**SKELETAL MUSCLE PROPERTIES**

## **Background** :

In this experiment, you will investigate the physiological properties of skeletal muscle from the isolated amphibian gastrocnemius. You will examine skeletal muscle functions such as the single twitch, multiple motor unit summation, the relationship between muscle length and generated tension, wave summation and tetanus, and muscle fatigue.

The physiology of skeletal muscle was not fully understood until the early 20$^{th}$ century. Some of the earliest experiments on muscle physiology were performed between 1661 and 1665 by Jan Swammerdam, who demonstrated that an isolated frog muscle could be made to contract when the sciatic nerve was irritated with a metal object. Later, Luigi Galvani (1737–1798) demonstrated that frog muscle responded to electrical currents (Fulton and Wilson, 1966).

The basic unit of a muscle is the muscle cell or myofiber, and whole muscles are made up of bundles of myofibers. A single muscle fiber has a very regular structure, and is composed of contractile units called myofibrils. Each myofibril consists of an orderly repeated arrangement of the contractile proteins actin and myosin (sarcomere), which slide past each other in the presence of $Ca^{2+}$ and ATP.

A motor unit is a motor neuron and all the myofibers it innervates. The greater the number of motor neurons associated with a muscle, the finer the control over the muscle. Motor neurons release the neurotransmitter acetylcholine from their synaptic bulbs onto muscle cells. This junction between a nerve and a muscle is called the neuromuscular junction. The release of acetylcholine at the motor end plate depolarizes the muscle tissue and in turn leads to the release of intracellular calcium from the sarcoplasmic reticulum, a variant of smooth endoplasmic reticulum. This release of intracellular calcium sets in motion the biochemical events that allow actin and myosin to interact and slide past each other, a process ultimately driven by ATP hydrolysis.

Skeletal muscle is similar to nerve tissue in that it responds to a stimulus in an all-or-none fashion. This response is called a twitch. Depending on the intensity and frequency of stimulation, greater numbers of myofibers are activated and contract. By increasing the number of active muscle fibers, the muscle is able to increase the force it generates. Muscle contractions are graded in size by wave summation, which depends on stimulus frequency, or by recruitment of more motor units. Thus, muscles with large cross-sectional areas are able to generate larger forces than those with small cross-sectional areas.

In this experiment, you will examine the basic principles of skeletal muscle physiology, including the all-or-none response, the effect of stimulus intensity and frequency on contraction force, the relationship between muscle length and generated tension, and the phenomenon of muscle fatigue. These experiments illustrate the collective understanding

of muscle physiology gained from over 400 years of research (adapted from AD Instruments Lab Protocols, for complete document see http://www.powerlab-teaching.com/experiments/TE04a_Frog_Muscle.html).

## Experimental apparatus and tissue preparation :

**Dissection**.

1. Your instructor will give you a double-pithed 3-4" Northern Grass Frog (*Rana pipiens*) or bullfrog (*Rana catsebiana*). Cut the skin all the way around the abdomen, and peel away the skin over the legs by pulling down with a pair of tissue-gripping forceps.

2. With a blunt probe and a glass hook, separate the quadriceps muscles from the hamstrings so that you can see the femur.

3. Cut the hamstrings proximally, close to the hip joint. Do the same to the quadriceps.

4. Cut the quadriceps and hamstrings distally, above the knee joint. A large segment of femur should be exposed.

5. Identify the gastrocnemius and **measure its resting length**.

6. Using a glass hook separate the gastrocnemius muscle from other muscles of the calf, and cut through the Achilles tendon above the ankle **leaving most of the tendon attached to the muscle**.

7. Cut the tibiofibula below the knee, along with the other calf muscles. You should be left with the gastrocnemius and the Achilles tendon at the distal end, and the previously exposed femur.

8. Using bone scissors cut the femur proximally, close to the hip joint.

9. Place the muscle in a bath of room temperature Ringer's solution at pH 7.2-7.4.

The muscle will contract in vitro when stimulated by an adequate electrical stimulus, just as it would in vivo when a motor unit is stimulated by a motor neuron.

**Transducer**. The device on your ring stand is a semi-isometric strain gage transducer (UFI Inc. Model 1030) which converts the mechanical tension produced by muscle contraction into an electronic signal that the recording system can detect. Other kinds of transducers exist for pressure, temperature, fluid flow, sound, and light – each of these forms of energy can be converted by a transducer to an electrical signal.

**Bridge amplifier and PowerLab.** The PowerLab units contain 4 banks of analog-to-digital conversion circuits which will record electrical signals from our preparation. The bridge amplifier is a Wheatstone bridge circuit which collects data from the strain gauge

transducer and provides a signal to PowerLab proportional to tension produced by contracting muscle. In some of the experimental stations the transducer may be connected directly to the PowerLab unit (the amplification is built into the PowerLab unit).

The PowerLab unit will also act as a physiological stimulator. Using Chart 5.0 software you will be able to set the intensity (voltage), duration (time in msec), and frequency (number of stimulation pulses/sec) of muscle stimulation.

**PC**. At your table is a desktop computer that can be used to record experimental data, make graphs, analyze experimental results, and work with text. You will record data using Chart 5.0 PowerLab software. This software simulates a pen-and-paper chart recorder.

