Muscle

In this experiment, you will explore how muscles work. You will also examine some of the properties of muscle fatigue. In this experiment, you will electrically stimulate the nerves in the forearm to demonstrate recruitment, summation, and tetanus.

Background

The skeleton provides support and articulation for the body. Bones act as support structures and joints function as pivot points. Skeletal, or striated, muscles are connected to the bones either directly or by tendons, strong bundles of collagen fibers. Two or more muscles usually work antagonistically. In this arrangement, a contraction of one muscle stretches, or elongates, the other. Skeletal muscle is composed of long, multinucleate cells called fibers. Motor nerves innervate these muscle fibers. An action potential in a motor axon produces an action potential in the muscle fibers it innervates. This muscle action potential allows for a brief increase in the intracellular concentration of calcium ions ($[Ca^{2+}]$), and activates the contractile molecular machinery inside the fiber. The result is a brief contraction called a twitch.

The firing of hundreds of motor axons controls a whole muscle. These motor nerves control movement in a variety of ways. One way the nervous system controls a muscle is by adjusting the *number* of motor axons firing, thus controlling the number of twitching muscle fibers. This process is called recruitment. A second way the nervous system controls a muscle contraction is to vary the *frequency* of action potentials in the motor axons. At stimulation frequencies of less than 5 Hz, intracellular $[Ca^{2+}]$ returns to normal levels between action potentials: the contraction consists of separate twitches. At stimulation frequencies between 5 and 15 Hz, $[Ca^{2+}]$ in the muscle has only partly recovered when the next action potential arrives. The muscle fiber produces a pulsing tension called a summation response with a force greater than that of a single twitch and that does not decay completely to zero between pulses. At even higher stimulation frequencies, the pulsing component becomes hard to discern and the muscle enters tetanus, a smooth contraction many times stronger than that in a single twitch.

In Exercise 1, you will observe muscular responses without recording them. In Exercises 2 and 3, you will use a force transducer to measure small forces generated by the adductor pollicis muscle. In the later exercises, the grip force exerted by the hand is recorded with a different transducer as you investigate the phenomenon of muscle fatigue.

Required Equipment

A computer system
Chart software
PowerLab
Finger pulse transducer
Bar stimulus electrode
Electrode cream
Hand dynamometer
Adhesive tape

Procedures

Warning

Some of these exercises involve application of electrical stimulation to muscle through electrodes placed on the skin. People who have cardiac pacemakers or who suffer from neurological or cardiac disorders should not volunteer for those exercises. If the volunteer feels major discomfort during the exercises, discontinue the exercise and consult your instructor.

Set up and equipment calibration

1. Make sure the PowerLab is connected and computer is turned on.

2. Connect the finger pulse transducer to the BNC socket on Input 1 of the PowerLab.

3. Place the finger pulse transducer diaphragm-side up on the top of the lab bench; tape the transducer in place along the Velcro strap (Figure 1).

4. Connect the bar stimulus electrode to the isolated stimulator output of the PowerLab. The leads are color-coded; plug the red lead into the red socket and the black lead into the black socket (Figure 1).

