

## **Skeletal Muscle and Twitch Response**

**Introduction:**

In order for muscle in the body to move, a series of events takes place within the body. These events involve both muscles and neurons. When a stimulus is present, the central nervous system (CNS) sends a signal or an action potential (AP) through the neurons. When this action potential reaches the muscle junction,  $\text{Ca}^{2+}$  is able to enter the terminal axon, which causes a neurotransmitter, acetylcholine (ACh), to be released and bind to the muscle fiber (Sherwood, 2010, pg. 246-247). When ACh is bound to the muscle fiber, the fiber depolarizes and initiates an AP. This action potential can be blocked from proceeding if ACh is not able to bind with its receptors. Tubocurarine is an antagonist which competes with ACh in binding to the receptors and when this drug out competes ACh, twitch tension decreases, contraction response decreases and paralysis can occur (Sherwood, 2010, pg. 252-253).

Without an inhibitor, ACh can bind to the receptors and an action potential is generated. This AP then travels down the transverse tubules (T tubules), which run on the surface of the muscle fiber, and activates the sarcoplasmic reticulum, which then releases intracellular calcium into the muscle cytosol. This  $\text{Ca}^{2+}$  causes a transformation in the troponin-tropomyosin complex covering the actin molecules. This change exposes the binding sites on actin, allowing myosin to bind. Once these molecules have bound, or formed a cross-bridge, the myosin pulls on the actin molecule causing a "power stroke" or a contraction (Matsuo et. al, 2008, p1019). It takes many myosin and actin units working together to contract one muscle fiber and cause a twitch.

Twitch tension, or the strength of the twitch, can vary depending on the number of twitches occurring together, the more twitches together the stronger the tension. Tension can also be increased by recruiting more motor units. When many muscle fibers act simultaneously, they stimulate a muscle unit, and when more strength is required muscle units summate, which is

known as muscle summation (Sherwood, 2010, pg. 270). When multiple twitches occur, due to increased stimuli and therefore more than one action potential, the twitches add on top of each other until maximum tension or tetanus occurs.

The goals of this experiment were to understand how changes in stimulus intensity and frequency impacts contraction, as well as to understand how neurotransmitters, such as ACh, impact twitch response. Also to see the effects on muscle contraction when directly stimulated by an electrode.

The experimental subject is a frog that is double-pithed or brain dead so that the muscles still function but the frog cannot stimulate its own muscles.

In this experiment it was hypothesized that when stimulation in the form of voltage increases, twitch magnitude also increases until a maximum force is achieved. As stimulation frequency increases, it was expected that the twitches will build upon each other leading to tetanic contraction. When adding tubocurare, it was expected to inhibit the neurotransmitter ACh and therefore no contractions would occur. Lastly, when directly stimulated by an electrical charge, the neurotransmitter pathway would be bypassed and a contraction would occur despite the inhibitor.

### **Methods:**

The procedure and methods used for the experiment can be found in "NPB 101L Physiology Lab Manual" (2009) Exercise 2, Properties of Skeletal Muscle on pg. 9-17. For the experiment a small frog was studied to examine skeletal muscle function and how electrical impulses to the sciatic nerve leads to muscle tension. Prior to the experiment, the frog was pithed. The central nervous system was destroyed so that the frog could not control the movements of its own muscles. Throughout the experiment the frog was kept wet with distilled water and once the

frog's leg muscle was exposed to the air, Ringer saline solution was applied. This solution had high levels of  $\text{Ca}^{2+}$ ,  $\text{Na}^{+}$  and  $\text{K}^{+}$  ions and used to delay the dying of the muscle tissue.

In order to expose the gastrocnemius muscle, the skin was removed. The gastrocnemius was separated from the leg but remained attached at the heel. The muscle was then hung at a  $90^{\circ}$  angle to the force transducer. The transducer was altered so that the tension was set to approximately twenty grams throughout the experiment. The sciatic nerve was identified and an electrode was placed under the nerve.

The next step was to identify threshold and maximum voltage. To do this, voltage was increased until a twitch was observed, this point was marked threshold. Voltage continued to increase until there was no longer an increase in observed force, this was marked maximum voltage. To observe graded response the voltage was increased by a calculated value,  $\text{max voltage} - \text{threshold voltage} / 5$ , and increased every ten seconds. To see twitch summation, stimulation frequency was increased. To examine paralysis, the muscle was stimulated then tubocurare was injected and observed for ten minutes. Lastly, to see direct electrical stimulation, two electrodes were inserted directed onto the gastrocnemius and voltage was increased until a twitch appeared.

