

**Introduction Overview**

The overall purpose of this lab was to take a closer look at the various properties of skeletal muscle and prove or disprove the physiological mechanisms that they have been suggested to have. To do this, 3 experiments designed to test different aspects of skeletal muscle were formed; the effect of stimulus intensity and frequency, the effect of injecting a muscle relaxant (tubocurare), and the effect of bypassing a neuron and directly stimulating the muscle. By the end of the experiment, a deeper understanding of why the results collected should be acquired, why changing variables had the effect they did, as well as the physiological principles that are responsible for them. Throughout the experiment, force was consistent as a dependent variable in order to compare and contrast different variables that could have an impact on muscle.

**Introduction: Stimulus Intensity and Frequency**

The first part of the experiment dealt with two independent variables, stimulus intensity and frequency. This experiment is based off of the proposed muscle property that muscle tension depends on both the number of muscle fibers contracting and the individual contracting force each of these fibers can provide (Sherwood, 2013, 275). With this in mind, it was expected for muscular contraction, measured in force (g), to increase in proportion with either stimulus intensity or frequency due to action potentials being summed together (Sherwood, 2013, 276).

**Introduction: Muscle Relaxants**

The second part of the experiment tests the effects of a muscle relaxant, tubocurare in this case, on the activity of skeletal muscle in order to highlight physiological mechanisms behind the signal transmission between nerves and skeletal muscle and what may occur when that signaling pathway is interrupted (Heier, 2010, 398). It is expected for muscle activity, measured in force (g) to decrease as a result of less than normal conditions under which a muscle operates.

**Introduction: Direct Stimulation of Skeletal Muscle**

In the last portion of this experiment, the effects of bypassing a neuromuscular junction in order to compare and contrast the differences, if any, between the threshold and maximum threshold of direct stimulation versus indirect stimulation, as tested during the stimulus frequency and intensity portion of the lab. Since neural pathways are being completely bypassed and an action potential has no way of spreading across a motor unit, it is expected for the direct stimulation muscle to be inefficient relative to indirect stimulation (Saladin, 2007, 412).

## **Materials and Methods**

### **Stimulus Intensity and Frequency**

The effect of stimulus intensity and frequency on skeletal muscle was observed by using the stimulator to provide stimulus to the sciatic nerve. An in-depth procedures list can be found in the lab manual listed under the section “The Effect of Stimulus Intensity on Muscle Activity: Threshold and Maximum” and “The Effect of Stimulus Frequency on Muscle Activity: Summation”.

### **Materials and Methods: Muscle Relaxants**

Tubocurarine into the frog's calf muscles and force was measured without the injection, start of the injection, and end of the injection. A more detailed procedure can be found in the lab manual under the section “The Effect of Tubocurarine on Muscle Activity: Paralysis”.

### **Materials and Methods: Direct Stimulation of Skeletal Muscle Activity**

The effects of direct muscle stimulation were observed by using needle electrodes implanted into the frogs calves and inputting stimulus via a stimulator. A more detailed procedure can be found in the lab manual under the section “The Effect of Direct Electrical Stimulation on Muscle Activity”.

### Experimental Deviations:

At the beginning of the experiment, the sciatic nerve of the frog specimen was stretched and extended to a high degree during the dissection of the lab. This could potentially have skewed results or not allowed for as much longevity of the nerve during stimulation tests.

### Results

#### Differences in threshold between Indirect vs. Direct Stimulation

Both threshold and maximum threshold were relatively low when a muscle was indirectly stimulated through the sciatic nerve (Table 1).

Table 1. Threshold and maximum threshold tested through indirect stimulation via a frog's sciatic nerve using a force transducer on single-fire mode, producing single twitches. Data was collected and analyzed through Biopac software. Max value functions were used to produce both the threshold and maximum threshold values.

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	Threshold	Maximum Threshold
<b>Voltage (V)</b>	0.8	1.25
<b>Force (g)</b>	29.81	130.24

When compared to direct stimulation (Table 2), 5-40 times more voltage was needed to reach the threshold and max threshold values. Note that the force generated was lower in direct stimulation for both threshold and maximum threshold values.

Table 2. Threshold and maximum threshold tested through direct stimulation of a frog's thighs connected to hook electrodes using a force transducer and stimulator on single-fire mode, producing single twitches. Data was collected and analyzed through Biopac software. Max value functions were used to produce both the threshold and maximum threshold values.

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	Threshold	Maximum Threshold
<b>Voltage (V)</b>	5.1	42.5
<b>Force (g)</b>	32.46	55.91

### The Effect of Increased Stimulus Frequency on Skeletal Muscle Activity

As stimulus frequency increased, force generated increased proportionately until a plateau at 8pps was reached. When frequency was faster than 8pps, the plateau remained constant until a slight drop in force could be observed beginning at 15pps (Figure 1).