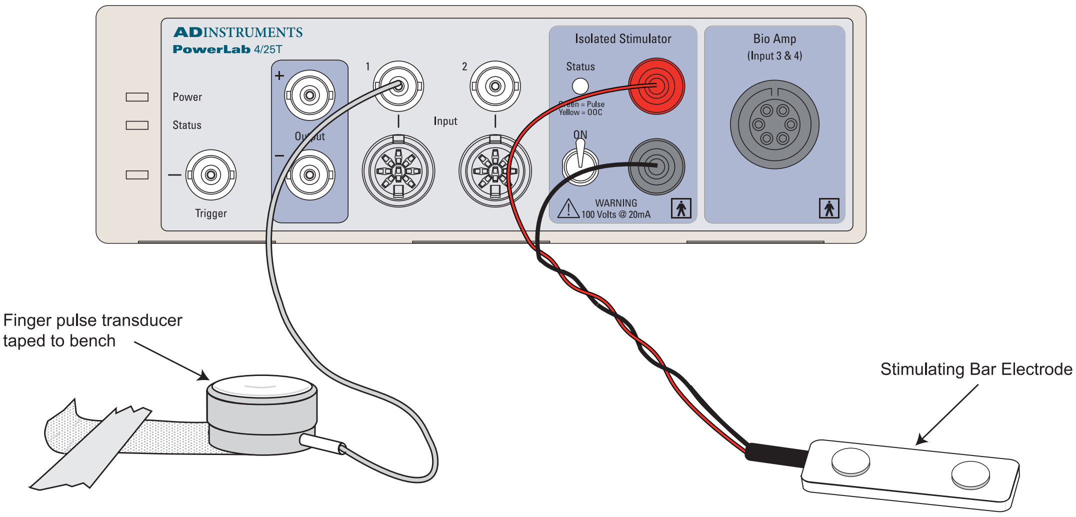

Figure 1

5. Place a small amount of electrode cream on the two silver contacts of the stimulating bar.

6. Turn on the PowerLab.

7. Locate Chart on the computer and launch the program. If the Experiments Gallery dialog window does not appear in front of the Chart window, choose the Experiments Gallery… command from the File menu.

8. In the Experiments Gallery dialog window, select **Muscle** from the left-hand list. Select the settings file "Nerve Effect Settings" in the right-hand list and click the **Open** button to apply those settings.

9. After a short time, the Chart window on the computer screen should be set up for the first exercise. Only one channel should be showing, Channel 1, and there should be a red cross through the Record/Monitor button next to the Start button at the bottom right of the Chart window.

Exercise 1: The effects of nerve stimulation

Objectives

To explore the motor and sensory effects of electrical stimuli on a volunteer, using the nerves of the forearm.

Procedure

In this first exercise, the PowerLab unit is used as a stimulator, instead of recorder, muscular responses will be observed by watching the hand of the volunteer. The finger pulse transducer is not used until Exercise 2. Chart is active only to control the Isolated Stimulator.

1. Choose the Isolated Stimulator command from Chart's Setup menu (Stimulator in Mac OS), and in the mini-window that appears, enter the following settings:
    - Frequency: 1 Hz
    - Pulse Duration: 200 µs
    - Amplitude: 10 mA
    - On button: highlighted

2. Turn the stimulator switch **OFF** on the front of the PowerLab(the switch should be pointing down). This disconnects the Isolated Stimulator terminals.

3. Click Start. Chart has been set up to monitor, not record. In this state, the Isolated Stimulator is active, but

no recording is made (the data display area is grayed out to indicate that any signal appearing there is temporary). Observe the Isolated Stimulator Status light on the PowerLab. It should flash yellow, once per second.

Safety Note: The yellow light on the Isolated Stimulator indicates that the stimulator cannot pass the stimulus current requested, a condition known as "out of compliance". This light flashes green when the stimulator switch is on, stimulus electrodes are correctly placed on the skin, and current is flowing properly.

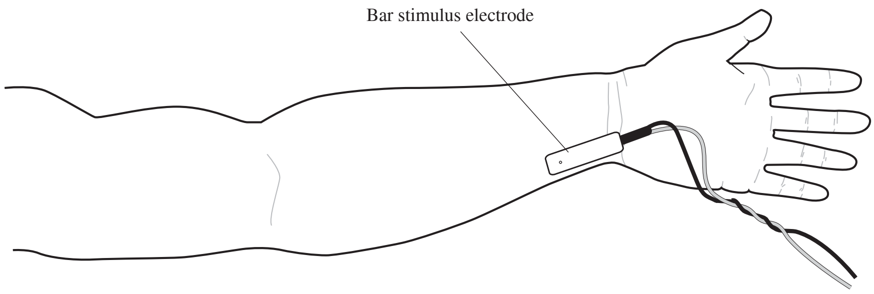

Figure 2.Placement of the bar stimulus electrode on the left wrist.

4. Place the bar stimulus electrode over the volunteer's ulnar nerve at the wrist (the approximate placing is shown in Figure 2). The bar stimulus electrode should be held in place along the axis of the arm, with the leads pointing towards the hand. The red dot on the back of the electrode indicates the anode (positive). The nerve impulse will be generated at the cathode end.

5. Set the stimulator switch on the PowerLab unit to the **ON** position (up). The stimulator status light should now flash green, indicating that the requested stimulus current is being passed through the subject's skin.

6. Note the twitch contractions affecting the thumb and fingers. Examine the effect of small adjustments to the placing of the electrodes, and locate the position giving the largest twitches.

