Lab 6: MUSCLE PHYSIOLOGY

Due to their attachment to the bones by tendons, skeletal muscles are used to produce movement and are also used for support. Skeletal muscles consist of many muscle fibers (muscle cells) that run the length of the muscle. The muscle fibers are packed with the contractile proteins, actin and myosin, which generate the force of a muscle contraction.

Skeletal muscles are under voluntary control and are stimulated to contract by somatic motor neurons. A somatic motor neuron and the muscle fibers it innervates make up a motor unit. The response of a motor unit to stimulation is a muscle twitch. The size and speed of the twitch is affected by the size of the load on the muscle. The amount of force generated by a muscle depends on the number of motor units activated (recruitment) and the amount of force produced by the individual muscle fibers (for example, the summation of muscle twitches, which occurs with increased frequency of stimulation).

Objectives

At the conclusion of this laboratory the student will understand and be able to describe:

1. the terms motor point, motor unit, threshold stimulus, and tetanus.
2. the characteristics which differentiate motor unit recruitment from summation.
3. the nerves and muscles that are used in this lab.
4. the effect of muscle fatigue on contraction and relaxation.
5. a single muscle twitch (be able to diagram and label one).
6. the importance of asynchronous firing of motor units during contraction of the whole muscle.

VOCABULARY LIST

Use this list to prepare for quizzes and lab practicals.

motor point	abductor digiti minimi	ulnar nerve	flexor digitorum superficialis
median nerve	muscle fiber	myofibrils	actin
myosin	sarcomeres	somatic motor neuron	sarcolemma
T tubules	sarcoplasmic reticulum	sarcoplasm	muscle twitch
contraction phase	relaxation phase	latent period	isometric twitch
isotonic twitch	load	motor unit	recruitment
fast-twitch fibers	slow-twitch fibers	fast glycolytic fibers	fast oxidative fibers
slow oxidative fibers	latent period	summation	incomplete tetanus
complete tetanus	muscle fatigue	threshold	maximal stimulus

This lab consists of 4 activities:

ACTIVITY 1. Single Muscle Twitch

Students will record a muscle twitch and analyze the latent period, twitch amplitude, and the periods of contraction and relaxation.

ACTIVITY 2. Threshold Stimulus and Motor Unit Recruitment: The Effect of Stimulus Strength on Tension Development (Twitch amplitude)

Students will determine the threshold stimulus required to cause a skeletal muscle to contract and observe recruitment when stimulus strength is increased.

ACTIVITY 3. Effect of Load on Isotonic Contractions

Students will observe how the size of a load affects the latent period and the speed and size of an isotonic muscle twitch.

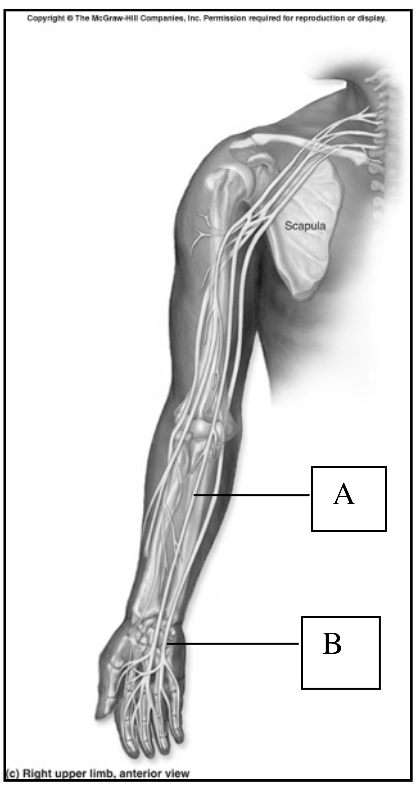
ACTIVITY 4. Summation and Tetanus

Students will demonstrate how summation increases the strength of contraction. Tetanus will also be observed.

BACKGROUND AND REFERENCES

Motor Point Stimulation

Skeletal muscle can be directly stimulated to contract through the skin using an electrical stimulus. Certain sensitive spots that elicit a very strong response are known as **motor points**. Motor points are usually found on the belly of the muscle where the nerve enters.



The muscles you will be stimulating include the **abductor digiti minimi** (innervated by the **ulnar nerve**) and the **flexor digitorum superficialis** (innervated by the **median nerve**). The abductor digiti minimi is located on the medial surface of the palm while the flexor digitorum superficialis is located on the anterior surface of the forearm. The function of the abductor digiti minimi is to abduct the little finger. The flexor digitorum superficialis flexes first the middle phalanges and then with continued action, the proximal phalanges and wrist. This muscle is involved only in rapid, forceful flexion of the digits in grasping movements. The motor point (median nerve) for the flexor digitorum superficialis is found on the lateral side of the anterior forearm (A in Figure 1). The motor point for the abductor digiti minimi is found on the medial side of the hand, just distal to the wrist (B in Figure 1). You will begin with the abductor digiti minimi motor point; however, if you have difficulty finding this motor point, you can attempt to find the motor point for the flexor digitorum superficialis.

Figure 1. The nerves of the forearm. A. The motor point for the flexor digitorum superficialis (median nerve). B. The motor point for the abductor digiti minimi (ulnar nerve). Taken from McKinley, 1st edition.

Checkpoint: With your group, answer the following questions.

1. What movement does the abductor digiti minimi cause? Demonstrate the movement.

2. What movement(s) does the flexor digitorum superficialis cause? Demonstrate the movements.

ACTIVITY 1. Single Muscle Twitch

In this activity, you will find your abductor digiti minimi motor point and use this motor point to cause a single muscle twitch. This twitch will be recorded by the computer, and you will then analyze the latent period, twitch amplitude, period of contraction, and period of relaxation.

A skeletal muscle is made up of many **muscle fibers** (muscle cells) that run the length of the muscle. Each muscle fiber contains many **myofibrils** (see Figures 2 and 3), which are "bundles" of the contractile proteins **actin** (thin filaments) and **myosin** (thick filaments) arranged into functional contractile units called **sarcomeres**. When the muscle fiber is stimulated to contract by a **somatic motor neuron**, an action potential is generated in the **sarcolemma** (the muscle fiber's plasma membrane). The action potential is conducted over the sarcolemma and deep into the muscle fiber by **transverse tubules** or **T tubules**, which are tubular extensions of the sarcolemma. As the action potential travels down the T tubules, it triggers the release of calcium ions from the **sarcoplasmic reticulum** (smooth ER) into the **sarcoplasm** (cytoplasm). The calcium ions bind to troponin complex, which moves tropomyosin away from where it was blocking the binding site on actin, thus allowing the myosin heads (crossbridges) to bind to the actin. Using the energy of ATP, the myosin crossbridges pivot and pull the actin filaments toward the center of the sarcomere. The sarcomeres shorten and pull on the tendons, which then pull on the bone. The muscle fiber relaxes when the calcium ions are pumped back into the sarcoplasmic reticulum. The myosin crossbridges can no longer bind to the actin, and the actin filaments slide back, returning the sarcomere to its original length.