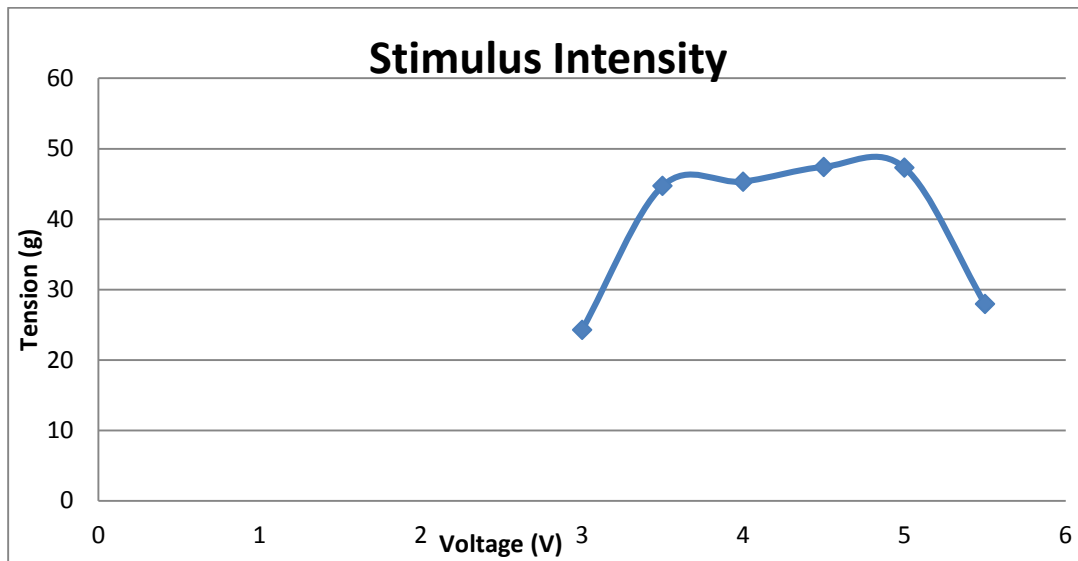
## **Results:**

### **Part 1: Effects of stimulus intensity on muscle activity.**

The initial tension from the frog leg was about 19.95grams. Voltage was increased until a twitch was observed, this was recorded as the threshold twitch, which was seen at 3.0V and the force was recorded at 24.26 grams.  $V_{\text{max}}$  was found at 5.5V and the force generated was 98.52 grams. To observe the effect of stimulus intensity on the activity of the muscle, voltage was set to threshold voltage (3.0V) and the volts were increased in increments of 0.5V. This increment was calculated by subtracting threshold voltage from maximum voltage and dividing by 5.

$$\Delta V = \frac{V_{\text{maximum}} - V_{\text{threshold}}}{5} = \frac{5.5 - 3.0}{5} = 0.5V$$

As the voltage stimulating the sciatic nerve increased, the tension increased until a drop in tension was observed, this can be seen in figure 1. The data for the threshold, Vmax and  $\Delta V$  can be found in table 2.



**Figure 1:** Graph of voltage (V) vs. tension (g). As voltage increased, the force, also known as tension of the twitch, increased until approximately where Vmax should have appeared and then tension dropped off. The voltages were recorded in increments of 0.5V