Note: If no twitch occurs, check that the electrodes are connected, and that the Isolated Stimulator is switched on. You may need to increase the stimulus amplitude to observe a twitch: do this in the Isolated Stimulator Panel. If the subject feels discomfort, you can at any time immediately stop stimulation by either turning the stimulator switch off on the PowerLab, or by removing the bar stimulus electrode from the subject's wrist.

7. Explore the results of stimulating at other places in the forearm. Each time you move the electrode to another location, wipe away the residual electrode cream from the skin to prevent short-circuiting. (Stimulation will be ineffective if the current flows along a surface layer of electrode cream rather than through the arm.)

Note: Effective stimulation will only occur when the two pads of the bar electrode are aligned along the arm's length. If the stimulus status light changes in color from green to yellow, to put more electrode cream on the pads.

Motor effects that may be observed include:

- Bending of the wrist (due to the flexor carpi radialis and flexor carpi ulnaris muscles)
- Bending of the last segments of the fingers (due to the long finger flexor muscles)
- Movement of all the fingers, combined with a pulling of the thumb towards the index finger (due to the intrinsic muscles of the hand innervated by the ulnar nerve)
- Lifting of the thumb (due to the muscles at the base of the thumb; innervated by the median nerve)

Note: Stimulation in most places gives rise to little discomfort. In some places, there is a substantial sensory effect: there may be a painful sensation in the forearm or hand, away from the site of stimulation (towards the fingers). At these places, a cutaneous sensory nerve is being stimulated.

8. Try stimulating the ulnar nerve at the level of the elbow. The nerve passes behind a bony prominence (the medial epicondyle) on the humerus. At this location, the nerve is exposed to minor mechanical injury and is known to children as the "funny bone". Stimulation at this site gives large and obvious motor effects.

9. Click the Stop button to stop Chart monitoring, and turn the stimulator switch **OFF** on the PowerLab. It doesn't matter if you close the Chart window or not.

Exercise 2: Twitch response and recruitment

Objectives

In this part of the laboratory, the muscular twitch response to nerve stimulation will be measured and show recruitment in the twitch response as the stimulus strength increases.

Procedure

1. .You should connect the finger pulse transducer and stimulus to channel 1 and 2 of your power lab, respectively. Then name channel 1 as 'Force' and channel 2 as 'Stimulus'.

2. The volunteer should place their hand as shown in Figure 3, with the fingers under the edge of the table, and the edge of the thumb resting lightly on the pulse transducer. If the table edge is too thick for the subject's hand, a plank or shelf may have to be used.

3. Choose Input Amplifier… from the "Force" Channel Function pop-up menu. The Input Amplifier dialog window should show a stable baseline reading in its display. A deflection of the trace should be seen when pressing lightly on the pulse transducer.

4. Wipe the electrode cream from the subject's wrist. Apply a small amount of electrode cream to the pads of the bar stimulus electrode, and place the electrode at the site for stimulation of the ulnar nerve at the wrist (Figure 3), and hold it firmly in place. (The subject can hold the electrode in place with their other hand if this is easier.) Ensure that the edge of the subject's thumb is resting lightly on the transducer.

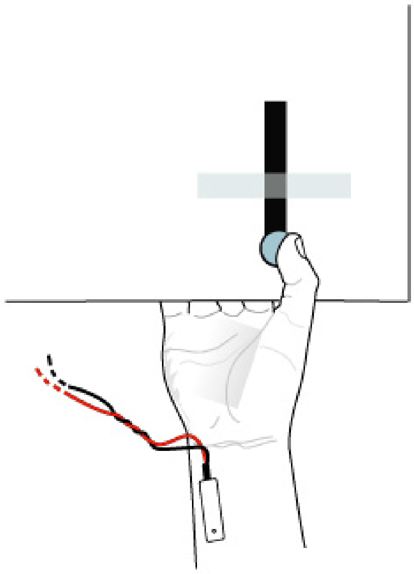

Figure 3. Using the finger pulse transducer to record muscle twitches of the thumb.

5. Turn the stimulator switch located on the front of the PowerLab, to **ON** (the up position).

6. The Isolated Stimulator mini-window should be floating in front of the Chart window. If not, choose the **Isolated Stimulator** command from the Setup menu (Win), or the **Stimulator Panel** command (Mac). The stimulus amplitude should be set to 0 mA. If necessary, move the mini-window to a convenient position that does not obscure view of the Chart window.