# Getting Started:

**1. Starting up and working with PowerLab**.

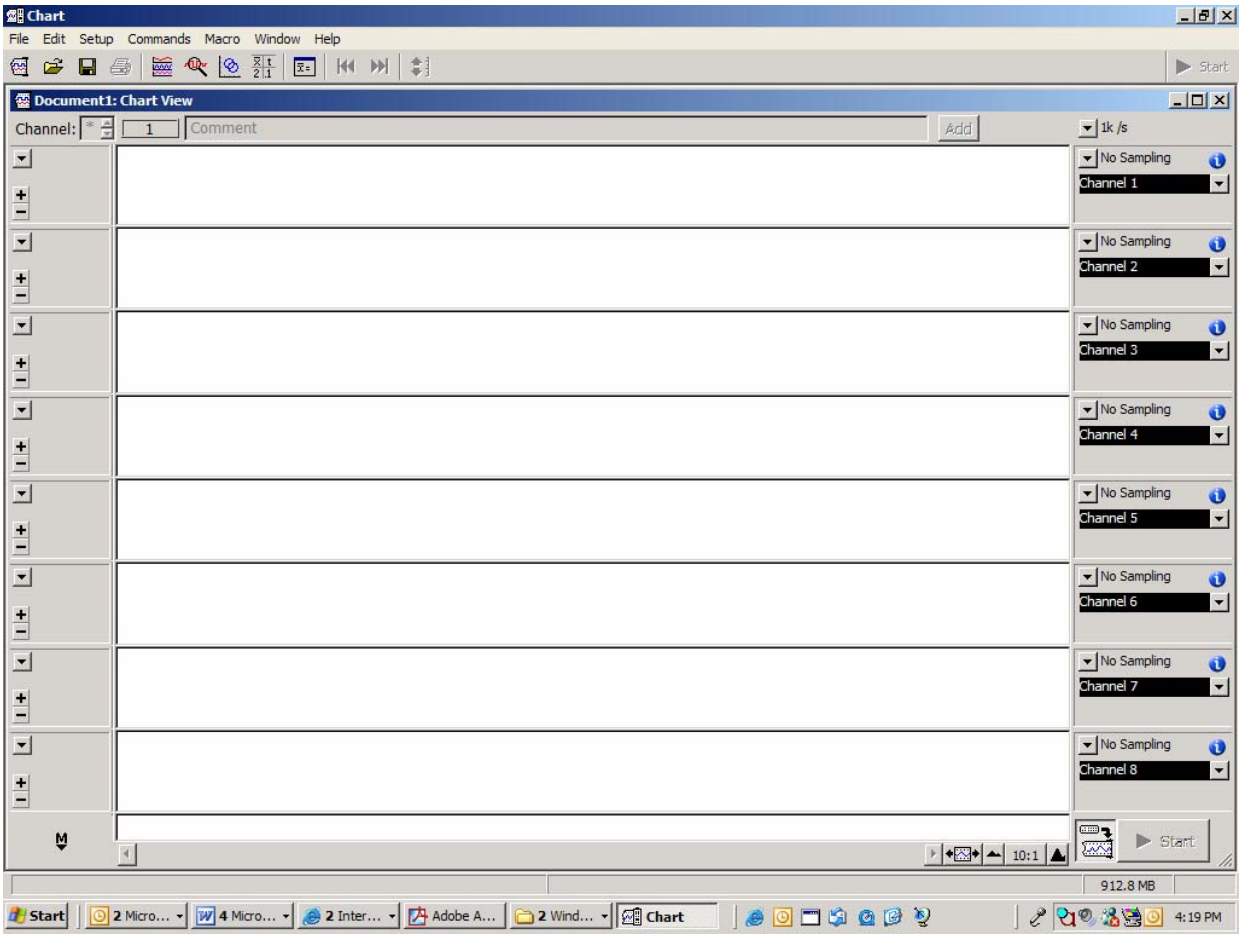
**a.** Make sure the PowerLab unit in your station is turned on – to turn it on use the power switch on the back of the unit.