**Table 1:** Voltages produced various amounts of tension (g) when the sciatic nerve of the frog was stimulated.

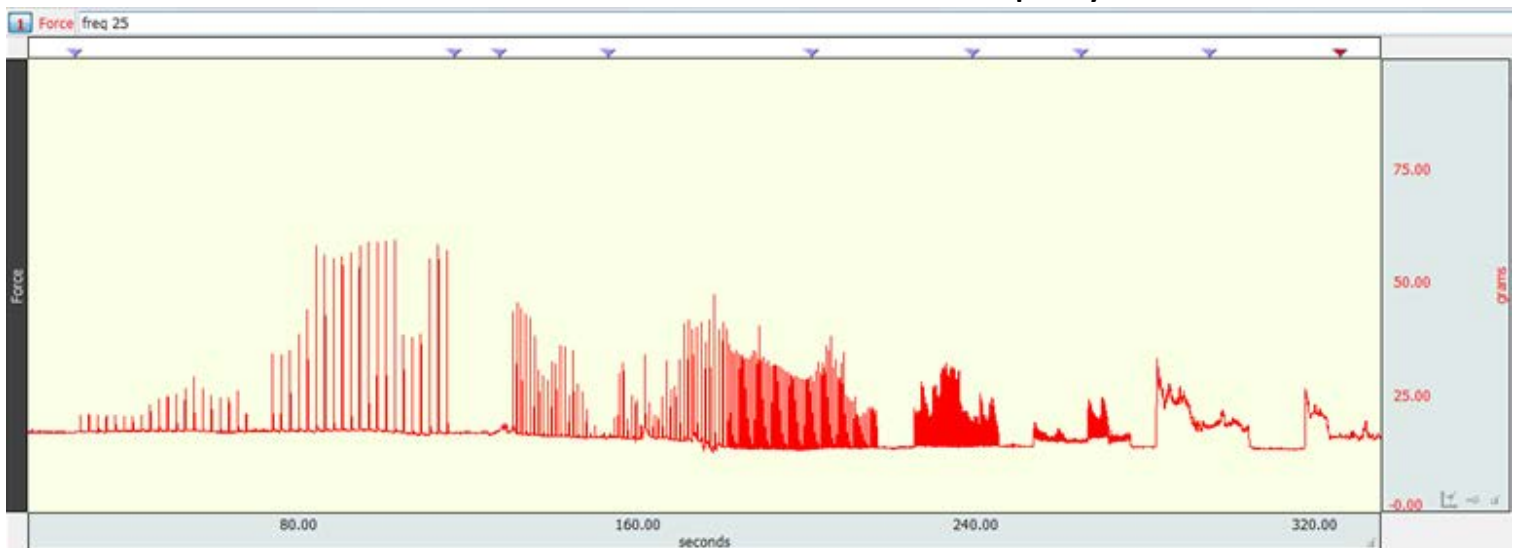
Voltage (V)	Tension (g)
3	24.26
3.5	44.7
4	45.32
4.5	47.41
5	47.29
5.5	27.96

**Table 2:** Threshold voltage, maximum voltage and the change in the voltage

V <sub>threshold</sub>	3 Volts
V <sub>maximum</sub>	5.5 Volts
$\Delta V$	0.5 Volts

**Part 2: Effects of stimulus frequency on muscle activity**

When the frequency of the stimulus increased, the twitches occurred closer together until waves appeared. Frequency was administered in increasing increments that can be seen in table 3 and wave formation can be seen in figure 2.

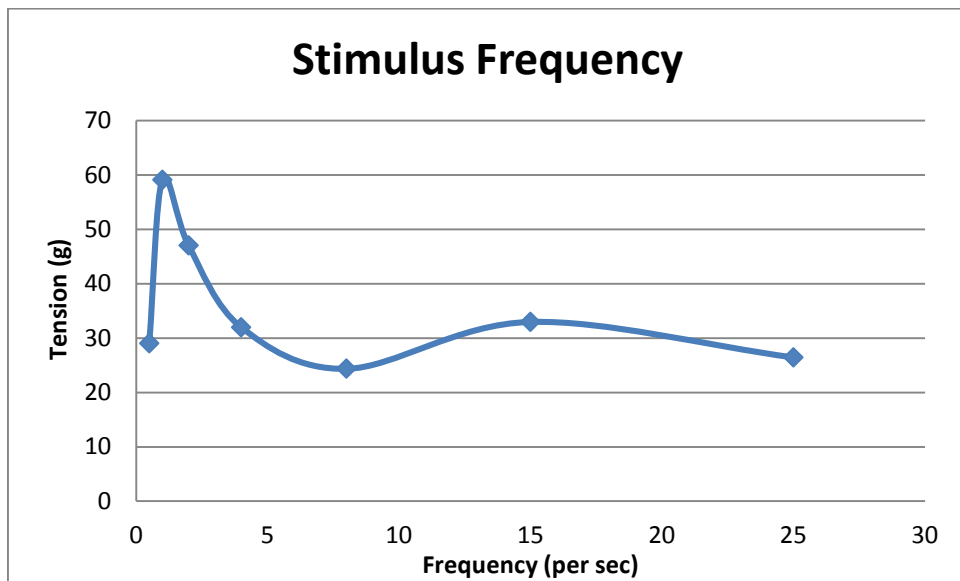
**Raw Data: Increased Stimulus Frequency**

**Figure 2:** Raw data of muscle twitches at increasing frequencies of 0.5,1,2,4,8,15 and 25pps. The y-axis represents force of each contraction in grams and time is seen on the x-axis. As the frequency increased, twitches occurred more often and merged together to eventually form a wave.