7. Click Start. Chart will record for a fixed duration of 0.5 seconds, then stop automatically.

8. Increase the amplitude to 1.0 mA and click Start. Continue to increase the amplitude in 1 mA steps, and click Start until a response is recorded. For most subjects, this *threshold stimulus* is in the range 3–8 mA. When the first response is seen, add a **comment** to the recording to note the stimulus amplitude used.

9. Reduce the amplitude by 1 mA, and then increase it in 0.5 mA steps, adding a comment each time to note the amplitude used. Continue this until the response no longer increases. For most subjects, this *maximal stimulus* is in the range 6–15 mA.

10. Close the Isolated Stimulator Panel. Turn the stimulator switch **OFF** on the PowerLab.

11. Choose Save from the File menu to save the recording. The data recorded should resemble that in Figure 4. (If you find the block boundaries distracting for such short blocks, they can be hidden. Choose the **Display Settings...** command from the Setup menu and alter the settings in the dialog that appears.)

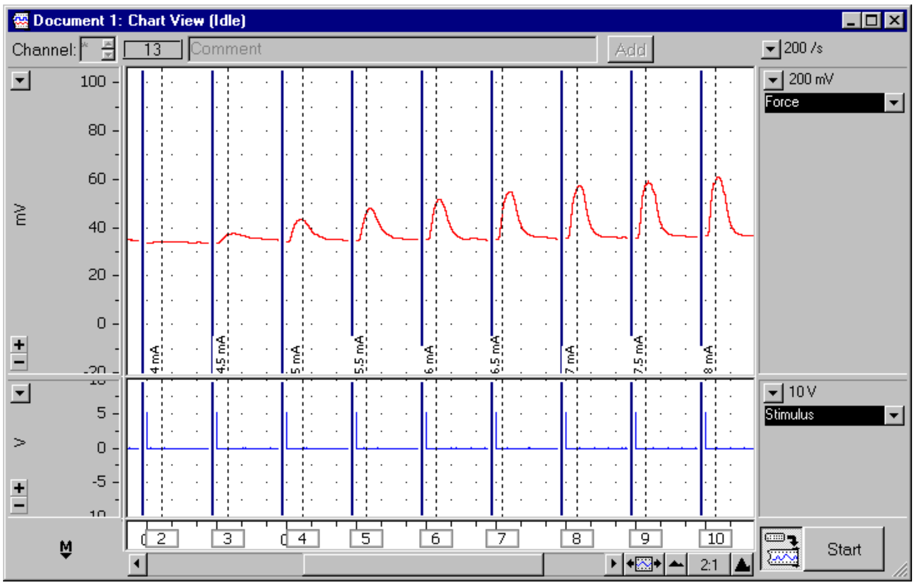

Figure 4. Typical results from Exercise 2, showing the effect of increasing stimulus strength.

Exercise 3: Summation and tetanus

Objectives

In this exercise, the effects of changing the interval between paired stimulus pulses will be demonstrated and observe a short tetanic contraction.

Procedure

12. Again,you should connect the finger pulse transducer and stimulus to channel 1 and 2 of your power lab, respectively. Then name channel 1 as 'Force' and channel 2 as 'Stimulus'. Windows users: From the Setup menu, choose **Isolated Stimulator**. This will open the IsolatedStimulator window (Mac OS users choose the **Stimulator** command to open the Stimulator window). Ensure that the settings are the same as in Figure 5.

13. Windows users: Proceed to step 4. Mac users: Close the Isolated Stimulator window, choose the **Stimulator Panel** command from the Setup menu, open the Isolated Stimulator Panel and then proceed to step 5.

14. Ensure that the volunteer’s hand and stimulus electrodes are placed as shown in Figure 2, and turn the stimulator switch **ON**.