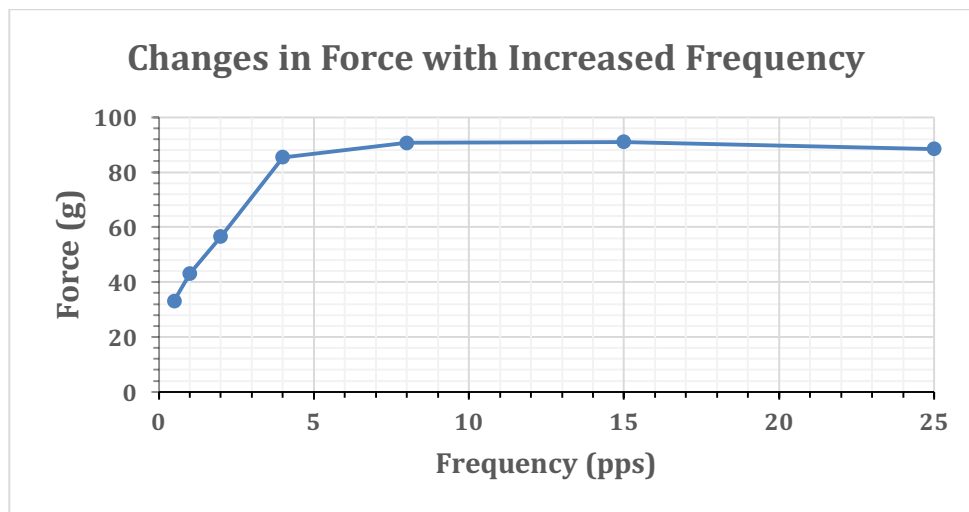
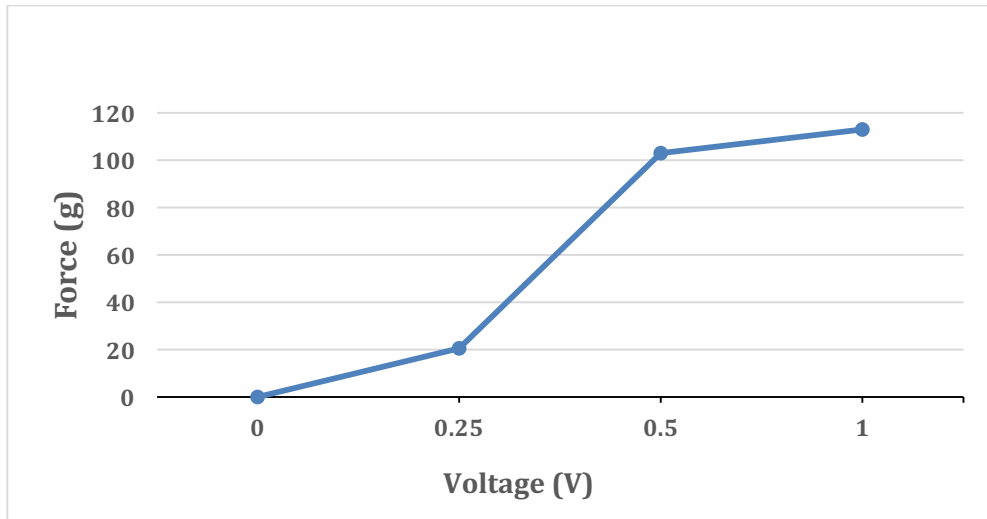


Figure 1. Changes in max force produced with increasing stimulus frequency tested indirectly through a frog's sciatic nerve via a force transducer, stimulator, and recorded and analyzed using Biopac software. Max force produced increased between 0.5pps, 2pps, 4pps, with a plateau being reached at 8pps and sustained at 10pps, with a gradual decrease at 15pps and 25pps.

