Modeling Physiological Systems and Laboratory
Fischell Department of Bioengineering
University of Maryland
College Park, MD

Lab 3: Frog Muscle Stimulation

Introduction

As a review, the three major types of muscle are smooth, cardiac, and skeletal. Skeletal muscles are controlled through the somatic nervous system and are under voluntary control. Muscle is made up of actin and myosin filaments that are arranged into a sarcomere- the basic unit of muscle. The actin and myosin make up myofibrils which combine to make muscle fibers which are grouped together and termed the muscle fasciculus. When the muscle is stimulated by a nerve, the sarcoplasmic reticulum releases the calcium, which triggers several mechanisms and allows the actin and myosin to slide along each other. When this happens the sarcomere shortens and the whole muscle contracts until the calcium is stored again and the muscle relaxes. Usually, calcium is released in response to an action potential. There is a minimum voltage necessary to generate a release of calcium (and thus a contraction). Once the muscle is stimulated over the minimum voltage, the tension generated by the muscle contraction will increase with stimulation amplitude until the muscle reaches a maximum value possible. When the stimulation ceases, the muscle will relax. However, if consecutive two stimuli are close enough together, the tension generated will be greater than if the muscle was given time to fully relax in between. When two stimuli together produce a higher force response this is called summation. An incomplete tetanus occurs when the tension generated is higher than that of a single twitch but the muscle is allowed to partially relax between each stimulus. When the frequency is high enough, the individual twitches will sum together and eventually create a sustained contraction, with no time for the muscle to relax in between. This is called complete tetanus, where there is no relaxation between stimuli and the tension generated is at a maximum.

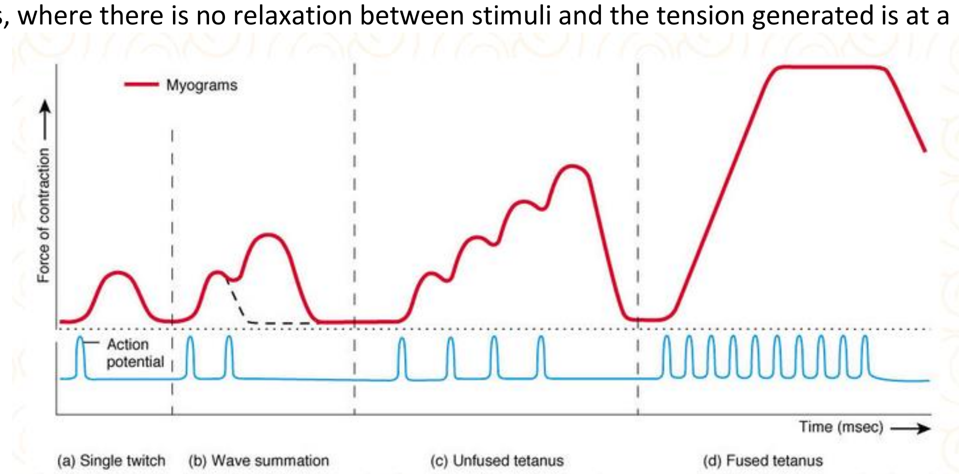

Fig 9. Typical myogram of a skeletal muscle. Chapter 8. Zoo145 San Diego Mesa College. 2014.

In this lab, you will observe the effect of various stimuli on the gastronemius muscle of a leopard frog (*rana pipiens).* In order to isolate the muscle, your lab group will begin with a dissection of a double-pithed frog. Pithing destroys the central nervous system activity, but leaves the peripheral nerves and organs intact. Since the muscle will be isolated from the body, for this dissection and experiment the source of calcium ions for muscle contraction are supplied in the Frog Ringer's solution provided. The Ringer's also keeps the tissue moist and must be used throughout the experiment. The goal of this experiment is to observe twitch, summation, incomplete tetanic, and complete or fused tetanic contractions. Using iWorx equipment, you will alter the external stimulus provided to the muscle, and record resulting force of contraction. From the recorded data, contraction time, relaxation time, and latent period can be calculated. The contraction time and relaxation time should be apparent from the positive and negative slopes of the tension graph, and the latent period is defined as the time between the start of the stimulus and the start of the muscle twitch.