When increasing stimulus frequency, voltage was set to V<sub>max</sub>. At this point in the experiment V<sub>max</sub> of 5.5V, that was established previously, did not produce maximum tension therefore a new V<sub>max</sub> was determined at 35V. During the first two frequency increments the twitches occurred more often but also returned to base tension of 16.6 grams between each

twitch. During frequency 2 and 4pps the twitches started not returning to base tension and began fusing together and by 15 and 25pps a wave shape was seen.

As frequency increased, it was expected that the force of each twitch would build on each other and tetanus would occur. Instead, tension initially increased with increasing stimulus then dropped back down as stimulus frequency continued to increase. This trend can be observed in figure 3.



**Figure 3:** Graph of the maximum tension, in grams, at each frequency increment. A sharp initial increase in tension is seen then a gradual decline in tension with increasing frequency.

**Table 3:** maximum tension, in grams, observed at each increasing frequency increment.

Frequency (per sec)	Tension (g)
0.5	29.06
1	59.11
2	47.04
4	32.02
8	24.38
15	33
25	26.47

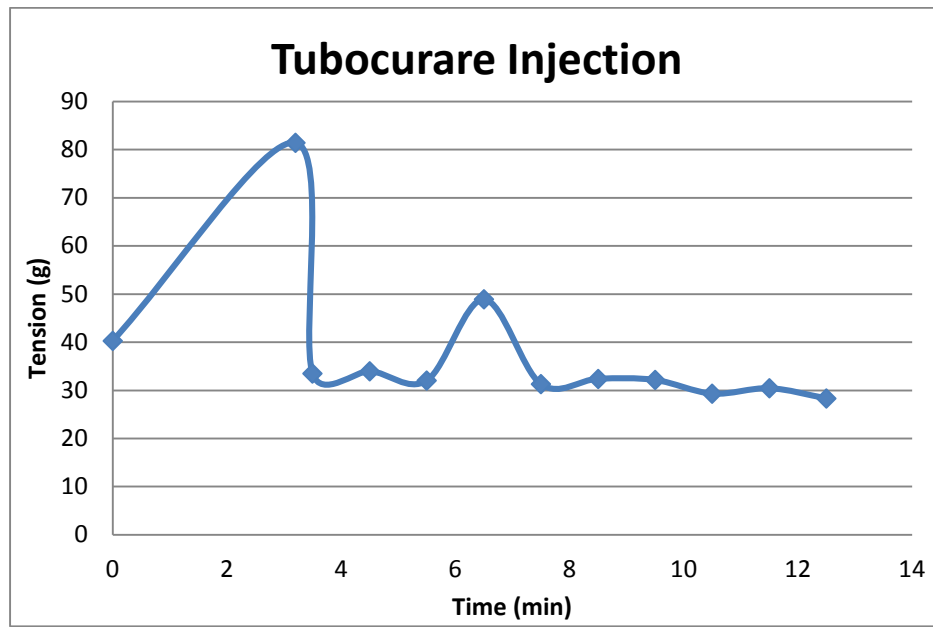
### Part 3: Effect of a competitive inhibitor on muscle activity

Tubocurare was injected into the frog's gastrocnemius. Prior to the injection, baseline tension was set to 25grams. From 3.2 minutes to 3.5 minutes tubocurare was administered to the frog's muscle. This time period is marked with a sharp increase in tension due to interference from injecting the muscle. After the injection period, tension was recorded at one minute intervals. This can be seen in table 4. The tension in the leg remained roughly constant for the following ten minutes with a slightly decrease towards the end. Also, an outlier appears to be present at 6.5 minutes where a jump in tension occurred. This trend can be seen in figure 4.

**Table 4:** Tension (g) before, during and after administration of the tubocurare injection into the gastrocnemius of the frog.