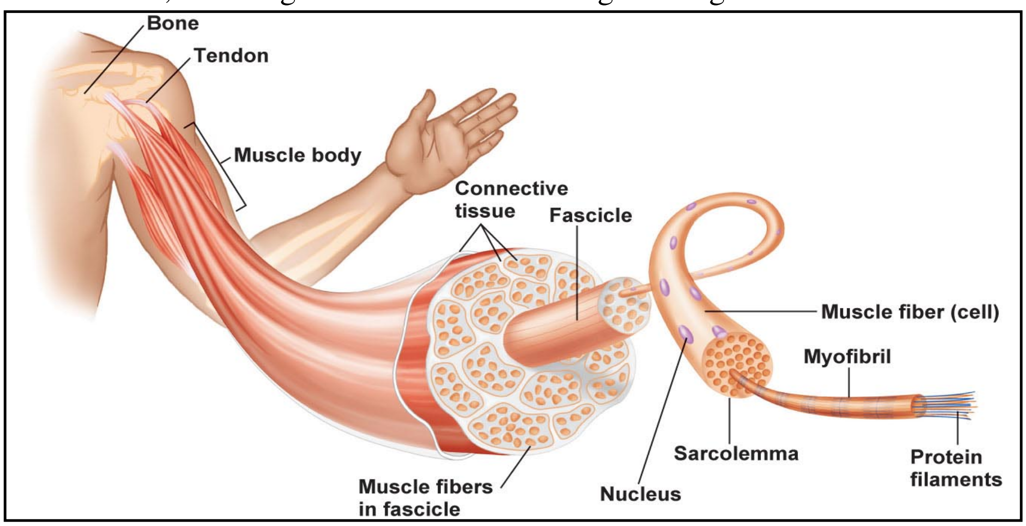


Figure 2. Structure of a skeletal muscle. Taken from Stanfield, 4th edition.

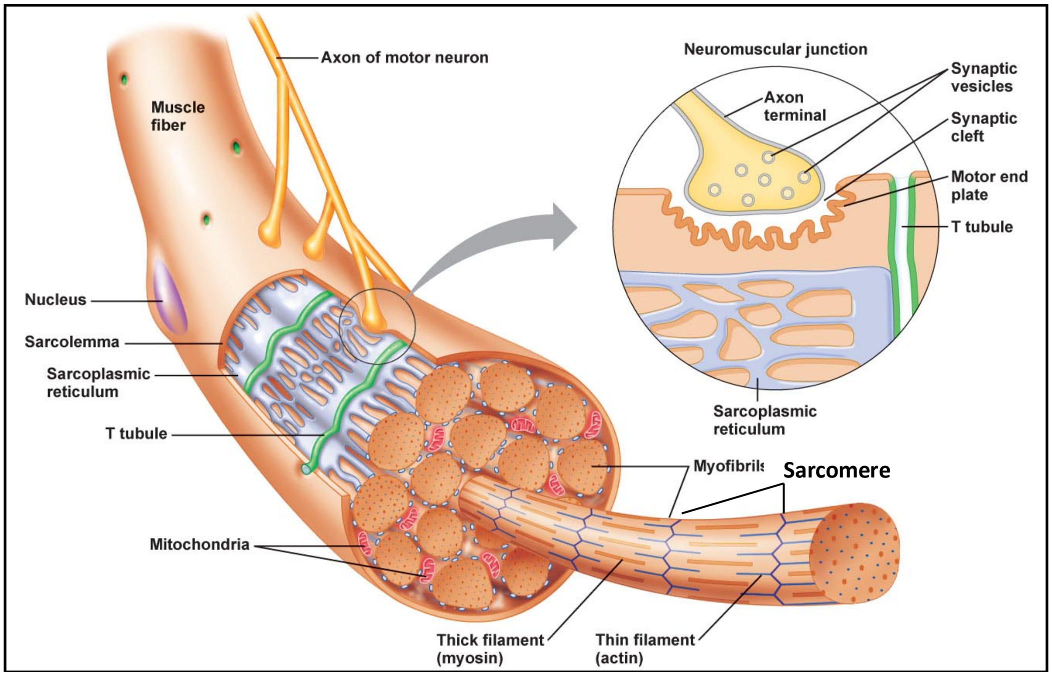


Figure 3. Structure of a skeletal muscle fiber. Taken from Stanfield, 4th edition.

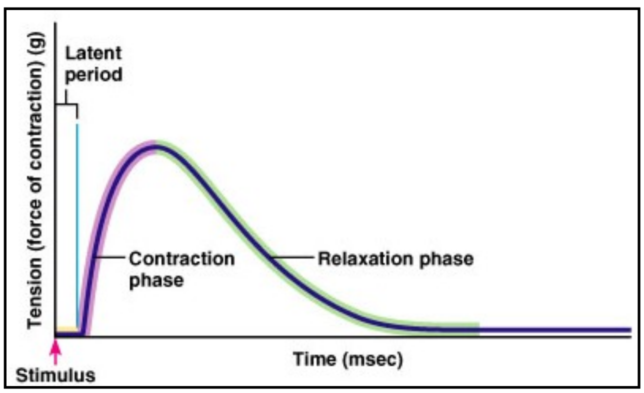


Figure 4. An isometric muscle twitch. From Germann, 1st edition.

A **muscle twitch** is the mechanical response of a muscle cell or whole muscle to a single action potential. If you look closely at a single muscle twitch (see Figure 4), you will see that the **contraction phase** occurs faster than the **relaxation phase**. Also note the **latent period**, which is the period of time it takes the muscle to start generating tension after it has been stimulated to contract. The latent period exists because the events that result in a contraction take time. These events include (1) depolarization of the sarcolemma and the T tubules, (2) release of calcium ions into the sarcoplasm from the sarcoplasmic reticulum, (3) attachment of myosin crossbridges to actin, and (4) tightening of elastic elements within the muscle cell.

There are two kinds of muscle twitches: **isometric twitches** and **isotonic twitches**. These two types of twitches differ based on whether the muscle shortens in length or not. In an isometric contraction, tension develops, but the muscle is not able to overcome the **load** (force opposing the contraction) and does not shorten. An example of an isometric contraction would be trying to lift a two-ton boulder. When a muscle contracts isotonically, the muscle generates enough tension to overcome the load, and thus the muscle shortens and lifts the load. An important point to remember is that all isotonic twitches start out as an isometric twitch. When a muscle first starts to contract, it does not shorten because it has not generated enough force to move the load (isometric contraction). With continued contraction, the muscle will produce enough tension to overcome the load and shorten (isotonic contraction).

Checkpoint:

1. With your group, graph and label the following portions of an isometric muscle twitch: latent period, contraction phase, relaxation phase.

2. Distinguish between an isometric and isotonic contraction.

ACTIVITY 2. Threshold Stimulus and Motor Unit Recruitment: The Effect of Stimulus Strength on Tension Development (Twitch Amplitude)

In this activity, you will determine the threshold stimulus required to cause your abductor digiti minimi or your flexor digitorum superficialis muscle to contract. You will also demonstrate the consequences of increasing the stimulus strength (increased force of contraction due solely to recruitment).

Skeletal muscles contain functional units called **motor units**. Each motor unit consists of a single **somatic motor neuron** that branches to innervate one or more muscle fibers. When a motor unit is stimulated, all of the muscle cells within that motor unit will contract with all of the force they can develop. In order to maintain smooth contraction of the whole muscle and to decrease fatigue of the individual motor units, the motor units are activated asynchronously. All the motor units of the muscle do not contract at the same time – while some motor units are contracting, others are relaxing.