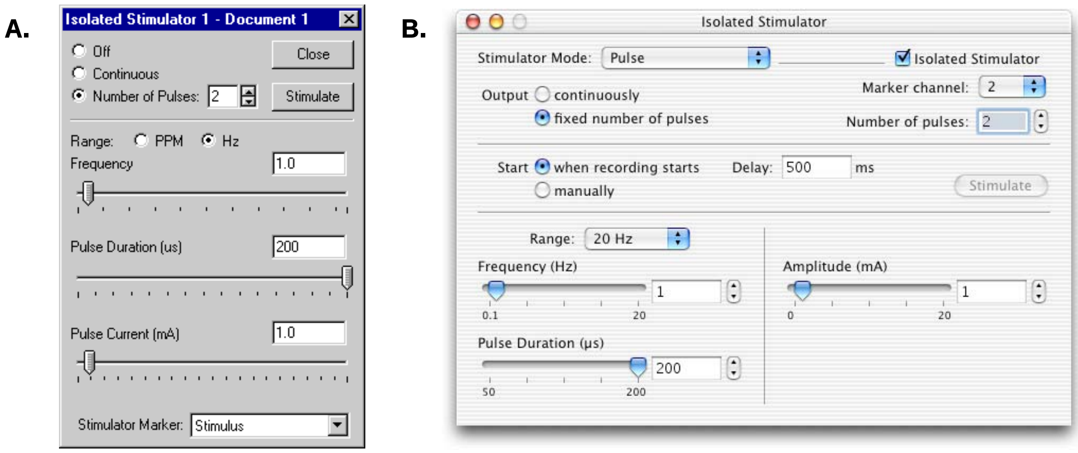

Figure 5. The Isolated Stimulator, showing the initial stimulation settings for Exercise 3. A - Windows, B - Mac OS X.

15. In the Isolated Stimulator Panel, set the pulse current to about 5 mA greater than the maximal stimulus value you determined in Exercise 2.

16. **Windows users:** Click Start and then immediately click the “Stimulate” button in the Isolated Stimulator window. Chart will record for a fixed duration of 3 seconds, and then stop automatically.
Mac OS users: The stimulus will automatically occur 0.5 seconds after the recording begins and Chart will record for 3 seconds. When the recording has stopped, add the **comment** “1 Hz” in the new block of data to note the stimulus frequency used.

17. Increase the stimulus frequency to 2 Hz in the Stimulator Panel, and then click Start. Note the stimulus frequency (2 Hz) in a **comment**, as in the previous step.

18. Repeat the stimulation for the frequencies 5, 10 and 20 Hz, noting the values in comments as above.

19. Open the Isolated Stimulator window once more, change the number of pulses from 2 to 3, then close the dialog window. Be careful with this setting: a prolonged tetanus with a large number of pulses may be painful for the subject.

20. Click Start. The volunteer should receive a burst of three stimuli at 20 Hz. Add the **comment** “Tetanic stimuli (3)” to the new block of data. If it is not causing too much discomfort, try again with four pulses.

21. Set the stimulus pulse current to 0 mA in the Isolated Stimulator Panel, then close it.

22. From the front of the PowerLab, turn the stimulator switch **OFF**. Disconnect the finger pulse transducer and stimulus electrodes from the PowerLab.

23. Choose Save from the File menu to save the recording. The data obtained should resemble Figure 6.

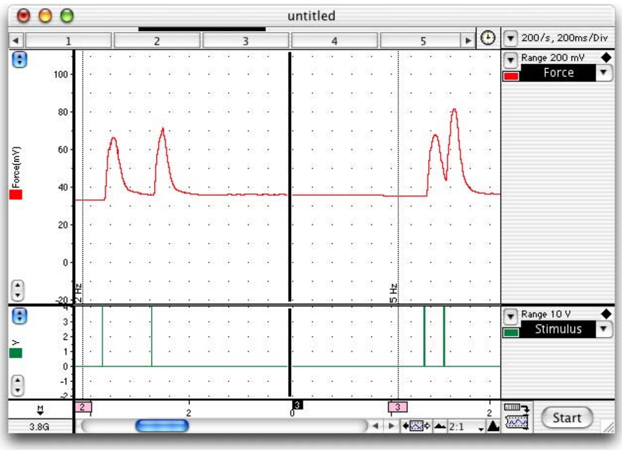

Figure 6.Typical results from paired stimuli, viewed with 2:1 horizontal compression (the stimulus delay is approximately one second).

Exercise 4: Grip force measurement

Objective

In this part of the experiment the hand dynamometer will be calibrated with respect to a volunteer's maximal grip strength.

Procedure

1. Remove any transducers and electrodes from the PowerLab, and then connect the plug of the grip force transducer to the Pod Input 1 (Figure 7).

2. Close the open Chart data file. If a warning dialog appears, click the No button. From the Experiments Gallery, open the settings file "Grip Settings". After a short time, the Chart View on the computer screen should be set up, with one channel appearing. Channel 1 should be labeled "Grip". Name the channel 1 as 'Grip' and prepare its necessary settings.

