

# Lab 6: Chick Embryo Skeletal Muscle

## Overview

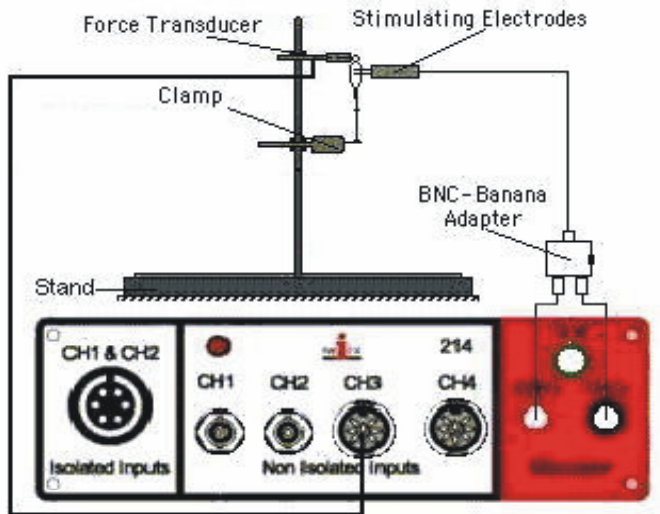
About 40% of the total body mass of a human is skeletal muscle. Skeletal muscle is intimately associated with the skeletal system and, combined, these muscles and bones are responsible for supporting and moving the body. While skeletal muscle fibers have sarcomeres and the same banded appearance, different muscles can function in different ways. For example, some are relatively weak and fatigue resistant, while others are strong but fatigue quickly. These features may be explained in terms of the biochemical properties of muscles.

The muscle fibers found in most mammalian skeletal muscles are either fast or slow twitch-types. Each type has a different myosin isoform, with different rates of ATPase activity and cross-bridge binding. Within the group of fast-twitch fibers, there are fibers that use glycolysis and oxidative phosphorylation. There are also fast twitch fibers that just use glycolysis; this group is less reliant upon oxygen and is much stronger than the fibers using phosphorylation. However, these stronger “glycolytic” fibers breakdown glucose very inefficiently; so, that a burst of contractile activity diminishes glucose levels, causes lactic acid to accumulate, and leads to fatigue.

Most skeletal muscles are composed of some combination of the different twitch-type fibers. Interestingly, a motorneuron makes only one synapse on each of their target fibers, and the muscle fibers innervated by a motorneuron are all of the same type. Therefore, stimulation of a particular motorneuron will create a contraction of only one type of muscle fibers; this property is used by the brain to recruit different muscle fibers into a contraction. Activity in descending tracts excites the spinal motorneurons; but, the size of the cell bodies and the activation thresholds of these neurons are different. Motorneurons that supply weak, slow, oxidative fibers have the lowest threshold; those innervating fast, intermediate-strength oxidative fibers have higher thresholds; and those that supply the fast, strong, glycolytic fibers have the highest thresholds. In this way increasing the amount of activity descending from the brain activates progressively more motorneurons, and more of the stronger muscle fibers, into the response. This will be simulated in the following experiment by slowly increasing the voltage applied directly to the muscle to recruit more muscle fibers into the contraction. In addition, the amount of contraction is dependent upon stimulus frequency.

## Equipment Required

Computer  
iWorx/214 data acquisition unit and USB cable  
FT-100 force transducer  
Stimulating electrodes and BNC-Banana adapter  
Ring stand and clamps  
Thread  
6" Ruler  
Ringer solution (bubbled with air before experiments)



*Figure 4-9: The equipment used to evoke and record contractions crayfish muscle using the iWorx/214.*

### Equipment Setup

- 1 Connect the iWorx/214 to the computer (described in Chapter 1).
- 2 Plug the DIN connector on the cable of the FT-100 Force Transducer into Channel 3 of the iWorx/214 unit (Figure 4-9 on page 73).
- 3 Plug the BNC-double banana adapter into the positive (red) and negative (black) sockets of the iWorx 214 stimulator. Check the side of the double banana adapter for a tab, often embossed with the letters GND. This is the side of adapter that goes into the negative (black) socket of the stimulator.
- 4 Attach the BNC connector of the stimulator cable to the adapter on the iWorx 214 stimulator.
- 5 Arrange the clamps on the ring stand so the clamp holding the transducer is on top, the clamp holding the stimulating electrodes is in the middle, and the clamp with the tread is on the bottom.