In a given muscle (for example, the abductor digiti minimi), there are many motor units which differ in their size and ease of stimulation. Small motor units, which consist of a small number of thinner diameter muscle fibers, respond to weaker stimuli and contract first, while large motor units, which consist of a larger number of thicker diameter muscle fibers, are harder to stimulate and require a much stronger stimulus. The overall contraction strength of a muscle is the summation of the pull of all of the motor units operating at any one time. As the stimulus strength increases, more motor units are stimulated to contract, generating a stronger force of contraction. This process of increasing the number of motor units that are active is known as **recruitment**. Besides recruitment, another way to increase muscle contraction strength is **summation**, which will be discussed in activity 4. In summation, the force produced by an individual muscle fiber increases when it is stimulated to contract more frequently, which causes the twitches to add together, generating more force.

The muscle fibers found in most mammalian skeletal muscles are either **fast-twitch fibers** or **slow-twitch fibers**. Each type has a unique form of myosin, which differs in the rates of ATPase activity and crossbridge cycling and, thus, results in differing contraction speeds. Within the group of fast-twitch fibers, **fast glycolytic fibers** use mainly fermentation (glycolysis) to produce ATP, while **fast oxidative fibers** generate ATP mainly by cellular (aerobic) respiration. The fast glycolytic fibers have the largest diameter and generate the strongest force of contraction, while fast oxidative fibers are intermediate in diameter and are hence intermediate in contraction strength. Fast glycolytic fibers are the quickest to fatigue because they break down glucose very inefficiently. A burst of contractile activity diminishes glucose levels and causes lactic acid to accumulate, which both lead to fatigue. The least powerful (due to their smallest diameter) but least likely to fatigue fiber types are the **slow oxidative fibers**, which rely almost entirely on cellular respiration for their energy source.

Skeletal muscles contain all three types of these fibers intermixed with each other. A given motor unit, however, only contains one fiber type, and thus, the stimulation of a particular motor neuron will cause contraction of only one type of muscle fiber. When muscle tension is required, the brain will recruit the small motor units containing slow oxidative fibers first. Increased signals will stimulate the fast oxidative fibers in the intermediate motor units to contract next to generate more force if needed. The fast glycolytic fibers in the large motor units are recruited last when a large amount of force is required, such as in high-intensity exercise like weight lifting. To summarize, as muscle tension requirements increase, the order of recruitment for these fibers is slow oxidative fibers first, fast oxidative fibers next, and fast glycolytic fibers last.

Checkpoint:
With your group, differentiate between fast glycolytic, fast oxidative, and slow oxidative fibers in terms of strength of contraction, resistance to fatigue, and order of stimulation.

Fiber type	Contraction strength	Resistance to fatigue	Order of stimulation

ACTIVITY 3. Effect of Load on Isotonic Contractions

In this activity, you will observe how the size of a load affects the latent period, the speed, and the force of an isotonic muscle twitch.

When a muscle is stimulated to lift a weight, it first develops tension without shortening (isometric contraction). If the muscle is able to create enough tension to overcome the load, the muscle shortens and the load is lifted (isotonic contraction). The time between when the muscle is stimulated and the beginning of shortening is called the **latent period**. The size of the load affects isotonic contractions in several ways. As the load becomes heavier, (1) the latent period becomes longer (it takes more time to start lifting the load); (2) the duration of muscle shortening decreases (the load cannot be lifted as long); (3) the velocity of shortening is slower (the load is not lifted as quickly); and (4) the distance shortened decreases (the load is not lifted as far).

The size of the load affects the amount of work that a muscle can perform. By definition, work is the product of a force being moved and the distance through which that force is moved. The equation for work is written as WORK = Force x distance. Based on this definition, a muscle does no work when it does a completely isometric contraction because nothing is being moved through a distance. In this lab, you will measure work as the mass of a weight being moved by your finger times the amplitude of the muscle twitch that occurs in your finger.

ACTIVITY 4. Summation and Tetanus

In this activity, you will demonstrate an increase in the strength of contraction due solely to summation. You will also demonstrate tetanus.

Remember that there are two factors that determine the amount of force generated by a muscle: (1) the number of muscle fibers contracting (recruitment) and (2) the amount of force produced by the individual muscle fibers (summation). Recruitment was discussed in activity 2. Activity 4 will focus on summation.

When an individual muscle fiber is stimulated before it has completed its current muscle twitch, the next twitch will add to the previous twitch in a process called **summation** (see Figure 5). When the stimuli arrive close together in this fashion, each individual muscle fiber contracts more powerfully than it did in response to a single stimulus. This increased contraction strength is caused by the increased amount of calcium ions present in the sarcoplasm. The calcium ions released from the sarcoplasmic reticulum in response to the first action potential have not all been pumped back into the sarcoplasmic reticulum when the next action potential arrives. This higher level of calcium ions in the sarcoplasm causes the exposure of more myosin-binding sites on the actin, allowing more myosin crossbridges to bind and greater tension to be produced. One way to think of this is to imagine a tug of war with a rope. If you can add more people to your end of the rope, your team will be able to pull more strongly.

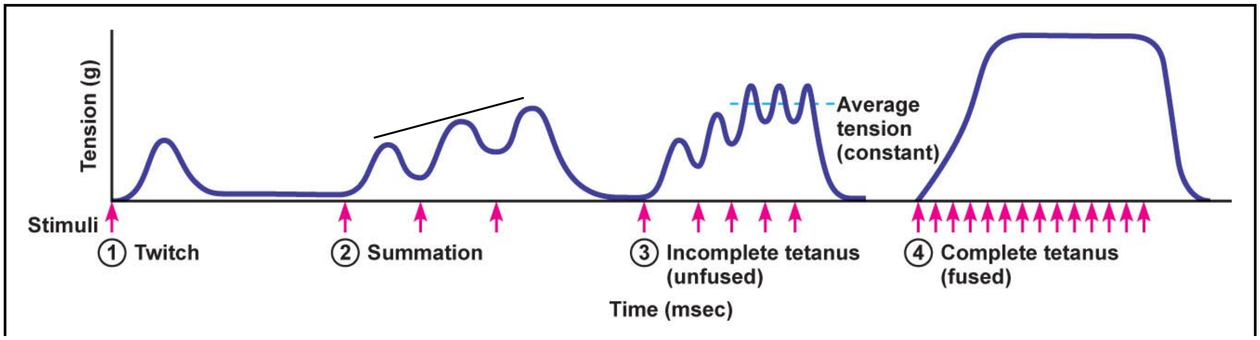


Figure 5. Effects of high stimulation frequency: summation and tetanus. Taken from Stanfield, 4th edition

As the frequency of stimulation increases further, the muscle fiber has even less time to relax and summation leads to **incomplete tetanus** (see Figure 5). Incomplete tetanus is characterized by a higher average tension than that generated by a single muscle twitch; however, the individual twitches are still observable. When the stimulus frequency is increased even further, summation produces a plateau called **complete tetanus** (see Figure 5). During complete tetanus, no relaxation occurs between stimulations and thus a sustained, maximum amount of tension is developed. Once the frequency for a tetanic contraction has been reached, increasing the stimulus rate any further will not increase the force of the contraction. The rate of muscle relaxation is much slower after tetanus than after a single muscle twitch because it takes more time to pump the excess calcium back into the sarcoplasmic reticulum.

Under normal conditions, motor units are not activated by single stimulus but instead by many stimuli in rapid succession. Thus, tetanic contractions are produced by each motor unit. The muscle as a whole is able to produce a smooth, sustained contraction because of asynchronous firing of the many motor units within that muscle.