3. The volunteer should loosely grip the hand dynamometer in the fist, as shown in Figure 7.

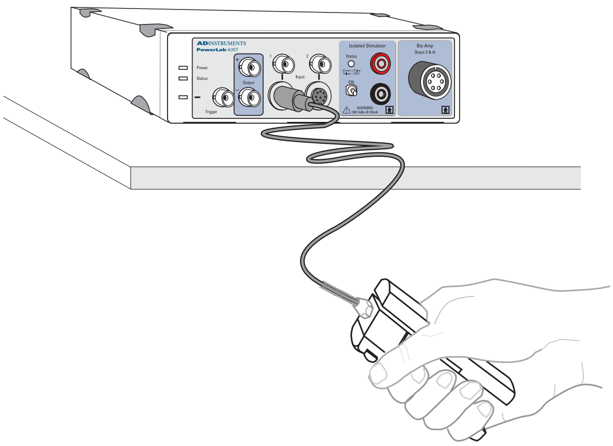

Figure 7.Connections for measuring grip strength and muscle fatigue.

4. Click Start to begin recording. The volunteer should squeeze the dynamometer as hard as possible for a second or two, and then relax their grip. After recording for a few seconds, have the volunteer repeat the maximum grip and then relax. Click the Stop button.

5. Drag over the largest response to **select** a range of data that includes both the relaxed and maximum-force signals (Figure 8). Choose the **Units Conversion…** item from the Channel Function pop-up menu for Channel 1.

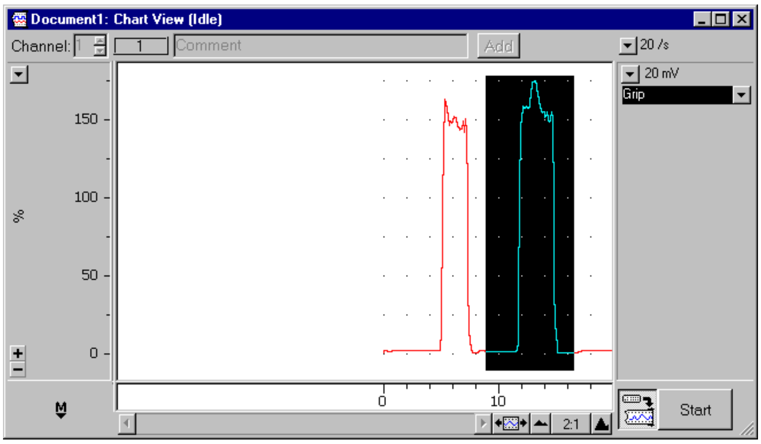

Figure 8.Selection of trace for calibrating to relative grip strength.

6. In the **Units Conversion** dialog (Figure 9) a roughly correct conversion has already been set. Calibrate correctly for the strength of the volunteer. Select part of the trace where the force was zero, and click the top Point 1 arrow button. Then select part of the trace at the peak, and click the bottom Point 2 arrow button. Click the OK button to return to the Chart View.

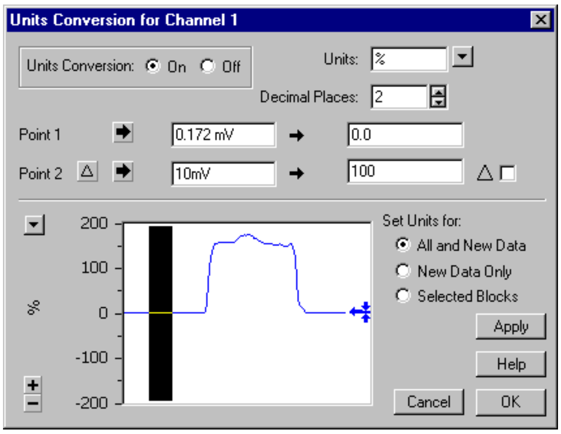

Figure 9.The Units Conversion dialog, with relaxed signal selected.

Exercise 5: Muscle fatigue

Objectives

In this part of the experiment, the decline in maximal force during a sustained contraction will be observed as well as examining some properties of muscular fatigue.

Procedure

The grip force transducer should already be calibrated for the volunteer, as described in Exercise 4.