### Start the Software

- 1 Click the Windows **Start** menu, move the cursor to **Programs** and then to the **iWorx** folder and select **LabScribe**; or click on the LabScribe icon on the Desktop.
- 2 When the program opens, select **Load Group** from the **Settings** menu.
- 3 When the dialog box appears, select **ahk214.iws** and then click **Load**.

4 Click on the **Settings** menu again and select the **Muscle-Sum-Tetanus-214** settings file.

5 After a short time, LabScribe will appear on the computer screen as configured by the **Muscle-Sum-Tetanus-214** settings.

6 The **Muscle-Sum-Tetanus-214** settings file adjusts:

- the stimulus amplitude to **0.00V**, with adjustable increments of **0.10V**.
  - The stimulus delay to **50ms**, with increments of **1ms**.
  - the stimulus duration to **10.0ms**, with adjustable increments of **1.0ms**.
  - the sampling rate to **200** per second.
  - the frequency to **0.5Hz**.
  - the number of pulses to **1**.
  - the stimulus pulse (**Out1**) to be displayed on Channel 4 (**Stimulus**) by selecting the **Stimulator Dspl** function, in the **right-click** menu of the **Stimulus** channel.
- These settings can be changed by selecting **Preferences** from the **Edit** menu.

## The Dissection

1 Remove chick embryo from egg.

2 Remove the skin from the legs by making an incision through the skin around the entire lower abdomen. Cut the connections between the skin and the body—especially around the base of the pelvic girdle. Use stout forceps to pull the skin off the frog in one piece (like a pair of pants).

3 Place the chick embryo in a dissection tray with its dorsal side up.

**Note:** *Moisten the exposed limbs muscles with Ringer's solution every five minutes or so.*

4 Identify the biggest muscle on the **foreleg**.



5 Use a glass hook to separate the muscle from the bone and other muscles of the foreleg.

6 Use scissors to free the tendon from the connective tissue around the knee. Double up a 24" piece of thread. Firmly tie the doubled thread around the tendon,

leaving the ends of the thread long enough to attach the muscle to the displacement transducer.

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

**7** Cut the tendon as close to the knee as possible, so the thread is still attached to the muscle.

**8** Move the muscle away from the rest of the knee. Cut the tibia just below the knee to separate the rest of the lower leg from the preparation. Rinse the preparation with Ringer's solution to moisten the tissue and rinse away any blood.

**9** Dissect away the tissue of the pelvic girdle. Use a pair of scissors to cut the muscle as close to the pelvis as possible. Rinse the preparation with Ringer's solution to moisten the tissue and rinse away any blood.

**10** Firmly tie the doubled thread around the anterior portion of the muscle, leaving the ends of the thread long enough to attach the muscle to a fix clamp.

### **The Preparation**

**1** Use the clamps to mount the preparation on the ringstand

**2** Attach the thread on the tendon to the hole on the end of the blade of the force transducer.

**3** Attach the thread on the anterior end of the muscle to fix clamp. There should be no slack in the thread, but do not stretch the muscle past its *in situ* length.

**4** Position the stimulating electrodes so they lay against the muscle about midway between the knee and the tendon. The two electrodes should not touch one another.

### **Exercise 1: Stimulus-Response**

**Aim:** To make sure that all muscle fibers contract when stimulated.

### **Procedure**

**1** Check values listed the stimulator panel, which is below the LabScribe toolbar (Figure 4-12 on page 78). The stimulus amplitude should be **0.00V** and the pulse width should be **10ms**.