Muscle Fatigue

When a muscle fiber is frequently stimulated to contract, the tension produced eventually decreases. This decline in muscle tension due to repetitive stimulation is called **muscle fatigue**. Muscle fatigue is influenced by the type of exercise, that is, the rate and force of contraction, and by the quantity and quality of the blood supply to the muscle. In high-intensity exercise, such as weight lifting or sprinting, fast glycolytic fibers are recruited, and they use fermentation (glycolysis) to produce ATP, resulting in the buildup of lactic acid. The change in pH alters the shape and activity of the contractile proteins and sarcoplasmic reticulum calcium pumps. Very high-intensity exercise can also cause neuromuscular fatigue, where the somatic motor neurons run out of neurotransmitter from being stimulated so frequently. In low-intensity, long-duration exercise where mostly slow oxidative and fast oxidative fibers are used, the depletion of fuel sources, such as glycogen, to run cellular respiration is an important cause of muscle fatigue.

While it may be obvious that energy is needed for muscle contraction, energy is also required for muscle relaxation. For relaxation to occur, the calcium ions must be pumped back into the sarcoplasmic reticulum from the cytoplasm, a process that takes abou 0.033 seconds. In the absence of ATP the calcium remains in the cytoplasm longer and the actin and myosin will continue to interact with each other. Also, in the absence of ATP, the myosin head cannot become uncoupled from the actin active site; thus, the muscle will not be able to relax.

Checkpoint:

List some reasons that muscles will fatigue in

- High-intensity exercise

- Very high-intensity exercise

- Low-intensity, long-duration exercise

EXPERIMENTAL PROCEDURES

In this experiment, you will use a specialized displacement transducer, the SMT-100 striated muscle transducer (finger twitch sensor), to demonstrate (1) the effect of increasing the stimulus intensity on the tension developed by a muscle; (2) the effect of increasing load on the shortening and work of a preloaded muscle; (3) the effect of increasing the frequency of stimulation on the contraction strength; and (4) the effect of muscle fatigue on contraction and relaxation. The effects listed above will all be measured as a function of twitch amplitude (mVolts) measured by the displacement transducer.

This experiment involves applying mild electrical shocks to the skin to produce a muscle contraction. While the sensation of evoked muscle contractions may initially feel peculiar, electrical stimulation of the muscles in the hand is safe and painless. Standard safety precautions need to be observed. **Persons with poor cardiac function, pacemakers, or any other condition that can be aggravated by electrical stimulation should not volunteer for this experiment**.

Equipment Required

- PC computer
- iWorx 214 unit and USB cable or serial cable

  This unit integrates the information being sent to your muscle by the stimulator with the muscle response that is recorded by the transducer. The iWorx unit then delivers this information to the PC computer so that you will be able to analyze the data.
- SMT-100 striated muscle transducer

  This is the twitch sensor, which records the tension developed when your muscle is stimulated
- SI-200 isolated stimulator
- HV stimulating electrodes and leads
- BNC-BNC cable and BNC-banana adapter

  These produce and deliver the electrical stimulation to your muscle. The event marker records when the stimulus is sent to your muscle so you can relate this to the timing of the muscle twitch.
- Thread and paper clips to hold weights
- Weights (10, 20, 30, 40, 50 grams)

  These are used to determine the effect of increasing loads on the muscle contraction.

Equipment Setup

1. Make sure you have the correct power supplies for the iWorx 214 unit and SI-200 isolated stimulator. The iWorx 214 power supply (Model D12-10-1000) is larger and has 3 prongs, while the SI-200 power supply (Model A12-1A) is smaller and has 2 prongs. The power supplies are not interchangeable.

2. Connect the iWorx 214 unit (Figure 7) to the computer via a USB cable that runs from the back of the unit to the front of the computer. Turn on the power switch, which is on the back of the unit (a red light on the front will light up).

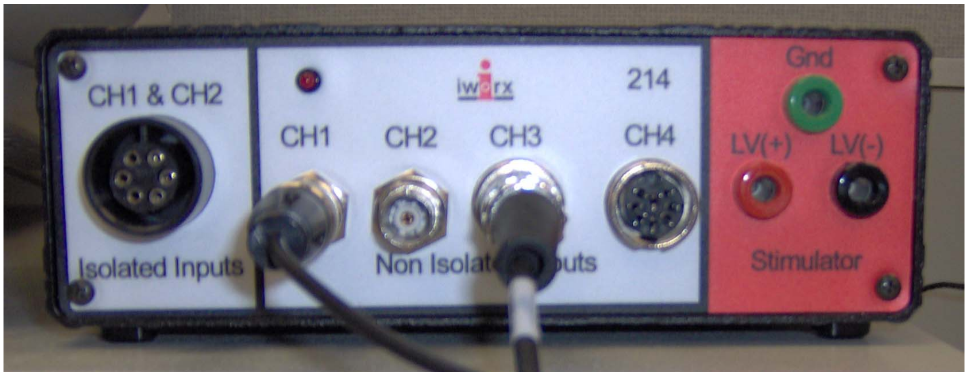


Figure 7. The iWorx 214 unit with cables attached. Channel 1 is receiving the cable from the "sync" channel of an older type stimulator. Channel 1 will NOT be used if you are using an iWorx stimulator. See Figure 10 for the connection of an iworx stimulator. Channel 3 is receiving the cable from the SMT-100 striated muscle transducer that is strapped to the subject's hand.

3. Connect the SI-200 isolated stimulator (Figure 8A) to the iWorx 214 unit. Twist the BNC cable (Figure 9) into the "Trigger" switch (Figure 8B) on the back of the SI-200. Place the banana prongs on the other end of the BNC cable into the front of the iWorx 214. The banana prong with the small nub on the side should be placed into the Gnd hole and the other should be placed into the LV(+) hole (Figure 10). The SI-200 isolated stimulator is now communicating with the iWorx 214 unit.

Figure 8A. The SI-200 isolated stimulator, front panel.

Figure 9. BNC-BNC cable with BNC-banana adapter.

Figure 8B. The SI-200 isolated stimulator, back panel.

Figure 10. BNC-banana adapter connected to the iWorx 214 unit.

4. On the SI-200 isolated stimulator, turn the Pulse Amplitude knob (Figure 8A) to “0”. Do this by rotating the Pulse Amplitude knob in a counterclockwise direction until the values on the dial and in the counter window read zero. The Pulse Amplitude knob controls the current output. Starting from zero, each 360° turn of the knob adds two milliamperes of current to the output. The knob can be turned a total of ten complete rotations to deliver a maximum output of twenty milliamperes.

5. Locate the two electrode stimulator lead wires, one colored black and the other colored red. On the front of the SI-200, plug the black connector into the black “High Voltage Current Output” (Negative) and the red connector into the red “High Voltage Current Output” (Positive). Push each connector into the appropriate socket as far as possible. Turn the power switch on the back of the unit to on. The green Power light should light up. Next, push the Arm button and the green Stimulator Ready light will turn on. (See Figure 8A)

6. Insert the connector of the SMT-100 striated muscle transducer (Figure 11) into the Channel 3 input of the iWorx 214 unit (Figure 7). The SMT-100 striated muscle transducer detects movement of the little finger when it is stimulated. This sensor will only deliver output when it is flexed in one direction. For maximum output, the sensor must be positioned with the side marked “OUTSIDE” facing away from the finger.

Figure 11. SMT-100 striated muscle transducer.