Time (min)	Tension (g)
0	40.27
3.2	81.42
3.5	33.5
4.5	33.99
5.5	32.02
6.5	48.89
7.5	31.28
8.5	32.39
9.5	32.14
10.5	29.31
11.5	30.42
12.5	28.32

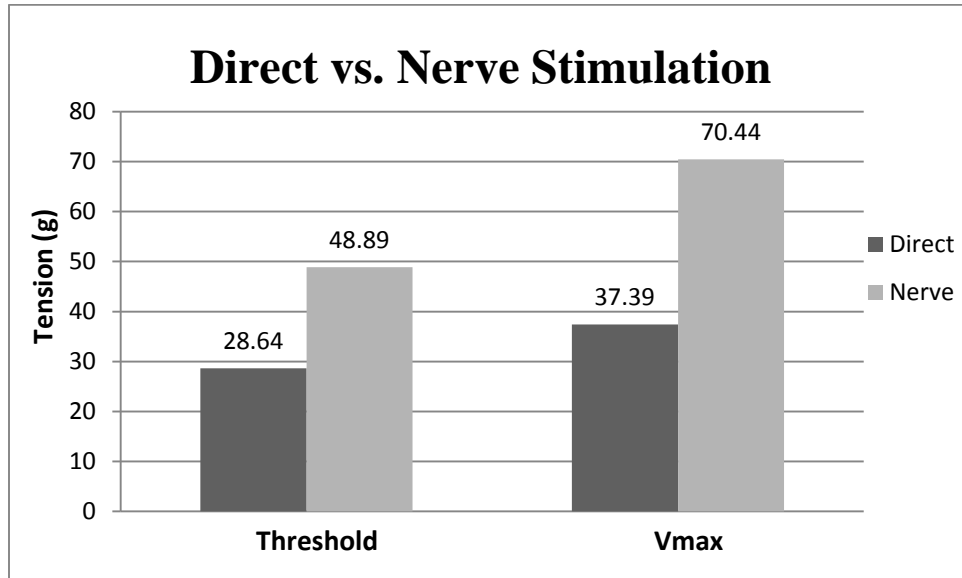




**Table 5:** Graph of the tension (g) vs. time (min) before, during and after the injection of tubocurare into the muscle. Time 0 is the tension prior to the injection, 3.2-3.5 is during the injection and all the data after 3.5min are the tensions recorded as time progressed. Tension during the time of the injection is irrelevant to impact of tubocurare on the muscle. Tension at 6.5min appears to be an outlier.

#### Part 4: Effect of direct stimulation of muscle activity

When the muscle is being directly stimulated by an electrode, only specific muscle fibers are being innervated. At voltage threshold, .9V, the muscle tension for direct stimulation was 28.64g while nerve stimulation required fewer volts to produce a muscle twitch. The same trend occurred for the Vmax. The Vmax for nerve stimulation was produced at 2V and the corresponding tension was 70.44g. Figure 6 shows that 10xVmax during direct stimulation produced a tension of 37.39g which is much less than the force produced via nerve stimulation.



**Figure 6:** Graph comparing the tension (g) produced at threshold and Vmax for direct and nerve stimulation. Note that for direct stimulation, Vmax is actually 10xVmax.

**Table 5:** The tension (g) of the frog's gastrocnemius when stimulated by different stimulation types.

Stimulation Type		Volts	Tension (g)
Direct	Threshold	0.9	28.64
	10xVmax	90	37.39
Nerve	Threshold	0.35	48.89
	Vmax	2	70.44

### Discussion:

#### **Part 1: Effects of stimulus intensity on muscle activity.**

When the sciatic nerve was stimulated by the electrode, the membrane potential was altered. This change allowed for an AP to be sent to the gastrocnemius causing a muscle twitch. A muscle twitch is an all-or-none response so if the action potential generated was not strong enough, no depolarization of the nerve would occur, and no response of the muscle cells would

have been seen. A threshold is the point that must be exceeded to elicit a response. It is the smallest amount of stimulus that can produce a response.

In part 1 of the experiment, the voltage threshold was the lowest voltage recorded that produced an action potential and a muscle twitch was observed. The voltage threshold was recorded at 3.0V and produced a force of 24.26 grams. In reality the threshold may have been lower but due to error, the force transducer may not have been able to record this threshold due to inaccurate set-up or a twitch at a lower voltage may not have been observed accurately by the human eye.