**b.** Log onto the PC.

**c.** Launch Chart 5.0., an application that simulates a pen-and-paper chart recorder.

**d.** The screen should look similar to **Figure 1** – a number of channels will be visible (probably eight).

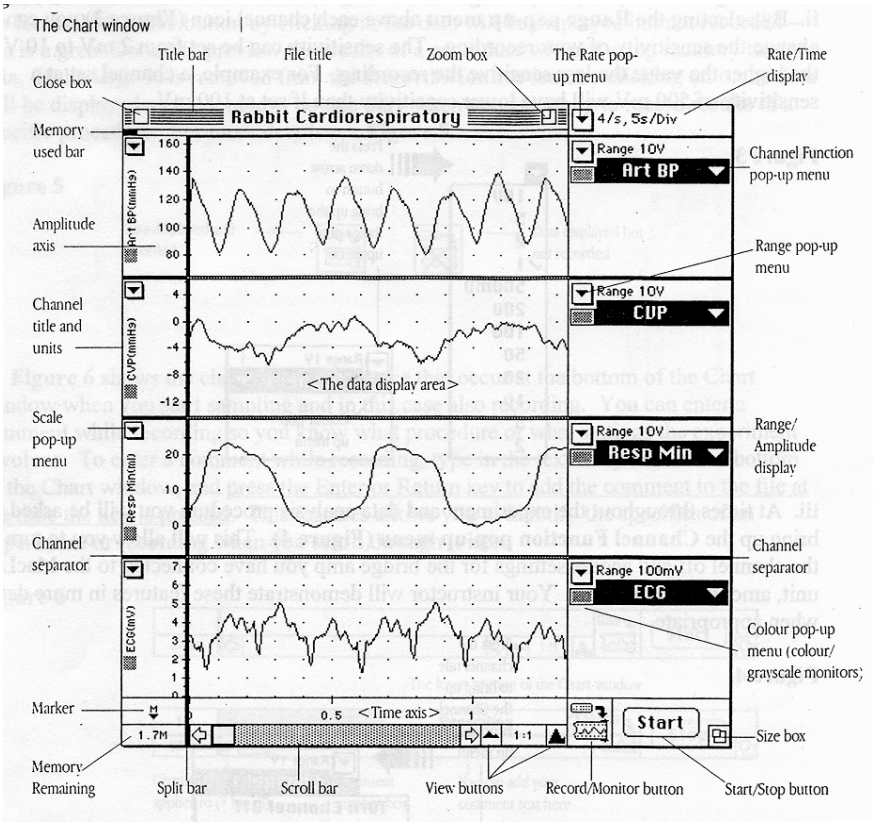
**Figure 1**




**e.** Go to the Setup menu, and select Channel Settings. In the lower right of the Channel settings dialog box select two active channels and press OK. The Chart window should reappear with only Channel 1 and Channel 2.

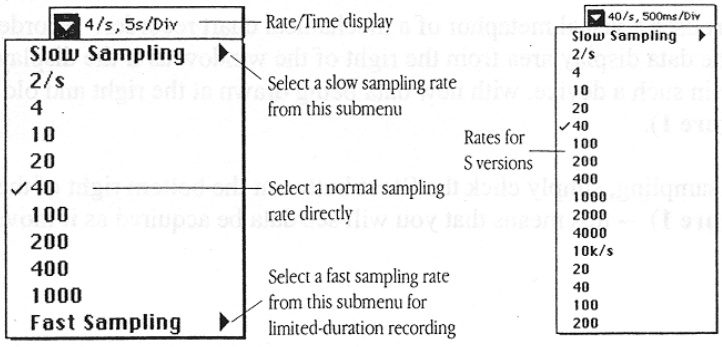
**f. Figure 2** below outlines a few features of the Chart window that allow you to collect and analyze data. We will use **Figure 2** to introduce you to a few key Chart commands, and you will gradually pick up other details as you work with the program.

**Figure 2**



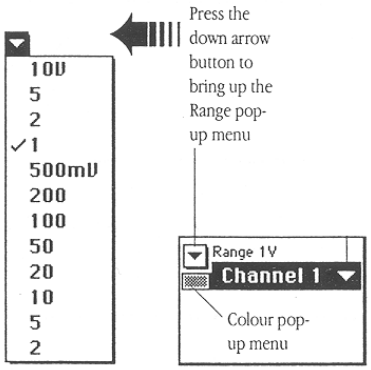

**i**. By selecting the **Rate pop-up menu** you can choose the sampling rate – the number of data samples that are stored per unit time, and the time taken by each sampling division to scroll past. For details see **Figure 3** below. By altering the value in this pop-up menu you are doing the same as controlling paper speed in an old-time pen-and-paper recorder.

**Figure 3**



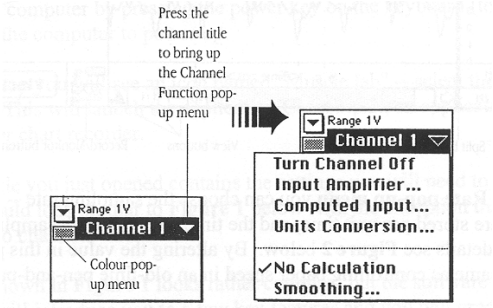
**ii.** By selecting the **Range pop-up menu** above each channel icon (**Figure 4**) you can change the sensitivity of your recording. The sensitivity can be set from 2 mV to 10 V – **the higher the value the less sensitive the recording**. For example, a channel set at a sensitivity of 500 mV will have lower sensitivity than if set at 100 mV.

**Figure 4**




**iii.** At times throughout the experiment and data analysis procedure you will be asked to bring up the **Channel Function pop-up menu** (**Figure 5**). This will allow you to turn the channel on and off and access settings for the bridge amp you have connected to the PowerLab unit, among other features. Your instructor will demonstrate these features in more detail when appropriate.

**Figure 5**




**iv.** Chart uses a visual metaphor of a mechanical chart recorder: recorded data scroll across the data display area from the right of the window as if the display area were a roll of paper in such a device, with new data being drawn at the right and old data moving left (see **Figure 2**).

