction Channel is the amplitude of the first muscle twitch in the series.

- Maximum Amplitude in Summation/Tetanus, which is the difference between the baseline tension of the muscle and the tension at the peak of the tallest twitch in the series. To measure this parameter, place one cursor at the beginning of the first twitch, and the second cursor on the peak of the tallest twitch in the series. The value for the V2-V1 function on the Muscle Contraction Channel is the amplitude due to mechanical summation.
- Change in Passive Tension, which is the difference between the baseline tension of the muscle and the tension at the highest relaxation point between the twitches in the series. To measure this parameter, place one cursor at the beginning of the first twitch, and the second cursor on the highest relaxation point between any pair twitches in the series. The value for the V2-V1 function on the Muscle Contraction Channel is the increase in the passive tension in the muscle during the series of twitches.

8. Record the values in the Journal using the one of the techniques described in the data analysis section for Exercise 1.
9. Select Save in the File menu.
10. Determine the frequencies at which the following first appear:
    - Mechanical summation.
    - Incomplete tetanus
    - Complete tetanus

Did your results match your hypotheses?

Note: You may want to keep the instructions on dissection, preparation and procedure listed in this manual, and bring them with you in the following three labs.

Bio 397NP Neurobiology and Physiology Lab

# Intro to Muscle Physiology
# Post Lab Assignment (Turn in 1 per group)

Student Names: _____

1. What effect do you think the increasing stimulus voltages might have on the force of contraction of the muscle? State this as a hypothesis:

2. What effect do you think the increasing frequency of stimulation might have on the force of contraction of the muscle? State this as a hypothesis:

3. Did your results match your hypotheses?

4. Come up with a new question and hypothesis for how this muscle functions. Make a prediction about how the mussel muscle would respond to a given test.