When twitch tension was at its highest point, voltage maximum ( $V_{max}$ ) was reached. At  $V_{max}$ , an increase in stimulus no longer produced an increase in force. Tension did not increase because all motor units had been recruited and all cross-bridges between myosin and actin had formed (Sherwood, 2010, pg. 270). Error in recording  $V_{max}$  may have also occurred. Once  $V_{max}$  was reached, an increase in voltage did not impact the amount of tension produced. This made it more challenging to accurately pinpoint the exact voltage required to reach  $V_{max}$ . With possible errors occurring when determining voltage threshold and maximum, the  $\Delta V$  calculated and used to produce a graded response may not have been accurate.

Muscle recruitment can be observed in figure 1. At 3.0V, tension was 24.26grams and at 3.5V the tension increased to 44.70g. This large increase indicated the recruitment of a motor unit. The small increases in tension that followed showed that although another motor unit wasn't being engaged, more cross-bridges were forming. The recruitment of large or small motor units along with increased formation of cross-bridges allowed for twitches to be graded in size (Sherwood, 2010, p.270).

When looking at figure 1, it can be seen that as voltage increased, tension increased. This positive relationship was expected to continue until  $V_{max}$ , at 5.5V, was reached. Instead, at about 5V, tension began to decrease as stimulus was increased. This result may have been due to combined errors. The initial recording of  $V_{max}$  may have been too high, which combined with possible failure of the muscle due to inadequate amounts of Ringer's solution, resulted in a drop in tension after a peak force around 5V was achieved.

## **Part 2: Effects of stimulus frequency on muscle activity**

A single AP in a muscle produces a single twitch but when more than one AP occurs before the muscle fiber has relaxed, the twitches add together or summate and a greater tension is produced (Sherwood, 2010, p.271). The rate at which action potentials occur is referred to as frequency so an increase in frequency should produce an increase in tension. Tension is produced as a result of increased cross-bridge formation but these bridges cannot be formed without calcium. As the frequency of action potentials occurs,  $Ca^{2+}$  levels increase. This calcium binds to troponin which pulls tropomyosin away from the actin binding sites, allowing myosin heads to bind and cross-bridge formation to occur (Despopoulos et al, 2008, p.66).

When APs are occurring at a rapid rate, the concentration of  $Ca^{2+}$  remains at high levels and the actin-myosin cross-bridges stay intact. These rapidly occurring action potentials don't allow the muscle fiber to relax at all between stimuli and as a result, tetanus occurs (Despopoulos et al, 2008, p.66).

Throughout the whole experiment tension developed at a constant muscle length. This is an isometric contraction and occurred due to the set up of the frog's gastrocnemius as the muscle was prevented from shortening (Sherwood, 2010, p. 274).

During the experiment it was expected that as stimulus frequency increased, tension would increase due to temporal summation and then possibly drop off at the end due to fatigue. Instead, as seen in figure 3, there was an initial sharp increase in tension and then tension dropped as frequency increased. As frequency increased, the twitches still summed up together to form a wave-like shape, which is seen in figure 2, but the force of each twitch did not sum together to add more tension. One problem that occurred during this part of the experiment was that when the voltage was set to the  $V_{max}$  of 5.5V, a maximum twitch was no longer produced. A new  $V_{max}$  was found and recorded at 35 volts. This new  $V_{max}$  was then used while frequency was increased.

Many errors could have occurred during this section of the experiment. The initial  $V_{max}$  of 5.5V may not have worked because the electrode was no longer stimulating the same part of the sciatic nerve or not enough Ringer's solution was applied so the muscle was not as viable. The new  $V_{max}$  of 35V may have negatively impacted the effect increased frequency may have had on muscle contraction and not allowed tetanus to occur. Also, stimulating the muscle at this high voltage for an extended period of time may have in the long-run shortened the life of the frog which impacted later parts of the experiment.

The data displayed in figure 3 does not illustrate the strength of a twitch summation versus that of a tetanus contraction. Tetanus is the maximum strength produced by a sustained contraction but instead of producing a higher force than a twitch, the tetanus frequency of 25pps produced a tension of 26.47g whereas a frequency of 2pps produced a tension of 59.11g. This decrease may have been due to fatigue because the muscle was not able to maintain the high voltage stimulus. Once again this data error may have been due to inaccurate set-up and stimulation which led to poor contraction and results.

In the study “The Tetanic Depression in Fast Motor Units...” by Celichowski et. al, the gastrocnemius muscle in a cat was stimulated first at low frequency then at higher frequencies. It was observed that when a low and high frequency stimulus occurred right after each other, the high frequency stimulus exhibited a lowered twitch tension after the occurrence of tetanus (Celichowski et. al, p 292). Although this is not visible in figure 2, if the experiment were to be repeated correctly, this phenomenon of tetanic depression should be seen when comparing two different frequencies, such as the jump from frequency 15 to 25.