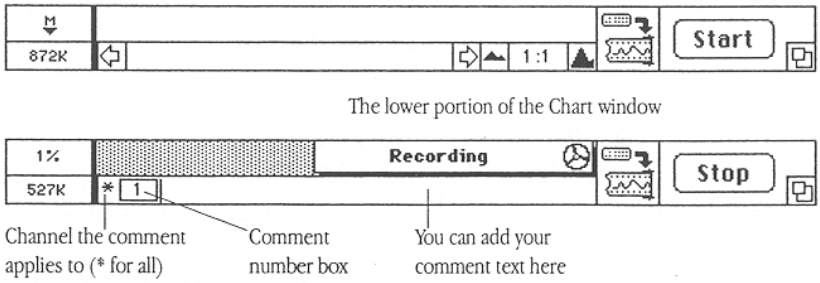
To start sampling, simply click the **Start** button at the bottom right of the Chart window (**see Figure 2**). – this means that you will see data be acquired as it moves across the screen. Whether the data is recorded or not depends on the status of the **Record/Monitor** icon to the left of the **Start button** (**Figure 2** and **Figure 6**). If you place a large red **X** over the **Record/Monitor** button by clicking it, the data will be displayed but not recorded – this is a great feature to preview the results of a procedure without using memory to store data. If the large **X** is removed from the **Record/Monitor** icon by

clicking on it, the data will be displayed and recorded – do this only when you are ready to record results of a specific procedure. For more details see **Figure 6**.

**Figure 6**


**v. Figure 7** shows the changes in appearance that occur at the bottom of the Chart window when you start sampling and in this case also recording. You can enter a comment while recording so you know what procedure or what settings the experiment involves (in some versions of Chart you enter comments on top of the Chart window). To enter a comment while recording, type in the text entry area at the top of the Chart window, and **press the Enter or Return key to add the comment to the file at the time the key is pressed**. This feature allows you to identify the specifics of an experiment or recording when you scroll through it later.

**Figure 7**

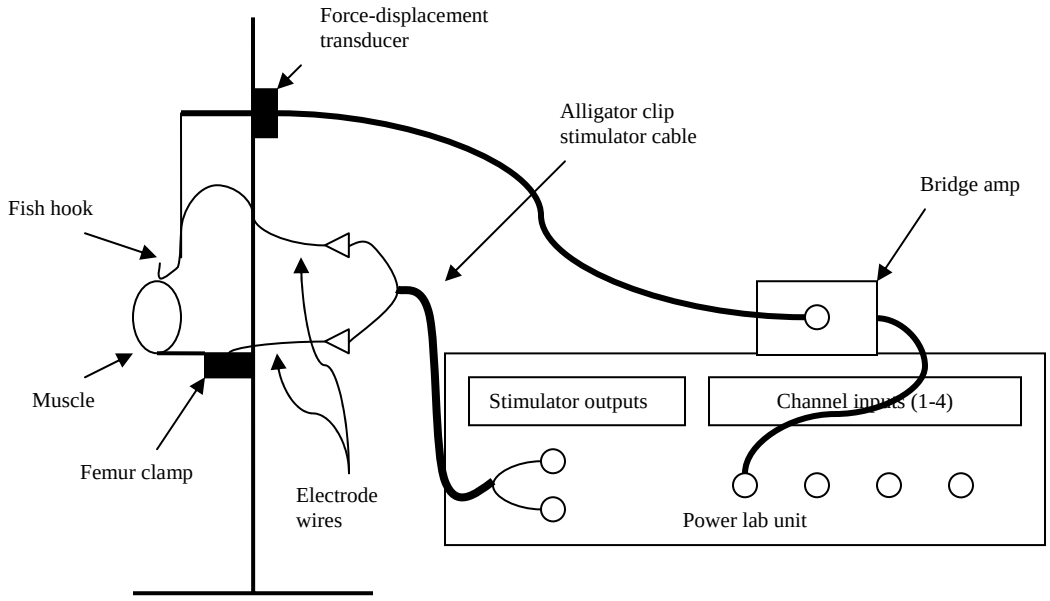



To look at several comments at once, locate a comment in a file, or delete or edit comments, choose Comments from the Windows menu to bring up the Comments window. The comments are listed in a scrolling field in the window in the order that they appear left to right across a file, with the comment numbers in boxes.

Try out some of the above features of CHART – don't hesitate to try anything! When you're done exploring, check with an instructor to see that your PowerLab is ready to record.

**2. Secure the muscle to the apparatus (Figure 8)**

**Figure 8: Experimental setup** (autographed at request)




**a.** Remove the muscle from its Ringer's bath. Attach the muscle to the femur clamp **with the bone attached parallel to the clamp and at right angles to the muscle**.

**b.** At the top end of the muscle, attach the preparation to the force-displacement transducer using the fishhook thrust through the Achilles' tendon and tied to the transducer lever. **Use two leaves of the transducer for best sensitivity and recording**. Stretch the muscle between the femur clamp and the transducer so that the muscle length is close to resting muscle length you measured prior to the dissection procedure.

**c.** Attach the alligator clip stimulator cable from the **Powerlab stimulator outputs** to the femur clamp electrode wire and to the fishhook electrode wire. Polarity (which one is + or -) doesn't matter. The ground alligator clip (green) can be left unconnected, or you can clip it to the ringstand.

**d.** Once you've got the muscle in place move the clamps apart so as to exert a slight tension on the resting muscle.

**e. Liberally douse the muscle with Ringer's saline solution now and throughout the procedure. Place a beaker underneath to catch the excess. It is extremely important that you keep the muscle wet throughout the procedure.**

**f.** In some units the transducer's output cable plugs into the bridge amplifier which in turn plugs into PowerLab Channel 1 by a BNC shielded cable. In other units the transducer's output cable plugs directly into PowerLab Channel 1.