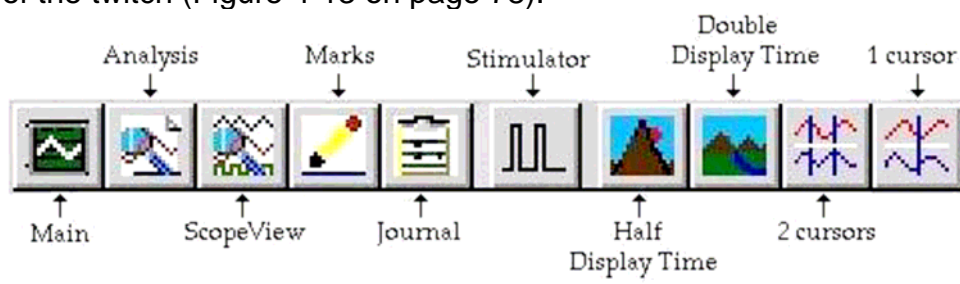
9 Moisten the muscle with frog Ringer's solution.

## Data Analysis

1 Scroll to the beginning of this section of data. Click **AutoScale** to maximize the size of the response on the window. Remember that at lower stimulus voltages, the amplitude of the muscle response may be zero.

2 Use the **Display Time (Half or Double)** icons in the LabScribe toolbar to adjust the **Main** window, so the twitch response spans about 50% of its width.

3 Click the **2-Cursor** icon (Figure 4-12 on page 77), so that two blue vertical lines appear over the **Muscle** channel on the **Main** window. Drag the cursors left and right so that one is on the baseline before the twitch and the other is on the peak of the twitch (Figure 4-13 on page 78).



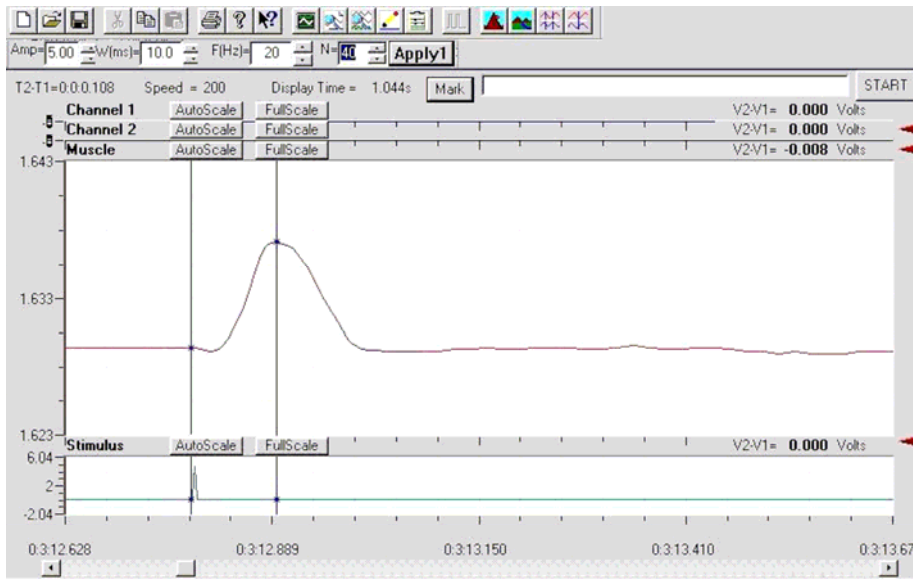
*Figure 4-12: The LabScribe toolbar*

4 The amplitude of the muscle twitch is displayed as the value for **V2-V1** in the upper right corner of the **Muscle** channel (CH3).

5 Data can be entered in the **Journal**, by clicking on the **Journal** icon in the LabScribe toolbar, and typing the amplitudes of the stimulus and the response in the **Journal** window.

6 Repeat these measurements for all the other responses.

7 Present your data in a table and a graph that relate the amplitude of the muscle response to the stimulus amplitude.



**Figure 4-13:** A recording of a muscle twitch (upper trace) and the stimulus pulse (lower trace). The cursors were placed on the baseline (left) and the peak of the twitch (right).