### The Effect of Stimulus Intensity on Skeletal Muscle Activity

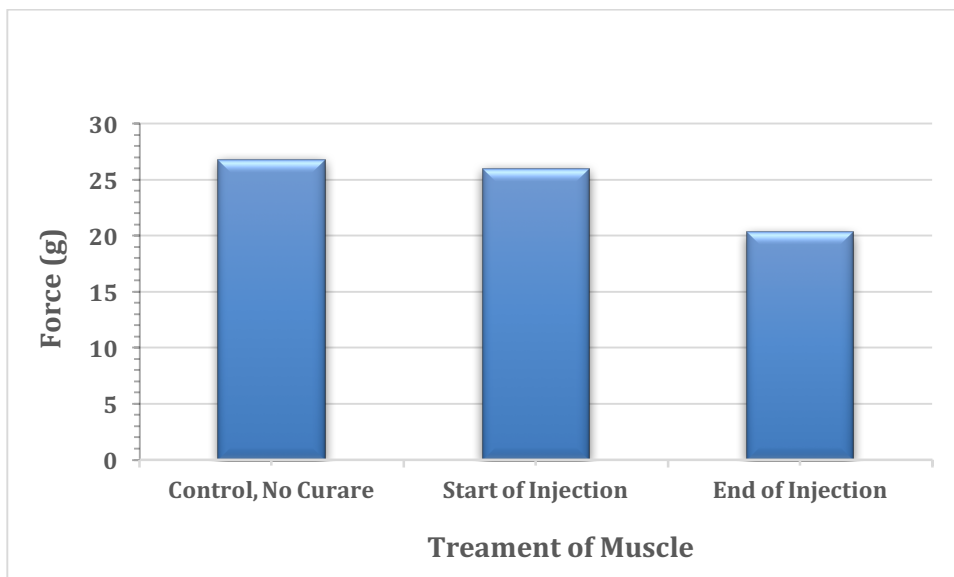
Stimulus intensity was increased by a calculated delta V (.10v) until maximum threshold was reached. Each successive increase by delta V produced exponential increases in force between .0, .1v, 2.v, 3.v, .5v until the amount of increase decreased between \_\_\_\_\_ ((Figure 2).



**Add Title** Figure 2. Changes in max force produced through increasing stimulus intensity tested indirectly through a frog's sciatic nerve using a force transducer, stimulator, and recorded and analyzed with Biopac software. Max force produce increased significantly between 0.25-5v with the increase being much smaller between 0.5v-1.0v.

### The Effect of Tubocurare on Skeletal Muscle Activity

The effect of tubocurare, a muscle relaxant, was observed by injecting the compound directly into the frog's calves and measuring force production. By the end of the treatment, force production had decreased.



**Add Title** Figure 3. Mean force recorded every 30 seconds in a frog's calves using a force transducer, stimulator, and recorded and analyzed with Biopac software. Recordings of the muscle made for no curare treatment, at the start of the treatment, and end of the treatment. Force production with the curare treatment resulted in lower force production.

## **Discussion**

### **Stimulus Intensity vs. Frequency on Skeletal Muscle Activity**

During the experiment, the effects of both stimulus intensity and stimulus frequency on skeletal muscle as a function of force were observed. Although the results seem similar in that both saw a proportional increase in force as intensity and frequency, the physiological mechanisms behind stimulus intensity versus frequency are quite different. In the case of stimulus intensity increasing, where voltage is increased via the stimulated, the result of higher voltage is that more muscle fibers in the motor unit are stimulated and contracted (Saladin, 2007, 424). Thus, more force is created, as seen in Figure 2. This is also known as motor unit recruitment (Saladin, 2007, 424). In other words, stronger contractions are a result of more motor units being stimulated and recruited to contract at the same time (Sherwood, 2013, 274).

However, even when stimulus intensity (voltage) remains constant, the contractile strength can still be increased through stimulus frequency (Saladin, 2007, 425). Increasing stimulus frequency increases force due to a muscle not being allowed to completely recuperate before it is restimulated with another action potential, which results in the potentials summing together and ultimately resulting in greater production of force in a process known as twitch summation (Sherwood, 2013, 275). This could be seen in Figure 1 between 0.5pps and 8pps, where increased stimulus frequencies of the stimulus resulted in greater force being generated as a result of many action potentials being summed together. At 8pps and 15pps, the contractile force peaks and remains constant. This is due to a physiological state called tetanus, where a muscle fiber is stimulated so rapidly that there is absolutely no time to recover between action potentials and a constant sustained contraction occurs (Sherwood, 2013, 276). During tetanus, all cross-

bridges have been binded in order to produce a maximum sustained contraction (Sherwood, 2013, 276). At 25pps, a decline in force is seen due to a physiological condition called muscle fatigue. At this point, there are no additional cross-bridge sites and not enough  $\text{Ca}^{2+}$  being released by the sarcoplasmic reticulum into the cytosol to bind with troponin and keep tropomyosin away from its blocking position, which is absolutely needed in order to create a muscular contraction in skeletal muscle and as a result, muscular fatigue and a decrease in contractile force occurs (Sherwood, 2013, 276).

It is important to note that the Henneman Size Principle, which states that in voluntary muscular contractions, smaller units composed of slow-twitch fibers and are more fatigue-resistant are fired first, could not be observed during this lab (Knaflitz, et al., 1990, 1662). This is due to the widely accepted principle that during artificially stimulated contractions, such as those through electrical stimulation, the principle is reversed, so that large-units that are less resistant to fatigue are fired first. This could be seen in Tables 1,2 and Figures 1,2 where relatively small increases in stimulus intensity or frequency resulted in large increases in force produced due to less resistant (larger) muscle fibers being fired first (Knaflitz, et al., 1990, 1664).