**2. Calibration**.

**Sampling Rate:** 400/sec
**View compression:** 10:1
**Channel settings:** Two channels displayed

**Channel 1:** Bridge amp
Range: 5 -10 mV
Low Pass: none or 300Hz

During the experiments, **after the muscle is set in place**, and between individual procedures, you will calibrate the transducer/PowerLab apparatus by placing a weight (10 or 20 grams) on the end of the transducer lever and recording the displacement caused by that weight. Record at a moderate speed of 400/sec, use **Rate/Time pop-up menu** to set this. You will use this calibration to compute the tension developed in your muscle under various conditions.

Repeat the calibration procedure at frequent intervals during your work, and record the new calibration on the PowerLab record as a comment or in your notebook for reference.

During experimentation, adjust sensitivity (use **Range pop-up menu**) as needed (except during an experimental procedure) to yield a good-sized contraction record.

Always record the settings (especially sensitivity) in your written notes and/or as a comment in CHART.

Liberally douse the muscle with Ringer's saline solution now and throughout the procedure. Place a beaker underneath to catch the excess.

## Experimental procedures :

**1. Threshold/maximal stimulus determination and recruitment of fibers**. For the first experiment, we will find the lowest stimulus voltage needed to start a muscle action potential and cause contraction.

**a.** General settings:

**Sampling Rate:** 400/sec
**View compression:** 10:1
**Channel settings:** Two channels displayed

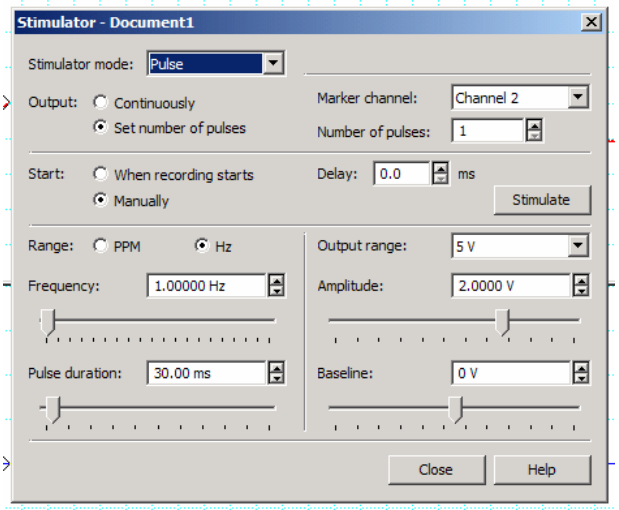
**Channel 1:** Bridge amp
Range: 5 -10 mV
Low Pass: none or 300Hz

**Channel 2:** will display a marking every time a stimulus is applied

**b.** Stimulator settings:

Make sure that the stimulator cable is properly attached to the muscle prep. In Chart choose **Setup: Stimulator** and input the settings shown in **Figure 9** below into the stimulator dialog box.

**Figure 9: Stimulator settings for threshold/maximal stimulus determination and recruitment of fibers.**




**Mode:** Pulse
**Output:** Set number of pulses
**Marker channel:** Channel 2
**Number of pulses:** 1
**Start:** Manually
**Delay:** 0 ms
**Range:** check Hz
**Frequency:** 1.0 Hz
**Output range:** 5V
**Amplitude:** start at 100 mV
**Pulse duration:** 30 msec
**Baseline:** 0 V

Close the stimulator dialog box. From the Setup menu select Stimulator panel. This will open an abbreviated stimulator panel that you can use to stimulate the muscle as you record data. You can also change stimulus properties such as amplitude and frequency.

**c.** With Chart recording, stimulate the muscle with a single pulse at each given stimulus intensity.

**d. Note in the Comments line the voltage applied for each stimulus – press Enter after typing each comment.**

**e.** Gradually increase the stimulus intensity in 50 mV increments, giving a single pulse at each voltage – keep increasing the voltage until you get a response. That stimulus intensity is the threshold for the most sensitive fibers in your muscle.

**f.** Continue increasing the stimulus intensity in 50 mV increments until a maximal contraction is obtained.

**g. Save your data** by click-dragging from the File menu down to SAVE or SAVE AS. Save to the M-drive.

**2. The muscle twitch**. The individual muscle contractions seen with a single stimulation are called twitch contractions. A maximal twitch involves all the muscle's fibers contracting at once and was observed above at the point when further increases in stimulus voltage didn't cause any further increase in tension.

During this procedure you will record a single maximal twitch at very high speed in Channel 1, also recording in Channel 2 the exact time when you applied the stimulus.