1. Adjust the **scale** for Channel 1 (Grip) to show –20 to 120%.

2. Allow the volunteer to view the computer screen. Click the Start button, and ask the volunteer to maintain 20% maximal grip strength while watching the recorded trace (the Range/Amplitude display for Channel 1 shows the percentage force applied).

3. After 20 seconds, tell the volunteer to relax.

4. Click Stop.

5. Wait for 30 seconds to allow recovery of muscle function.

6. Repeat steps 2–4 above for contractions of 40%, 60%, 80% and 100% of maximal grip strength.

7. Allow the volunteer to rest for two minutes.

8. Turn the volunteer away so that they cannot see the computer screen.

9. Click Start, and ask the volunteer to produce a sustained maximal contraction. After 8 to 10 seconds, or when the force has obviously declined, instruct them to try harder. After another 8 to 10 seconds, repeat the encouragement. After a few seconds, ask the volunteer to relax, and click Stop.

Note: Nearly all subjects can produce temporary increases in muscle force during a fatiguing contraction, when sufficiently motivated by verbal encouragement.

10. Click Start and ask the volunteer to produce a sustained maximal contraction. Every 8 to 10 seconds, allow the volunteer to relax very briefly (half a second), and then return to maximal contraction. Click the Stop button to terminate recording after 30 to 40 seconds.

Note: Even brief periods of relaxation allow substantial recovery from fatigue, but the recovery is only temporary (Figure 10).

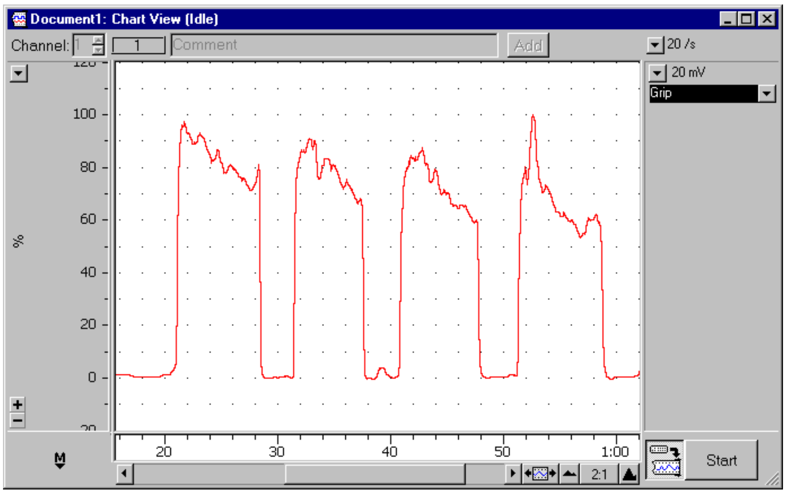

Figure 10.Fatiguing contraction, with brief periods of relaxation.

11. Allow the volunteer to use their other hand if gripping the transducer has become painful. Turn the volunteer so that they can see the computer screen. Click the Start button, and ask the volunteer to produce a 40% contraction while watching the trace. After 10 seconds, press the Enter key to enter a **comment** (to mark the time).

12. Have the volunteer close their eyes, and attempt to maintain exactly the same contraction force for the next 30 seconds.

13. After the elapsed time, the volunteer should open their eyes, and adjust the contraction force back to 40%.

14. Click Stop and examine the trace.

Note: Almost all subjects will show a declining force (pseudo-fatigue) while their eyes are shut, that is very similar to fatigue. This is, however, not true fatigue, because the full 40% force can be exerted easily, as can be seen when the subject's eyes are opened again.

Analysis

Exercise 2: Twitch response and recruitment

1. Use the Waveform Cursor to measure the amplitude of each peak: place it over the peak and read off the force in the Range/Amplitude display above the channel title. Use the View buttons to expand the horizontal display to 1:1.

2. Refer to the comments in the Chart View to determine the current applied to produce each response.

3. Write down the data in Table 1 of the Data Notebook. Graph the relationship between stimulus current and response size. Note the stimulus intensity at which the maximal response first appears.

Exercise 3: Summation and tetanus

1. Calculate the stimulus interval in seconds for each stimulation frequency. Enter the data in Table 2 of the Data Notebook.

2. Using the **Marker** and **Waveform Cursor**, measure the amplitude of the first two responses at each stimulus interval. Enter these results in Table 2 of the Data Notebook.

3. Examine the tetanic response. Calculate the stimulation interval in seconds and enter the value in Table 3 of the Data Notebook.