Wet Lab Protocol

Materials (provided):

Dissection kit- including hemostats, scissors and pick ups
Dissection tray
Frog Ringer's solution
Gauze
Thread
Ring stand and clamps
Force transducer
Stimulator
iWorx control module
Computer with iWorx LabScribe3 software
Calipers

Dissection: ****Make sure to keep the frog moist using the Frog Ringer's solution provided***

1. Retrieve a double pithed frog and place in dissection tray.
2. Using a hemostat and dissection scissors, cut the skin all the way around the center of the frog's body. Then, holding the top half of the frog steady, use the hemostat to pull the skin down and off of the legs so that the gastrocnemius muscle is showing.
3. Place the hemostat between the muscle and bone and use blunt dissection to separate as much as of the muscle from the bone while still leaving the muscle attached to the bone.
4. With the hemostat between the muscle and bone, pull a 6 inch piece of thread through the muscle and bone and tie it to the Achilles tendon. Tie as far down the tendon as possible but leave enough room to cut the tendon from the bone after the thread.
5. To get to the femur: cut the muscle away from the femur near the patella so that you can see the femur. Then, with the mayo scissors (for bone cutting) expose as much of the femur as possible by pushing the muscle up the bone and cut the femur as high up as possible.
6. Using the mayo scissors again, cut the tibia just below the patella.
7. Now the gastrocnemius muscle should be separated from the rest of the frog with one end attached to the femur and the other to the Achilles tendon.
8. Measure the muscle and the tendon below the thread at its resting length.

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Experiment Setup*:* *A picture of the software and the locations of several buttons is shown at the end of the section.*

1. The hardware should be set up on a ring stand in the following order from top to bottom: force transducer, stimulator, and then the femur clamp. Tie the Achilles tendon end of the excised muscle to the force transducer, and then clamp the femur at the opposite end. Make sure that the force transducer is level, the white numbers on the transducer face up, and the muscle is vertical. The thread should not have any slack but the muscle should also not be stretched past resting length.
2. Adjust the stimulator so that it is resting in the middle of the muscle and both ends are in contact with the muscle.

Keep the muscle moist with the Frog Ringer's solution, apply ~every 5 minutes

3. Plug the iWorx control module into your computer using the USB cable. Open LabScribe3.
4. To calibrate the transducer, begin recording in the software, and place a provided weight on the transducer. Stop recording after a few seconds. Click the Double Cursors button at the top of the screen and place the cursors at the bottom and top of the signal. Then, right click and select Simple. When the dialog box opens, set the first cursor to zero and the second to the known weight you used. Name the units g and click OK.

Experiment 1: Twitch Contractions

1. Locate the stimulator toolbar at the top of the iWorx software. If the settings are not visible, the Stimulator Preferences button will show them.
2. Adjust the setting to start out with Pulse; Amp: 0; # pulses: 1; W (ms) =10; F (Hz) = 0.5; HP =0
3. Click Apply to the left of the stimulator preferences toolbar.
4. You can comment on the recording by typing into the Mark bar under the Stimulator preferences. You can label each different amplitude for analysis later.
5. Make sure the stimulator is in contact with the muscle and press Record to administer the stimulus and begin collecting data. The stimulus will appear on the screen, as well as any fluctuation from the force transducer.
6. Watch both the muscle and the iWorx software for signs of a response, and then click Stop. You may have to autoscale (a magnifying glass with 2 arrows) since the amplitudes are expected to be low. Remember it is also possible that the voltage you have started with is not high enough to trigger a contraction.
7. Increase the amplitude by 0.05V, and leave the rest of the preferences the same. Click Apply.
8. Again, press record, and observe the muscle and iWorx software for signs of a response. Click Stop.
9. Continue increasing the voltage by 0.05V until the response reaches a maximum value. Do not continue past 5V.

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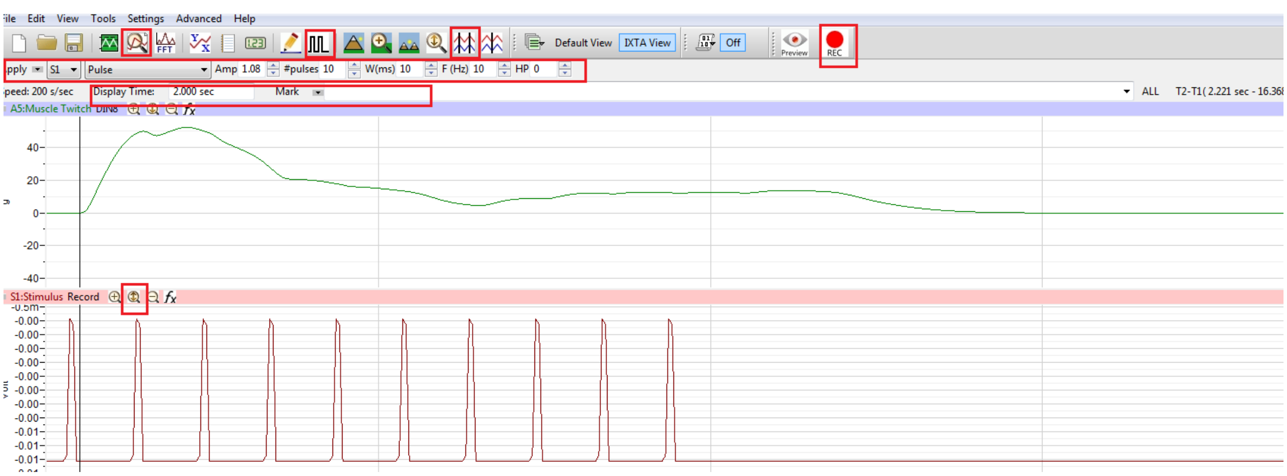
Experiment 2: Tetanic Contractions