## Questions

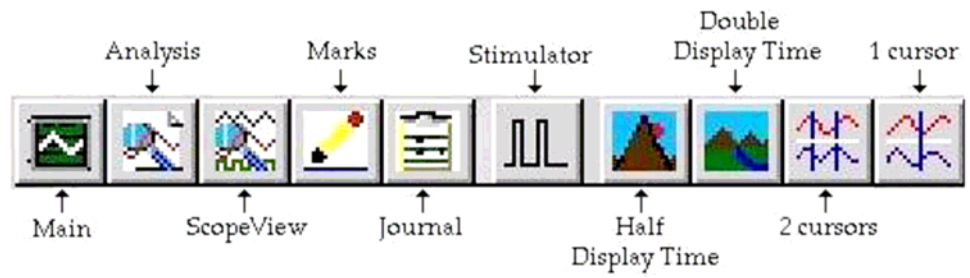
- 1 How does direct electrical stimulation produce contractions of the muscle?
- 2 Why doesn't the muscle respond to low stimulus voltages?
- 3 Why does the amplitude of the muscle response increase with increasing stimulus voltages?
- 4 At high stimulus voltages, the muscle response reaches a maximum amplitude. Why doesn't the muscle response continue to increase with increasing stimulus voltages?

## Exercise 2: Summation and Tetanus

**Aim:** To measure the amplitude of contraction produced in a muscle that is stimulated with repeated pulses delivered at progressively higher frequencies.

## Procedure

- 1 Check the values listed the stimulator panel, which is below the LabScribe toolbar (Figure 4-12 on page 77). Use the arrow buttons in the stimulator panel to change: the stimulus amplitude (**Amp**) to value that will create a maximum muscle response; the stimulus frequency (**F**) to **500mHz** (which is also 0.5Hz); and the number of pulse (**N**) to **15**. Click the **Apply1** button on the right of the stimulator panel to effect the change in the stimulus.



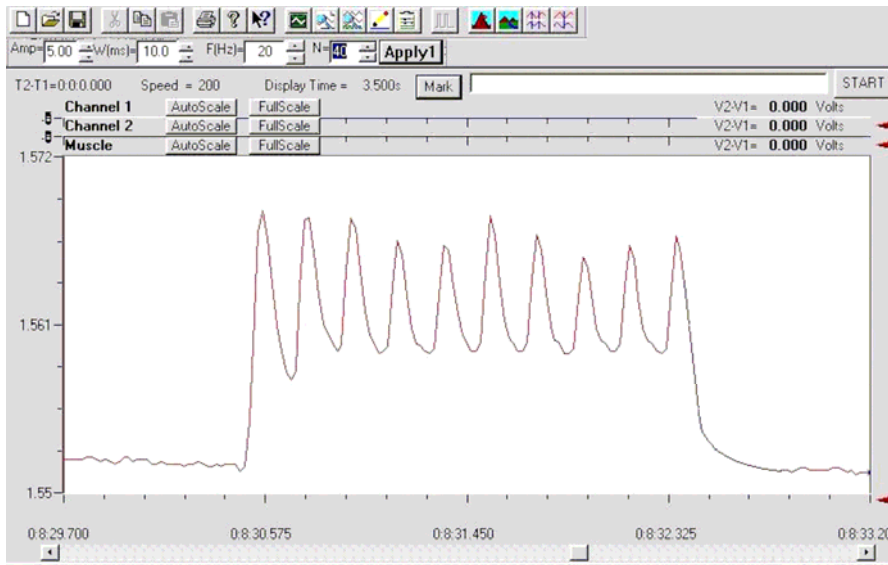
2 Click **Start**. Type " 0.5Hz" on the comment line to the right of the **Mark** button, and press the **Enter** key on the keyboard to mark your record. Record at this frequency for about 15 twitches. Click **Stop**.

3 Stimulate the muscle at higher frequencies. Use the arrow buttons in the stimulator panel to change increase the frequency to 1, 2, 3, 4, 5, 10, 20, and then 30Hz. Click the **Apply1** button on the right of the stimulator panel to effect the change in the stimulus. Type the value of each new frequency on the comment line. Record 15 twitches at each frequency. Annotate the muscle response for each new frequency.