**a.** General settings:

**Sampling Rate:** 20k/sec
**View compression:** 10:1
**Channel settings:** Two channels displayed

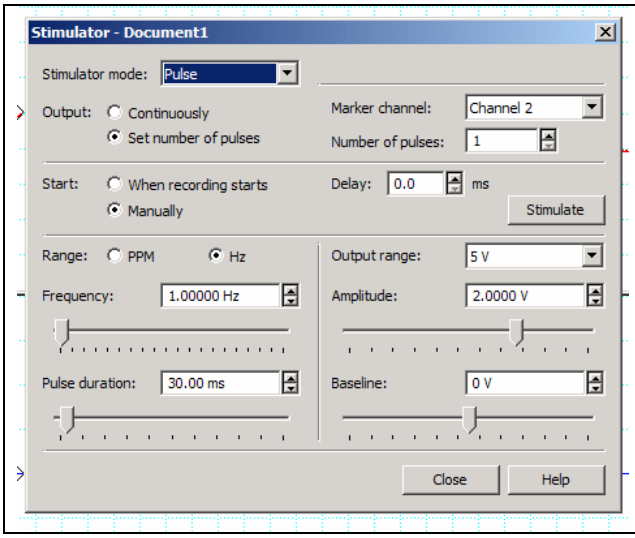
**Channel 1:** Bridge amp
Range: 5 -10 mV
Low Pass: none or 300Hz

**Channel 2:** will display a marking every time a stimulus is applied

**b.** Stimulator settings:

In Chart choose **Setup: Stimulator** and input the settings shown in **Figure 10** below into the stimulator dialog box. Settings are the same as before, with the exception that you will use a supramaximal stimulus intensity, in the example below it was 2 V.

**Figure 10: Stimulator settings for high speed muscle twitch recording**




**Mode:** Pulse
**Output:** Set number of pulses
**Marker channel:** Channel 2
**Number of pulses:** 1
**Start:** Manually
**Delay:** 0 ms
**Range:** check Hz
**Frequency:** 1.0 Hz
**Output range:** 5V
**Amplitude:** supramaximal stimulus
**Pulse duration:** 30 msec
**Baseline:** 0 V

**c.** Start Chart and record two muscle twitches (not very close together).

**d.** Don't forget to bathe the muscle periodically with Ringer's saline solution to rinse away waste products and keep the prep moist.

**e.** Save your data again by choosing SAVE from the File Menu.

**3. Twitch fusion and tetany**.

**a.** General settings:

**Sampling Rate:** 400/sec
**View compression:** 10:1
**Channel settings:** Two channels displayed

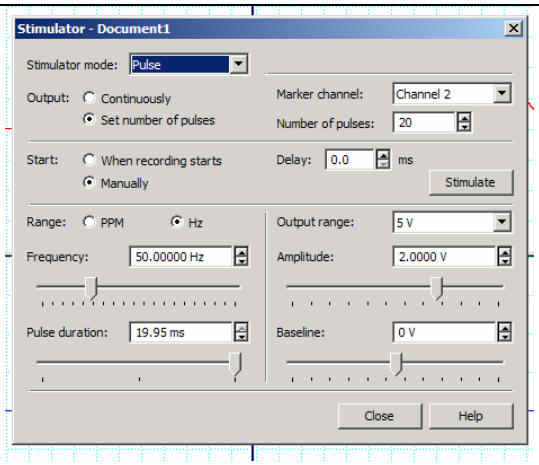
**Channel 1:** Bridge amp
Range: 5 -10 mV
Low Pass: none or 300Hz

**Channel 2:** will display a marking every time a stimulus is applied

**b.** Stimulator settings:

In Chart choose **Setup: Stimulator** and input the settings shown in **Figure 11** below into the stimulator dialog box.

**Figure 11: Initial stimulator settings for twitch fusion and tetany (note in text below that a number of parameters will be changed as frequency is increased)**




**Mode:** Pulse
**Output:** Set number of pulses
**Marker channel:** Channel 2
**Number of pulses:** 5 – 20 (see below)
**Start:** Manually
**Delay:** 0 ms
**Range:** check Hz
**Frequency:** 1.0 Hz – 50 Hz (see below)
**Output range:** 5V
**Amplitude:** supramaximal stimulus
**Pulse duration:** 30 msec
**Baseline:** 0 V

**c.** Calibrate your prep as described above (threshold/recruitment section).

**d.** Use a supramaximal stimulus amplitude (2 V in example above) that will produce a maximal contraction.

**e.** Stimulation at 1/sec. (change the highlighted fields)

**Mode:** Pulse
**Output:** Set number of pulses
**Marker channel:** Channel 2
**Number of pulses:** 5
**Start:** Manually
**Delay:** 0 ms
**Range:** check Hz
**Frequency:** 1.0 Hz
**Output range:** 5V
**Amplitude:** supramaximal stimulus
**Pulse duration:** 30 msec
**Baseline:** 0 V

Start Chart, stimulate the muscle using the Stimulator panel dialog box, make an appropriate comment indicating stimulus frequency, stop recording, and reset stimulator for higher frequency stimulation as outlined below.

**f.** Stimulation at 2/sec: change the stimulator settings shown below and repeat part **e.** above.

**Number of pulses:** 10 **
**Frequency:** 2.0 Hz **

**g.** Stimulation at 3/sec: change the stimulator settings shown below and repeat part **e.** above.

**Number of pulses:** 10
**Frequency:** 3.0 Hz **

**h.** Stimulation at 5/sec: change the stimulator settings shown below and repeat part **e.** above.

**Number of pulses:** 10
**Frequency:** 5.0 Hz **

**i.** Stimulation at 7/sec: change the stimulator settings shown below and repeat part **e.** above.

**Number of pulses:** 10
**Frequency:** 7.0 Hz **

**j.** Stimulation at 10/sec: change the stimulator settings shown below and repeat part **e.** above.

**Number of pulses:** 20 **
**Frequency:** 10.0 Hz **

**k.** Stimulation at 15/sec: change the stimulator settings shown below and repeat part **e.** above.

**Number of pulses:** 20
**Frequency:** 15.0 Hz **

**l.** Stimulation at 20/sec: change the stimulator settings shown below and repeat part **e.** above.

**Number of pulses:** 20
**Frequency:** 20.0 Hz **

The muscle twitches you observe will fuse together, and at high frequency stimulation will reach a condition of tetanic, or constant contraction. If the recording goes off scale you'll have to reduce the sensitivity, recalibrate, and repeat the experiment for all stimulation frequencies.