4. Drag across the tetanic response to **select** it, and examine the selected data in the **Zoom** window. Determine the maximum force amplitude using the **Marker** and **Waveform Cursor** and enter these results in Table 3 of the Data Notebook.

5. If the tetanus experiment with four pulses was repeated, repeat the analysis and add it to Table 3. Otherwise, leave the second line blank in Table 3.

Data Notebook

Table 1. The effects of varying stimulus strength on twitch force.

Stimulus	Response	Stimulus	Response	Stimulus	Response
0.0 mA		7.0 mA		14.0 mA	
0.5 mA		7.5 mA		14.5 mA	
1.0 mA		8.0 mA		15.0 mA	
1.5 mA		8.5 mA		15.5 mA	
2.0 mA		9.0 mA		16.0 mA	
2.5 mA		9.5 mA		16.5 mA	
3.0 mA		10.0 mA		17.0 mA	
3.5 mA		10.5 mA		17.5 mA	
4.0 mA		11.0 mA		18.0 mA	
4.5 mA		11.5 mA		18.5 mA	
5.0 mA		12.0 mA		19.0 mA	
5.5 mA		12.5 mA		19.5 mA	
6.0 mA		13.0 mA		20.0 mA	
6.5 mA		13.5 mA			

Table 2. Results for summation experiment in Exercise 3.

Stimulus frequency (Hz)	Stimulus interval (sec)	Amplitude of first response (mV)	Amplitude of second response (mV)
1			
2			
5			
10			
20			

Table 3. Results from the tetanus experiment in Exercise 3.

Stimulus frequency (Hz)	Stimulus interval (sec)	Number of pulses	Amplitude of response (mV)
20		3	
20		4	

Results

Exercise 1: The effects of nerve stimulation

Describe your observations and sensations experienced when the ulnar nerve was stimulated.

Exercise 2: Twitch response and recruitment

Draw a scatter plot graph of stimulus intensity (mA) versus twitch response (mV). Use the data from Table 6-1.

Exercise 3: Summation and tetanus

Attach a copy of the Zoom window showing a tetanic response.

Exercise 5: Muscle fatigue

Attach a copy of the Zoom window showing muscle fatigue using the hand dynamometer.

Conclusions

Answer the following questions in complete sentences.

1) What was the smallest current required to produce a contraction (the threshold current)? What proportion of the fibers in the muscle do you think were contracting to produce this small response? What was the smallest current required for a measurable muscle contraction?
2) What was the smallest current required to produce the maximum (largest) contraction? What proportion of the fibers in the muscle do you think were contracting to produce this maximal response?
3) What happened to the twitch force at the highest currents? Was the increase in contraction force linear? If not, how can you explain your result in terms of muscle physiology?
4) What was the stimulus interval required to cause tetanus?
5) How long did it take for your hand muscles to fatigue? Explain one mechanism that could cause muscle fatigue.
6) Did the time to reach muscle fatigue differ between the members of your group? Explain why different people may have different rates of muscle fatigue.

Study Questions

Write a paragraph briefly explaining how muscles work in the human body. What is muscle fatigue? What do you expect to learn from this experiment? Briefly describe the techniques you will use to measure muscle activity.

Exercise 2

1. Will you get a measurable twitch with a stimulus of 0 mA? What will this tell you about the number of muscle fibers contracting at this stimulus current?

2. What will happen to the number of fibers contracting as the current is raised from threshold to that required to produce a maximal contraction?

3. Why does varying the stimulus strength affect the twitch force?

Exercise 3

4. What are the two ways by which the nervous system can control the force generated by a muscle?

5. Electromyography, with needle electrodes inserted through the skin into a muscle, has been used to study the frequency of muscle fiber activation during voluntary contraction in humans. During weak contractions, the firing frequency is low, so that each fiber produces distinct twitches. The force produced by the whole muscle, however, is relatively smooth. How do you think this occurs?

Exercise 5

Fatigue is not well understood. Some factors that have been proposed to explain the fall in force during fatigue include: changes in the "sense of effort", loss of "central drive", failure of neuromuscular propagation, reduction in calcium release in excitation–contraction coupling, metabolic changes in the muscle, and reduction in muscle blood flow owing to compression of blood vessels.

6. Will your experiments help to decide which factors are important? How?

7. What explanations can you think of for pseudo-fatigue?