Start the Software

1. Click the Windows *Start* menu, click on *All Programs* and then on the *iWorx* folder and select *LabScribe2*; or click on the *LabScribe2* icon on the Desktop. Close the Tip of the Day that pops up. A pop up box should also appear indicating that the iWorx 214 unit has been found. If it says “Hardware not Found”, check to make sure that the USB cable is properly connected and that the iWorx214 unit is turned on (red light on). Next click on *Tools* and then *Find Hardware*.
2. When the program opens, click on *Settings* on the toolbar on top. Then select *Load Group*. Next, double click on *Settings* again. Now click on *IPLMVComplete.iwxgrp* followed by *Open*; or just double click on *IPLMVComplete.iwxgrp*.
3. Click on the *Settings* menu again and select *Human Muscle* and then *HumanMuscleTwitch-LS2*. This will pull up an instruction manual, which can be reduced and used if needed for reference.

4. Click the *Stimulator Preferences* icon on the Labscribe2 toolbar (Figure 12) to open the stimulator control panel (Figure 13); or click on *View* menu on top of page and then select *Stimulator Panel*. The stimulator control panel (Figure 13) will appear just below the Labscribe2 tool bar (Figure 12).

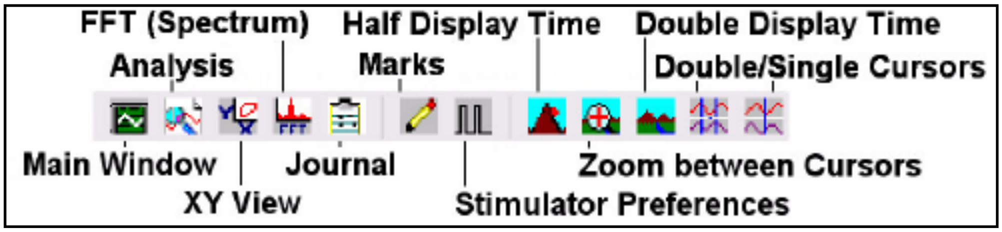


Figure 12. The LabScribe toolbar.

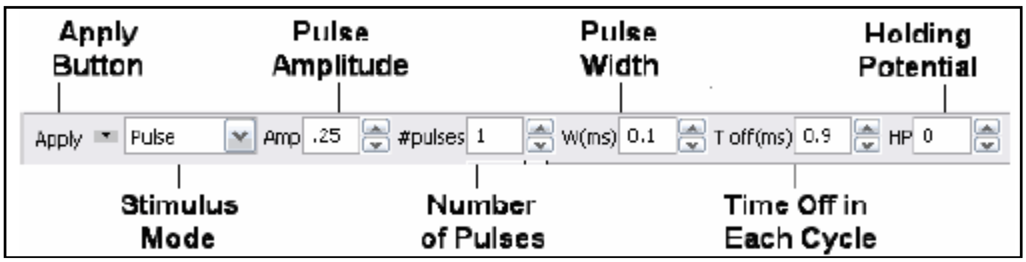


Figure 13. The stimulator control panel.

Positioning Stimulating Electrodes

1. The subject should remove all jewelry from his/her right hand and wrist.

2. Obtain two disposable electrodes, or use 1 disposable electrode and one probe electrode. Ask your lab instructor which type of electrode works best.

3. Peel the protective shield off one of the electrodes and attach it in the center of the back of the right hand, half way between the knuckle of the middle finger and the wrist (Figure 14A). This is the positive stimulating electrode and functions as a current sink or reference electrode. Connect the red lead from the Positive High Voltage Output of the stimulator to this electrode.

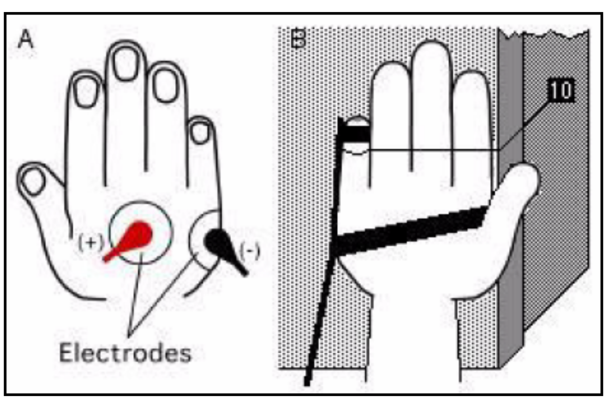


Figure 14.
A. Stimulating electrode placement on the back of the right hand.
B. Placement of the SMT-100 transducer and placement of a hanging weight across the inside of the hand.

4. Peel the protective shield off the other electrode (or use the probe electrode). Attach it slightly above the lateral edge of the right palm, about 3/8" or 1 cm onto the back of the hand and half way between the knuckle of the little finger and the wrist (Figure 14A). This electrode is the negative stimulating electrode. It is being placed where the stimulus usually elicits a large response from the little finger (the motor point). Connect the black lead from the Negative High Voltage Output of the stimulator to this electrode. If using a probe electrode, you will be able to move the electrode around to find the motor point.

5. Attach the SMT-100 striated muscle transducer (twitch sensor) along the side of the little finger (Figure 14B). Use the short Velcro strap or a piece of surgical tape to attach the tip of the sensor to the end of the little finger. Use a piece of surgical tape or a longer Velcro strap or to attach the base of the sensor along the lateral edge of the palm of the hand. Be sure the side of the transducer marked "OUTSIDE" faces away from the skin. **If using the Velcro strap across the palm, make sure that it does not slip upwards and interfere with the movement of the little finger.**

6. Click on the Stimulator Preferences icon (Figure 12) in the LabScribe toolbar to open the stimulator control panel (Figure 13). Set the parameters as seen in this table and click on the *Apply* button to complete the changes:

#pulses	pulse amplitude (Amp)	pulse width (W)	Frequency (F)
0	5.0 V	5 ms	1 Hz.

Warning: Make sure the Pulse Amplitude knob on the front of the SI-200 is set to zero.

7. Click on the *Record* button on the LabScribe Main window to activate the iWorx 214 unit and SI-200 stimulator. There should be no response from the subject's finger since the current output is zero. Continue to record.

8. Slowly rotate the Pulse Amplitude knob clockwise 1 turn, which is equal to a current output of 2 milliamperes (mA). Ask the subject to indicate the first occurrence of tingling. If no finger movement is seen, the stimulus current is below the threshold current of the most sensitive muscle fibers controlling the finger's movement. **Threshold** is the current level that is needed to create a muscle fiber contraction.

9. If a finger twitch does not occur at 2 mA, rotate the Pulse Amplitude knob an additional 1/4 turn to increase the stimulus current by 0.5 mA. Check for tingling and finger movement. Increase the current output in increments of 0.5 mA until the subject's finger twitches with the largest range of motion.

10. If the stimulus current has been raised to 5 mA and the subject feels tingling but no finger movement is observed, adjust the position of the negative stimulating electrode before making additional increases in the stimulus current.
    - Lower the stimulus current to 2.5 mA before moving the negative stimulating electrode.
    - Move the negative stimulating electrode a centimeter closer to the positive stimulating electrode.
    - Stimulate the subject's hand. Raise the stimulus current as high as 5 mA.
    - If the response from the subject's hand is miniscule, or non-existent, with 5 mA of current, place the negative electrode at different points along an imaginary line on the back of the hand, from the base of the little finger to the lateral edge of the wrist.

11. Find the lowest stimulus current that creates the largest twitch from the subject's finger, and then click *Stop* on the LabScribe Main window to turn off the stimulator.

12. Note the reading on the Pulse Amplitude knob and set the current at this level for Activity 1.

Note: The lowest stimulus that causes the largest possible response is called the **maximal stimulus**. Any stimulus that is above the maximal level is known as supra-maximal. Currents above threshold and below maximal are called sub-maximal. The amplitude required to cause a finger twitch will differ between subjects. Some subjects require as low as 3 mA of current to create a maximum response, while other subjects may require 7 mA or more to create the strongest response.