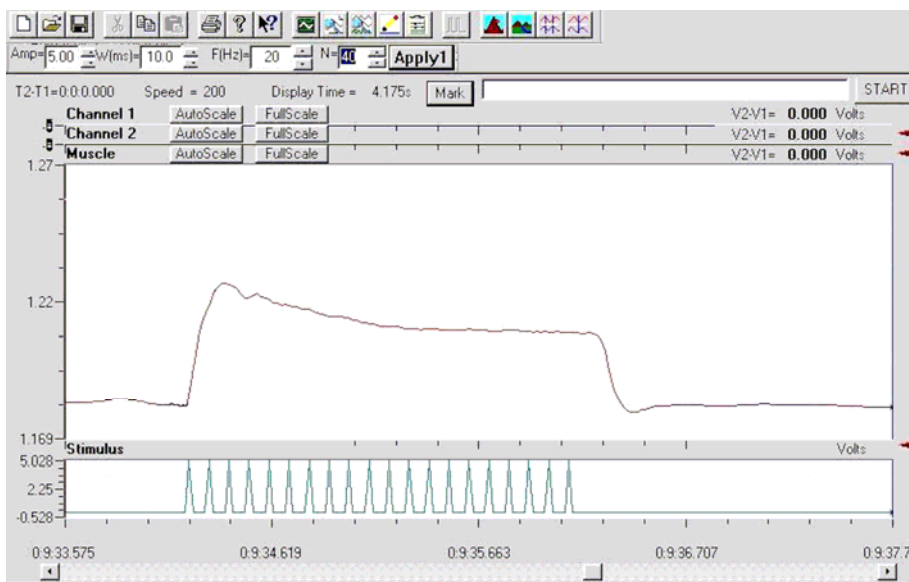
Frequency (Hz)	Contraction Amplitude (g)	Fuses Tetanus (Yes or No)
0.5		
1		
2		
3		
4		
5		
10		
20		
30		

4 Notice that at a certain frequency:

- The muscle does not have sufficient time to fully relax; the muscle response does not return to baseline (Figure 4-14 on page 79). This is mechanical summation.



**Figure 4-14:** A recording showing mechanical summation, where the muscle does not have time to return to “baseline” (resting length) between contractions. Notice that single twitches are not completely fused (unfused tetanus)



**Figure 4-15:** A recording showing muscle stimulation with a short burst of high frequency stimuli to produce complete tetanus. Notice that single twitches have fused completely (fused tetanus). Muscle fatigue is also evident in this example since the peak of the contraction at the beginning of the stimulation is higher than at the end of the pulse.

- The amount of tension produced by the muscle is greater than that seen during a single twitch (Figure 4-15 on page 79). This is tetanus.

5 Select **Save** in the **File** menu.

6 Moisten the muscle with Ringer's solution.

## Data Analysis

### Summation

1 Scroll to the data for the stimulus frequency where mechanical summation first appears.

2 Click the **2-Cursor** icon (Figure 4-12 on page 77), so that two blue vertical lines appear over the **Muscle** channel on the **Main** window. Drag the cursors left and right so that one is on the peak of a twitch and the other is on the peak of the adjacent twitch. The value for **T2-T1** is the period between twitches.

3 Calculate the frequency at which mechanical summation first appears. Remember that frequency is the inverse of the period: Frequency (Hz) = (1000 msec/sec) (msec/period)

### Tetanus

4 Scroll to the data for the stimulus frequency where complete tetanus first appears

5 Use the two cursors to measure the maximum amplitude of the complete persistence muscle contraction. Compare the amplitudes of this titanic contraction and a single twitch.

## Questions

1 If contraction amplitude is dependent upon the increases in concentration and persistence of intracellular calcium, why are the contraction amplitudes of single twitches the same?

2 Tetanus requires high stimulus frequencies. What does this tell you about calcium reuptake by the sarcoplasmic reticulum?

3 Why is the rate of muscle relaxation much slower after tetanus than after a single twitch?

4 At which frequency did you observe unfuse tetanus? fuse tetanus?

5 What differences you should expect to see if instead of directly stimulating the muscle you could stimulate the nerve innervating the muscle?

6 What are the most likely reasons for muscle fatigue?