1. Change the stimulator preferences to: Pulse; Amp= submaximal voltage (select based on your twitch contraction experiment); # pulses: 15; W (ms) =10; F (Hz) =0.5; HP =0.
2. Again, you can type the different frequencies into the Mark bar before recording to keep track for analysis.
3. Click Apply, and make sure the stimulator is in contact with the muscle before you click Record. Observe the contraction, and stop recording.
4. Repeat at F (Hz): 1,2,3,4,5,10,15,20,30. Remember to click Apply before starting to record and make sure the stimulator is in contact with the muscle at all times.

Experiment 3: Length Tension Curve

1. Set the stimulator settings back to a single pulse setting: Amp: submaximal; # pulses: 1; W (ms): 10; F (Hz): 0.5; HP: 0.
2. Apply those changes, and press record. Observe contractions, and then stop recording
3. Using the calipers to measure, stretch the muscle ~2mm by moving the femur clamp down. By moving the femur clamp instead of the force transducer, you can collect the increase in tension both before and after stimulation. This may be difficult to be precise but just try to measure the new length from using 2 consistent points. Choose points to measure from that will be recognizable through the stretching.
4. Click Record, observe, and then click Stop.
5. Continue stretching the muscle and recording the new length as well as the response.



Row 1 (left to right): analysis button, stimulator preferences, double cursor, and record/stop
Row 2: Stimulator preferences toolbar and Apply button

Row 3: Display time changes the time scale of the display, and Mark is where you can label the recording
Row 4: The autoscale button

In MATLAB, compile the data you have collected from each of these three experiments. It may be easiest to export the data into a txt or excel file. Create a plot describing the relationship between voltage of stimulus and resulting force generated by the muscle (Experiment 1). Additionally, provide one example plot showing tension in the muscle over time (to include several stimuli). Mark on the plot the times the stimulus was provided. Next, create a plot showing the relationship between frequency of pulses and max tension in the muscle (Experiment 2). Provide an example image of unfused and fused tetanic contraction. Finally, create a length tension curve (Experiment 3). Each figure should have axis labels and titles. Include these figures in the results section of your lab report.

Model Application

As discussed in lab, Hill's Muscle Model can be used to describe muscle contraction. By building a simplified simulation of Hill's model, we can describe the force and length relationship of a muscle. In this simplified model, we will assume length is prescribed as an input, and consider a case where a tetanizing stimulus is applied.

1. First create a model in MATLAB to solve for the length of the series elastic element and the series contractile element, as well as the tension in the muscle over time. Start with the variables we discussed in class (total length is 1mm, length of contractile element is 0.3mm).
2. Use the successful model you have developed to help investigate characteristics of the actual tetanic contraction you have observed in lab. Begin by plotting the time versus force data you have collected corresponding to the stimulation that resulted in fused tetanus. Adjust your model to account for the length of your muscle, and plot the resulting calculations. Finally, adjust constants (such as spring constant, series elastic length, etc.) to help your model of force over time fit the actual data.
3. Display on one plot your finalized model data and your actual collected force data for fused tetanic contractions. The figure should have axis labels, a legend, and a title. Include this figure in the results section of your lab report. Discuss the variables you chose to utilize.

Analysis Questions

You will need to turn in a complete lab report to accompany your program or code. You should follow the guidelines on how to write lab reports posted on ELMS. Specifically regarding this lab, it may be helpful to review your lecture slides and notes, and then consider answering the following questions:

1. Results
    a. Include figures and written description of what your program or code reported in response to each of the signals tested.

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   b. Discuss the measured contraction time, relaxation time, and latent period calculated from various voltages applied.
   c. Describe the differences observed between the plots representing unfused and fused tetanus.
2. Discussion
   a. What is the maximum force generated in the twitch contraction experiment? Why does it reach a max?
   b. Why does force increase with frequency in experiment 2? And why can't you detect individual contractions with complete tetanus?
   c. In the length-tension curve, explain the trend of the graph. Why does the force decrease with high lengths of muscle?
   d. Discuss the accuracy of your model in describing tetanic contractions when compared to your collected data.

Due December 4th: The assignment deliverable is a formal lab report with the necessary MATLAB code attached, due at the start of class on December 4th. Please also turn in on ELMS an electronic copy of the MATLAB code (.m file please!) along with the data set your code runs on. Both the electronic and hard copies are due by 3:30 PM on December 4th.