Checkpoint: Record the following measurements for the group member being stimulated.

1. What was the **threshold** stimulus needed to cause muscle contraction?

2. What was the **maximal stimulus** (the lowest stimulus that causes the larges possible response)? You will use this measurement in the following activities.

Activity 1: Single Muscle Twitch

Aim:

To become familiar with the SI-200 and SMT-100 transducer and to record a finger muscle twitch.

Procedure

1. Ask the subject to place his right hand on the bench with the palm up and relax.
2. Click the *Stimulator Preferences* icon on the LabScribe toolbar (Figure 12) to open the stimulator control panel (Figure 13) on the Main window.
3. Set the number of pulses to 15 by clicking on the arrow buttons to the right of the window or by typing in the number. The pulse amplitude (Amp) should be set to 5.0 V, the pulse width (W) to 5 ms, and the frequency (F) to 1 Hz. Click the *Apply* button to finalize the changes.
4. Set the Pulse Amplitude knob of the SI-200 to the maximal stimulus level as determined in the checkpoint above.
5. Click the *Record* button on the LabScribe Main window. Click on the *AutoScale* button on the top half (Finger Twitch channel) of the Main window to enlarge the muscle twitch tracing if needed. Click *Stop* when the stimulus pulse stops firing. There should be fifteen twitches on the recording.The bottom half of the Main window (Stimulus Trigger channel) shows the stimulus signal at a frequency of 1 Hz.

Data Analysis

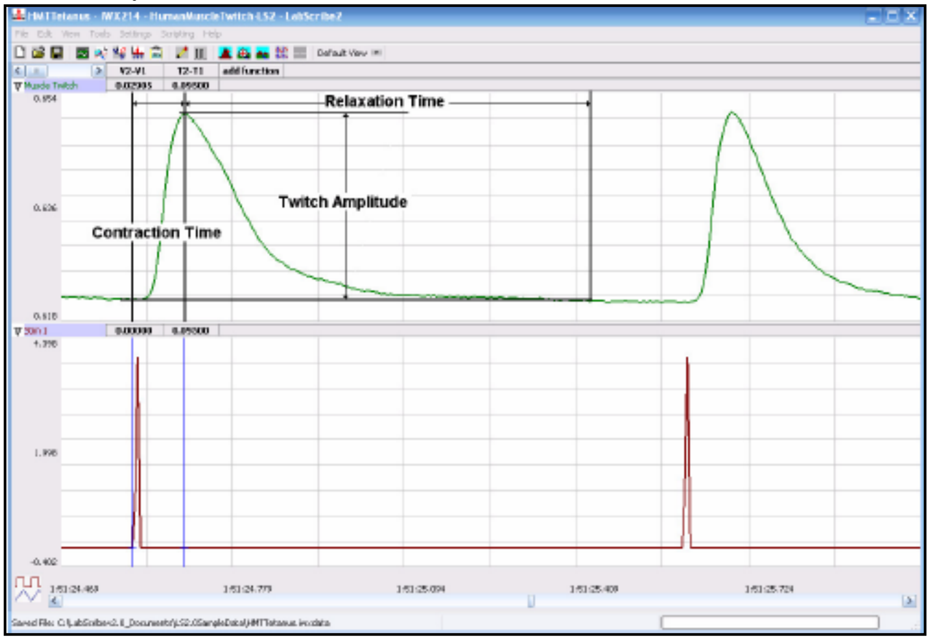


Figure 15. Finger twitches stimulated by maximal current at a frequency of 1 Hz.

1. Scroll through the recording of the finger twitches (Figure 15). Notice each finger twitch has two phases, a quick contraction phase followed by a slow relaxation phase. Since the duration of the stimulus pulses are short and their frequency is low, the muscles of the finger have the opportunity to relax between twitches as indicated by the return of the twitch amplitude to the same baseline between twitches. Use the *Display Time* (Half or Double) icons (Figure 12) to adjust the Display Time of the Main window to show at least three complete twitches on the Main window. Three adjacent twitches can also be selected by:

   • Placing the cursors on either side of a group of three complete twitches; and

   • Clicking the *Zoom between Cursors* button on the LabScribe toolbar to expand the three twitches to the width of the Main window.

2. Click on the *Analysis window* icon in the LabScribe toolbar (Figure 12) or select *Analysis* from the View menu.

3. On the Finger Twitch channel, use the mouse to click on and drag the cursors to specific points on the recording to measure the following parameters:

• **Latent Period**. To measure the latent period, place one cursor on the peak of the stimulus signal in the lower Stimulus Trigger channel, and the second cursor on the baseline at the point just before the twitch starts to develop in the upper Finger Twitch channel. On the Finger Twitch channel, the value for T2-T1 is the latent period of that twitch measured in seconds. Record this value in the Journal (click on *Journal* icon) or on the post lab data sheet. To record in the Journal after opening it, click on the value for T2-T1 and select *Add Title to Journal*. Change the Title to Latent Period. Next, click on the value for T2-T1 again and select *Add Ch. Data to Journal*. Erase the value for V2-V1 on the Journal.

• **Twitch Amplitude**. To measure the amplitude of the twitch, place one cursor on the baseline at the point just before the twitch starts to develop, and the second cursor on the peak of the twitch. On the Finger Twitch channel, the value for V2-V1 is the amplitude of that twitch measured in volts (Figure15). Record this value in the Journal (click on *Journal icon*) or on the post lab data sheet. To record in the Journal after opening it, click on the value for V2-V1 and select *Add Title to Journal*. Change the Title to Twitch Amplitude. Next, click on the value for V2-V1 again and select *Add Ch. Data to Journal*.

• **Contraction Time**, which is the time it takes the amplitude of the twitch to rise to its peak. To measure the contraction time of the twitch, keep the cursors in the same positions used to measure the twitch amplitude. On the Finger Twitch channel, the value for T2-T1 is the contraction time of that twitch (Figure 15). Follow the directions above to record the contraction time in the Journal.

• **Relaxation Time**, which is the time it takes the amplitude of the twitch to return to baseline. To measure the relaxation time of the twitch, place one cursor on the peak of the twitch, and the second cursor at the point where the amplitude returns to the baseline. On the Finger Twitch channel, the value for T2-T1 is the relaxation time of that twitch (Figure 15). Follow the directions above to record the relaxation time in the Journal.

• **Length of the Entire Twitch**. To measure the length of the entire twitch, place one cursor on the peak of the stimulus signal in the lower Stimulus Trigger channel, and the second cursor at the point where the amplitude returns to the baseline. On the Finger Twitch channel, the value for T2-T1 is the relaxation time of that twitch. Follow the directions above to record the length of the entire twitch in the Journal.

Activity 2: Threshold Stimulus and Motor Unit Recruitment: The Effect of Stimulus Strength on Tension Development (Twitch Amplitude)

Aims:

1. To determine the threshold stimulus strength for the abductor digiti minimi
2. To examine the effect of stimulus size on the amplitude of finger movement.