**m.** Save your data again by choosing SAVE from the File Menu.

**4. Length-tension curve**. You will examine the effects of changing the initial length of the muscle on the tension generated by stimulation with a maximal stimulus.

**a.** General settings:

**Sampling Rate:** 400/sec
**View compression:** 10:1
**Channel settings:** Two channels displayed

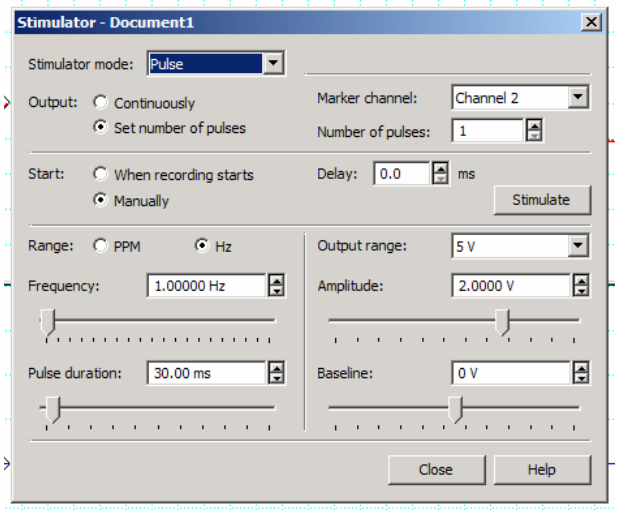
**Channel 1:** Bridge amp
Range: 5 -10 mV
Low Pass: none or 300Hz

**Channel 2:** will display a marking every time a stimulus is applied

**b.** Stimulator settings:

In Chart choose **Setup: Stimulator** and input the settings shown in **Figure 12** below into the stimulator dialog box.

**Figure 12: Stimulator settings for length-tension curve experiments.**




**Mode:** Pulse
**Output:** Set number of pulses
**Marker channel:** Channel 2
**Number of pulses:** 1
**Start:** Manually
**Delay:** 0 ms
**Range:** check Hz
**Frequency:** 1.0 Hz
**Output range:** 5V
**Amplitude:** supramaximal stimulus
**Pulse duration:** 30 msec
**Baseline:** 0 V

**c.** Calibrate your prep as described above (threshold/recruitment section).

**d.** Move the transducer up or down and measure the muscle length with a ruler or calipers.

**e.** At each length stimulate the muscle with a single pulse using a maximal stimulus to elicit the maximum muscle tension. Use only a short range of lengths in your experiment at most ± **0.1 – 1.0 cm** total. Collect 5-6 data points if possible. For each contraction, save the length value in a comment**.**

***Make sure you recalibrate every time you change muscle length.***

**f.** Save your data again by choosing SAVE from the File Menu.

**5. Fatigue**. When you have completed all the above experiments [not before!], look at the effects of a continuous, high frequency stimulation on muscle tension.

**a.** General settings:

**Sampling Rate:** 200/sec****
**View compression:** 20:1****
**Channel settings:** Two channels displayed

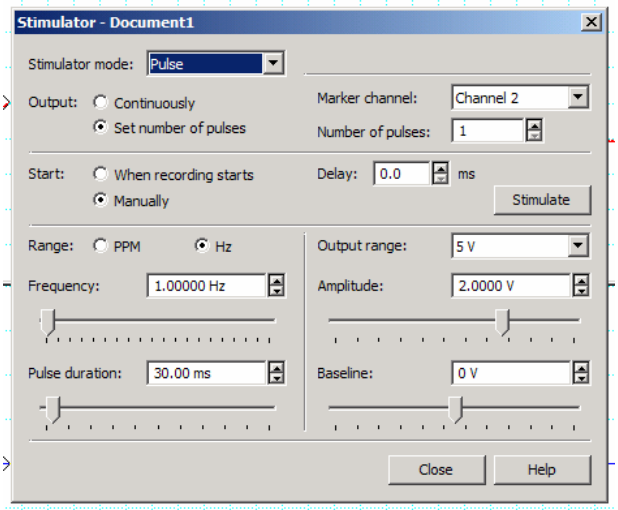
**Channel 1:** Bridge amp
Range: 5 -10 mV
Low Pass: none or 300Hz

**Channel 2:** will display a marking every time a stimulus is applied

**b.** Stimulator settings:

In Chart choose **Setup: Stimulator** and input the settings shown in **Figure 13** below into the stimulator dialog box.