### **The Effect of Tubocurare on Skeletal Muscle**

When an action potential is elicited in a motor neuron, it must first cross a physical gap between the neuron and muscle cell known as the synaptic cleft before it can be propagated to the muscles it innervates (Saladin, 2007, 431). Acetylcholine (Ach), located on the surface of muscle cells, is designed to overcome this physical barrier. Vesicles containing Ach release it into the synaptic cleft through exocytosis, essentially creating a chemical link between the nerve and muscle cells (Saladin, 2007, 465). In Figure 3, the control represents a frog's calf muscle which has not been injected with any curare and exhibits normal muscle function. At the start of the injection of

curare, a decrease in force generated was observed. When curare is injected into the muscle, it competitively competes with Ach to bind with the Ach receptors located in the neuromuscular junction (Saladin, 2007, 414). As a result, a deficiency in Ach will eventually lead to the complete paralysis of the muscle (Saladin, 2007, 414). In Dr. Heier's research on muscle relaxants, such as curare, it was discovered that the binding of one curare molecule is sufficient to prevent the binding of an ion channel necessary for muscular contraction to occur (Heier, 2010, 398). From the data collected during the experiment, force decreased from 26 grams to 20 grams. Based on the amount of curare injected, it was expected for the force to drop much more drastically and eventually not be able to generate force at all, or complete paralysis, but it did not do so. This could be due to a larger than average amount of Ach that may have already been present in the frog's calves prior to the treatment. This amount of Ach, in conjunction with the muscles continued natural production of the neurotransmitter, would counteract the effects of curare and eventually allow for normal function of the muscle after some time has passed (Heier, 2010, 398).

### **Direct Electrical Stimulation of Skeletal Muscle**

Using a hook electrode connected to the stimulator and directly into the frog's thighs, it was possible to elicit a muscular contraction. Compared to the indirect neural stimulation that was performed earlier in the experiment, it took a significant amount more stimulus in order to reach the observed threshold and maximum threshold. It took 5.1 volts via direct stimulation to reach threshold while only 0.8 volts was needed when stimulated indirectly through the sciatic nerve as seen in Tables 1 and 2. Likewise for maximum threshold, 42.5 volts via direct stimulation was needed while indirect stimulation required relatively a much lower 1.25 volts, as seen in Tables 1 and 2. In a review article by Doucet, Lam, and Griffin on neuromuscular electrical stimulation on

skeletal muscle function, it is proposed that although electrical stimulation provides an alternative for muscular contraction for those with neural damage, it is less efficient than the normal physiological pathway (Doucet, et al., 2012, 208). The reason behind this is because neurons are designed to be both the translators and messengers of physical and chemical signals to other parts of the body, whether it be muscle, the CNS, and so forth (Gemes et al., 2013, 1111). In Dr. Gemes' research on the failure of action potential propagation in sensory neurons, it was proposed that the failure for an action potential to propagate was linked with a decrease in somatic cell input resistance (Gemes et al., 2013, 1111). This decreased sensitivity to resistance highlights the importance of neurons and their vital role in normal function of physical processes. Motor neurons are the pathway for action potentials to travel by which the CNS is able to influence and control skeletal muscle and thus, if their pathways are blocked, damaged, or in this case, completely bypassed, optimal and efficient body processes, including skeletal muscle contraction, cannot be accomplished (Sherwood, 2013, 248).

In this experiment, by bypassing the neuron completely and stimulating muscular contraction directly to the muscle, all the efficiency a neuron has to offer in spreading an action potential throughout an entire motor unit is lost (Sherwood, 2013, 248). As a result, instead of only having a neuron brought to threshold and having the action potential spread throughout the entire motor unit and induce contraction, many individual muscle fibers had to be brought to threshold and thus, more stimulus was required to do so. In other words, it is much more efficient to induce muscular contraction via a neuron than direct stimulation to the muscle.

It is important to note that the force generated between threshold and max threshold between indirect stimulation and direct stimulation was much lower in the former. As seen in Figure 1, indirect stimulation yielded forces of 29.81 grams at threshold and 130.24 grams at max

threshold. Direct stimulation yielded values of 32.46 grams at threshold and 55.91 grams at maximum threshold. Several reasons may have been the cause for this discrepancy. First, the indirect stimulation portion of the lab was performed first, while the direct stimulation portion last. The lower force values may be the result of the frogs muscles already fatigued from the constant stimulation throughout the lab. Additionally, in conjunction with the proposed idea that direct electrical stimulation is not as efficient as indirect neural stimulation, the lower force values can be explained (Doucet, et al., 2012, 208).