Procedure

1. Remind the subject to relax and place the hand that is used for the experiment on the bench, with the palm up.
2. Open the stimulator control panel on the LabScribe Main window. Set the number of pulses to 0, the duration of the stimulus pulses to 5 milliseconds (ms), and the frequency to 1 Hz. Setting the number of pulses to zero makes the SI-200 stimulus isolator fire continuously. Click the *Apply* button on the stimulator control panel to save the changes.
3. Make sure the Pulse Amplitude knob on the front of the SI-200 is set to zero. Type Zero in the Mark box that is to the right of the *Mark* button. Click the *Record* button on the LabScribe Main window. Press the *Enter* key on the keyboard to mark the recording.
4. While recording, type 0.5 mA in the Mark box that is to the right of the Mark button. Increase the current output of the SI-200 by 0.5mA by rotating the Pulse Amplitude knob half a turn. Press the *Enter* key on the keyboard to mark the recording. Record at this stimulus current for ten to fifteen seconds.
5. Repeat Step 4, in increments of 0.5 mA, until the stimulus reaches the maximal level as determined previously (when the finger deflection reaches a maximum and stops increasing). Click the *Stop* button.

Data Analysis

1. Scroll to the beginning of the data for this activity. Locate the segment of the finger twitches that occurred at threshold stimulus current (where first see a muscle twitch recorded). Click *AutoScale* to maximize the size of the twitch on the window. For any stimulus currents that did not cause finger movement, report the amplitude of the finger twitch as zero.
2. Use the *Display Time* (Half or Double) icons to adjust the Display Time of the Main window to show a segment with four or five twitches on the Main window. The twitches can also be selected by:

   • Placing the cursors on either side of the selected twitches; and

   • Clicking the *Zoom between Cursors* button on the LabScribe toolbar to expand the segment of twitches to the width of the Main window.
3. Click on the *Analysis* window icon in the LabScribe toolbar (Figure 12) or select *Analysis* from the View menu to transfer the data displayed in the Main window to the Analysis window.

4. Use the same techniques used in Exercise 1 to measure and record the amplitudes (V2-V1) of three twitches recorded for each stimulus current that causes a response. Data can be entered in the Journal.

5. Repeat these amplitude measurements for each stimulus current tested. You will have three measurements for each stimulus current level.

6. On a piece of graph paper or in a graphing program, plot the average amplitude of the finger twitch at each stimulus current as a function of the stimulus current (Figure 16).

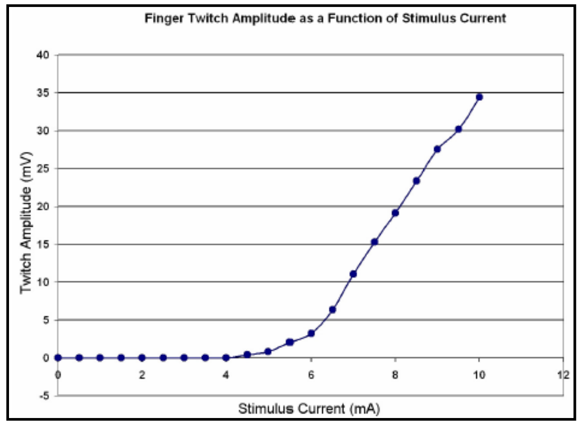


Figure 16. Average finger twitch amplitude as a function of the stimulus current.

Activity 3: The Effect of Load on Isotonic Contractions

Aim:

To determine the amount of work performed by the finger with different weights, or loads, attached to the finger.

Procedure

1. Remind the subject to relax and place the hand that is used for the experiment on the bench, with the palm up.

2. Click the *Stimulator Preferences* icon on the LabScribe toolbar (Figure 12) to open the stimulator control panel (Figure 13) on the Main window.

3. Set the number of pulses to 15 by clicking on the arrow buttons to the right of the window or by typing in the number. The pulse amplitude (Amp) should be set to 5.0 V, the pulse width (W) to 5 ms, and the frequency (F) to 1 Hz. Click the *Apply* button to finalize the changes.

4. Set the Pulse Amplitude knob of the SI-200 to the maximal stimulus level as used in Activity 1.

5. Attach a thread to the last joint of the finger being used in the experiment. The thread should be long enough to go across the ring, middle, and index fingers of the same hand, and also allow the weight to hang over the edge of the table. Attach a 5 g weight to the other end of the thread.

6. Have the subject move his or her hand to the edge of the table, so the palm is up and the thread with the weight is hanging along the side of the table (Figure 14B).

7. Type the size of the weight which is on the thread in the Mark box that is to the right of the *Mark* button. Click the *Record* button on the LabScribe Main window. Press the *Enter* key on the keyboard to mark the recording. Click *Stop* when the stimulus pulse stops firing. There should be fifteen twitches on the recording.

8. Change to a 10 g weight on the end of the thread and repeat Step 7. Repeat for each of the weights up through the 50 g weight or until the amplitude of the finger twitch in undetectable.

Data Analysis

1. Scroll to the beginning of this section of data, and locate the response of the finger while it was lifting the lowest weight. Click *AutoScale* to maximize the size of the response on the window.

2. Use the *Display Time* (Half or Double) icons in the LabScribe toolbar to adjust the Display Time of the Main window to show a segment with four or five twitches on the Main window. The twitches can also be selected by:

• Placing the cursors on either side of the selected twitches; and

• Clicking the *Zoom between Cursors* button on the LabScribe toolbar to expand the segment of twitches to the width of the Main window.

3. Click on the *Analysis* window icon in the LabScribe toolbar (Figure 12) or select Analysis from the *View* menu to transfer the data displayed in the Main window to the Analysis window.
4. For each weight, measure the twitch latent period and the total contraction time. Data can be recorded in the Journal.
5. For each weight, determine the average amplitude of three finger twitches. Use the same techniques used in Activity 1 to measure and record the amplitudes (V2-V1) of the twitches. Data can be recorded in the Journal.
6. Use the average twitch amplitude at each weight to calculate the work performed by the muscle on that weight. For this exercise, use the following equation:

   Work = Weight (g) x Average Amplitude of Twitch (mV) with that Weight

7. On a piece of graph paper or in a graphing program, plot the work performed at each weight as a function of the weight attached to the finger (Figure 17).

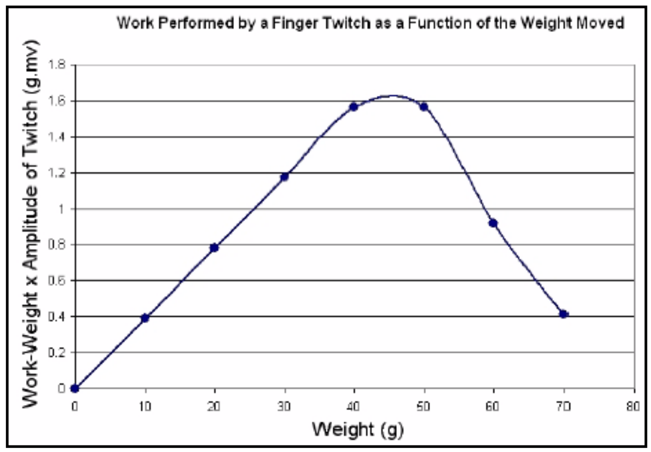


Figure 17. The relationship between work performed at each weight and the weight moved by a finger twitch.

Activity 4: Summation and Tetanus

Aim:
To monitor the contraction and relaxation of the finger twitch in relation to different stimulus frequencies.