**Figure 13: Stimulator settings for muscle fatigue experiment.**




**Mode:** Pulse
**Output:** Continously
**Marker channel:** Channel 2
**Number of pulses:** 1
**Start:** Manually
**Delay:** 0 ms
**Range:** check Hz
**Frequency:** 25.0 Hz
**Output range:** 5V
**Amplitude:** supramaximal stimulus
**Pulse duration:** 30 msec
**Baseline:** 0 V

**c.** Start Chart and begin stimulation using the stimulator panel dialog box. Watch the development of fatigue in the muscle. Apply several drops of Ringer's after tension has declined significantly. Does tension increase after a Ringer's wash?

**d.** Save your data again by choosing SAVE from the File Menu.

## Clean up

You have completed your experimental procedures. Before leaving do the following:

**1.** Remove the muscle from the apparatus and dispose of it in an animal waste bag.

**2.** Clean all instruments and equipment.

**3.** Close the Chart program, logoff the PC, and turn off the power in the back of the PowerLab unit.

**4.** Start data analysis.

## **Analysis** :

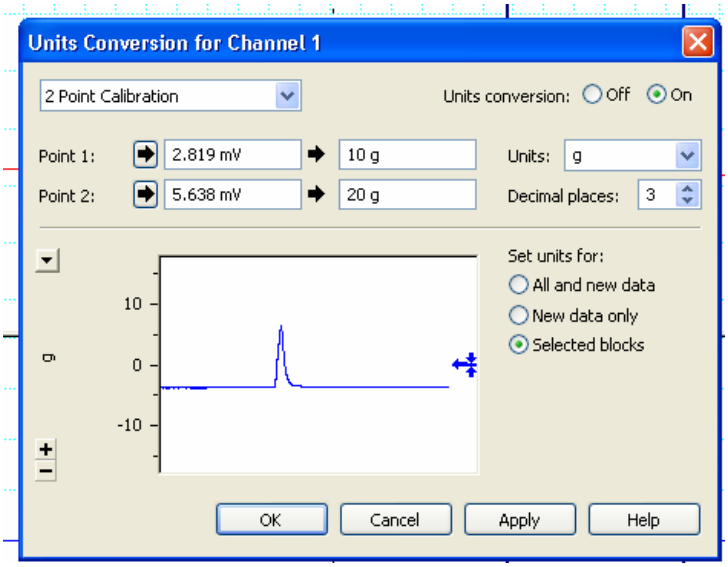
Before starting analysis covert your data to grams by using calibration waves you collected along the procedure and the Units Conversion feature of Chart 5.0.

**a.** Go to the calibration wave of interest, produced by a 10 g weight, for example. Highlight the wave and Zoom on it. Place the marker on the baseline and determine the displacement produced by the 10 g weight (2.819 mV, for example).

**b.** Highlight the segment of data that you want to convert to grams using these parameters – make sure to include the calibration wave of interest.

**c.** Select the Channel 1 pop-up menu and select units conversion. Enter the appropriate data into the Units conversion dialog box, as shown in **Figure 14** below – I used the example numbers mentioned above.

**Figure 14: Units conversion dialog box**




Make sure you check the Selected blocks option. Select Apply and OK.

**d.** Zoom back into the calibration wave, place the Marker on the baseline, and verify that the wave displacement corresponds to the correct weight you used for calibration (in the above example, 10 g).

**e.** You will repeat this procedure for any data segment that a different calibration wave applies to.

**1. Threshold and recruitment of fibers**.

Examine your data recording from Part 1.

**a.** Place the **marker (M)** on the baseline of the first visible muscle twitch in Channel 1. You may want to use the Zoom feature.

**b.** Using the mouse, place the **waveform cursor** at the top of the contraction peak. Record the stimulus intensity and corresponding contraction amplitude in **Table 1** of the data notebook.

**c.** Repeat this for every twitch recording in Part 1.

**d.** From your data, determine the minimum voltage required to elicit a maximal contraction. This is the maximum excitation voltage, a maximal stimulus. Determine your value for a supramaximal stimulus by multiplying this voltage by 1.5. Record these values in **Table 2** of the data notebook

**2. The muscle twitch**.

**a.** Go to a recording of a high speed twitch from Part 2. Highlight both channels so that you include the full twitch. Zoom into this window and using the **marker (M**) and the waveform cursor, measure the duration of the latent period, contraction period, and relaxation period. Record these values in Table 3.

**b.** Either copy the zoom window and paste onto a Word document. Identify the latent period, contraction period, relaxation period.

**3. Twitch fusion and tetany**.

**a.** Examine your data from Part 3. There will be eight blocks of recorded data. Calibrate appropriately.

**b.** For each data block, determine the maximum contraction force using the marker and waveform cursor.

**c.** Record your results in Table 4 of your data notebook.

**4. Length-tension curve**.

**a.** Examine your data from Part 4. Calibrate appropriately.

**b.** For each initial muscle length, measure force produced by muscle contraction – use **marker (M)** and waveform cursor. Don't forget to recalibrate after each trial.

**c.** Record your data in Table 5

**5. Muscle fatigue**

a. Examine your data from Part 5. Calibrate appropriately.

b. Place the **marker (M)** on the waveform immediately prior to stimulation.

c. Use the waveform cursor to determine maximum contraction force. Record this value and the time of maximal stimulation in Table 6 of your data notebook.

d. Determine the contraction force at t = 15, 30, 60, 90, 120, 150, and 180 seconds after stimulation.

f. Record your results in Table 6 of your data notebook.