Procedure

1. Remind the subject to relax and place the hand that is used for the experiment on the bench, with the palm up.
2. Open the stimulator control panel on the LabScribe Main window. Set the number of pulses to 15, the duration of the stimulus pulses to 5 milliseconds (ms), and the frequency to 1 Hz. Click the *Apply* button on the stimulator control panel to save the changes.
3. Use the Pulse Amplitude knob of the SI-200 to set the stimulus current to the maximal level as used in Activity 1.
4. Type 1 Hz in the Mark box to the right of the Mark button. Click the *Record* button on the LabScribe Main window. Press the *Enter* key on the keyboard. Click *Stop* when the stimulus pulse stops firing. There should be fifteen twitches on the recording.
5. Change the stimulus frequency to 2 Hz. Go to the stimulator control panel on the LabScribe Main window, and change the value in the stimulus frequency box, labeled F (Hz), from 1 to 2. Click the up arrow in the box to increase the frequency. The frequency can also be changed by typing the new value into box. Click the Apply button to put any frequency change into effect.

6. Repeat Step 5 with the stimulus frequency set to 2 Hz. Type 2 in the Mark box that is to the right of the Mark button.

7. Repeat Step 5 for stimulus frequencies of 3, 4, 5, 10, 15, and 20 Hz. At higher frequencies, you will also need to increase the number of pulses sent to the subject's finger to see the effects of summation and tetanus. For example, it may take a few seconds to see the complete effect of tetanus at 20 Hz. So, the total number of pulses may need to be set to 40 or higher to see the complete effect (Figure 18). Remember to click the *Apply* button to effect changes.

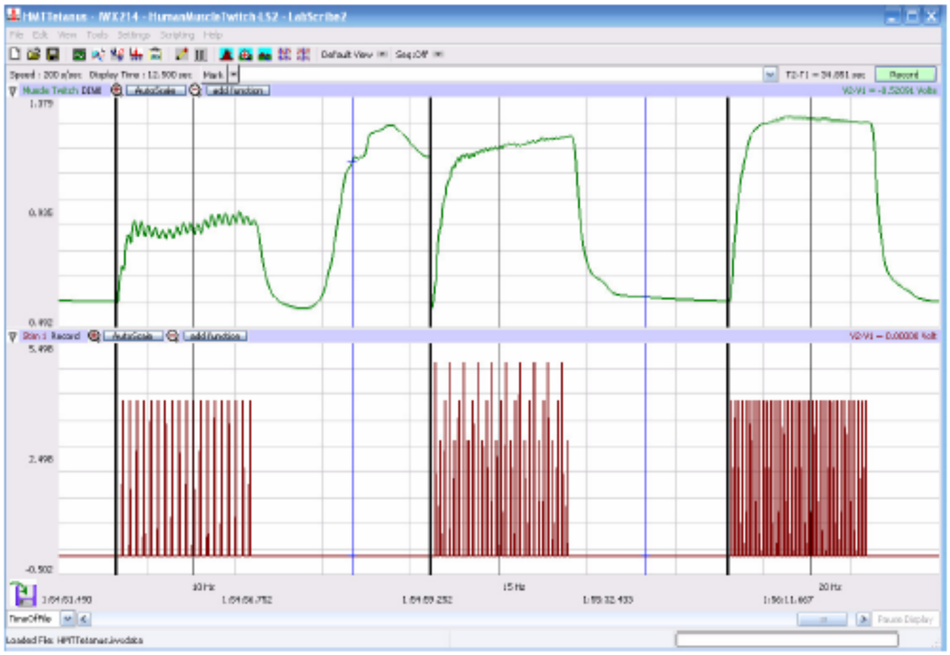


Figure 18. Amplitudes of finger twitches at 10, 15, and 20 Hz from left to right. Recordings from 10 and 15 Hz show incomplete tetanus. Complete tetanus occurs at 20 Hz.

8. When performing the experiment at the highest frequency of 20 Hz, keep recording after complete tetanus is reached. Remove the cable from the stimulator of the iWorx 214 to stop the SI-200 stimulus isolator from firing. Continue to record as the tension in the muscles of the finger begins to relax.

9. When the muscles in the finger are relaxed, click *Stop* to halt the recording.

Data Analysis

1. Locate the segment of the finger twitches that occurred at a frequency of 1 Hz. Click *AutoScale* to maximize the size of the twitches on the window.

2. Use the *Display Time* (Half or Double) icons in the LabScribe toolbar to adjust the Display Time of the Main window to show a segment with four or five twitches on the Main window. The twitches can also be selected by:
   - Placing the cursors on either side of the selected twitches; and
   - Clicking the Zoom between Cursors button on the LabScribe toolbar to expand the segment of twitches to the width of the Main window.

3. Click on the *Analysis* window icon in the LabScribe toolbar (Figure 12) or select *Analysis* from the View menu to transfer the data displayed in the Main window to the Analysis window.

4. Use the same techniques used in Activity 1 to measure and record the amplitudes (V2-V1) of two adjacent twitches recorded at a stimulus frequency of 1 Hz. Data can be recorded in the Journal.

5. Move to the segment of data recorded at the next stimulus frequency. If the twitches at this frequency are all about the same amplitude, measure and record the amplitudes of two adjacent twitches.
6. Repeat Step 5, until the recording of the finger twitches show summation. Summation occurs when the time between twitches is not long enough to allow the muscle to completely relax to its baseline level of tension. Another twitch or contraction, following in quick succession to the first, will add on the first twitch at the current level of tension for the first twitch. The third twitch will add on to the current tension of the second twitch, and so on (see Figure 19).
7. At stimulus frequencies where summation occurred, measure and record the amplitude of the first twitch in the series and the amplitude of the tallest twitch in the series (Figure19).
8. If a level of relatively constant tension occurs during contractions at high frequency, the phenomenon is called tetanus. If small relaxations are still detectable along the level of constant tension, a state of incomplete tetanus exists (5 Hz in Figure19; 10 and 15 Hz in Figure 18). If there are no detectable relaxations along the region of constant tension, a state of complete tetanus exists (20 Hz in Figure 18).

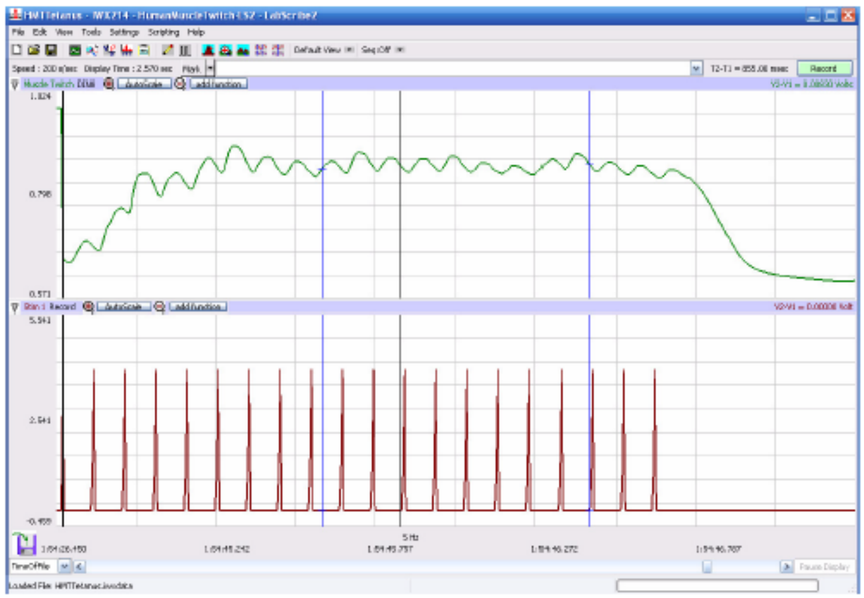


Figure 19. Summation of finger twitches occurring at a stimulus frequency of 5 Hz.