### **Part 3: Effect of a competitive inhibitor on muscle activity:**

When an action potential is sent down the nerve to the neuromuscular junction, a rise in calcium ions signals vesicles, located in the terminal button of the nerve axon, to release acetylcholine (ACh) which is a neurotransmitter that signals muscle fibers to open channels allowing  $\text{Na}^+$  to enter. The inward flux of  $\text{Na}^+$  causes a depolarization and a muscle contraction occurs (Sherwood, 2010, p. 247-249). Tubocurarine is a competitive inhibitor of ACh, meaning that it has a similar conformation to the neurotransmitter and competes for the same receptors. The presence of tubocurarine hinders ACh and therefore a muscle twitch does not occur (Sherwood, 2010, p253).

During the experiment it was expected that the tension of each muscle twitch would decrease with time after the injection of tubocurarine as the drug would block ACh receptors and no depolarization would occur. In figure 4, after the injection of tubocurarine, the tension of the muscle twitch remained roughly constant with one jump in tension at 6.5 minutes. This random increase in tension may have been caused by a movement of the stimulator on the sciatic nerve, or the addition of Ringer’s solution that may have impacted the electrode, or some other human error

and therefore is an outlier in the trend of data. A drop in tension was not observed. This may be due to inadequate amount of time for tubocurarine to act on the system as there is a slight observable decrease in tension towards to end of the ten minute period.

In the study, "The effect of Tubocurarine..." by Magleby et. al, tubocurarine was administered to frogs and it was once again determined that tubocurarine has an impact on the post-synaptic plate. Although this role is already known and understood, the experiment determined that under repetitive stimulation tubocurarine is thought to have an influence on the pre-synaptic area as well (Magleby et. al, 1981, p.97-98,110-111).

Tubocurarine is a paralytic agent and is lethal to most terrestrial vertebrates because it lowers and blocks twitch response and does not allow for proper function of the muscle. If tubocurarine is present in large amounts and blocks enough acetylcholine receptors, all muscle in the body will cease to function and paralysis can occur. Vital contractions such as the movement of the diaphragm can stop which impacts the respiratory system (Sherwood, 2010, 253). Ultimately, death ensues as the animal is no longer able to breathe and provide its body nutrients.

#### **Part 4: Effect of direct stimulation of muscle activity**

When directly stimulating the muscle with an electrode, the neuromuscular junction was not needed as the electrode is able to bypass that part. The bypass allowed for contractions to occur even though tubocurarine was still bound to ACh receptors and was blocking any possible APs. Unlike with the stimulation of the sciatic nerve, the direct stimulation only recruited the muscle fibers it was directly in contact with and as a result the tension produced at the same voltage was lower than it would have been with nerve stimulation.

For the direct stimulation part of the experiment, data was borrowed from a different group because the frog was no longer viable and produced no response when stimulated with maximum amount of voltage. This may have occurred because very high voltages were required to produce a  $V_{max}$  earlier in the experiment. Also, Ringer's solution may not have been applied in adequate amounts throughout the procedure.

In order to compare twitch tension in part 1 of the experiment and part 4, data was borrowed from a different group instead of using two different data sets for the comparison. Between the two different parts of the experiment, there was a noticeable difference in twitch tension. At the  $V_{max}$  during the electrode stimulation of the sciatic nerve, the tension produced was 70.44g. This was significantly different from the 37.39g of tension at 90V produced by the direct muscle stimulation. This difference in force illustrated the properties of motor unit recruitment. When the sciatic nerve was stimulated it caused a motor unit to innervate multiple muscle fibers. These fibers worked together to increase the tension when at a lower stimulus. On the other hand, direct stimulation only innervated a single muscle fiber and therefore caused a lower tension response.

Throughout the entire experiment the frog was required to be bathed in Ringer's solution. If it was not covered in the solution, contraction of the gastrocnemius would not occur because the solution contains ions necessary for contraction. Ringer's solution contains  $Ca^{2+}$ ,  $Na^{+}$ , and  $K^{+}$ . All these cations are necessary for AP, ACh, and myosin-actin cross-bridge formation. Even via direct stimulation these components are required for contraction. Calcium is specifically important because it is necessary to allow myosin to bind to actin.



**Conclusion:**

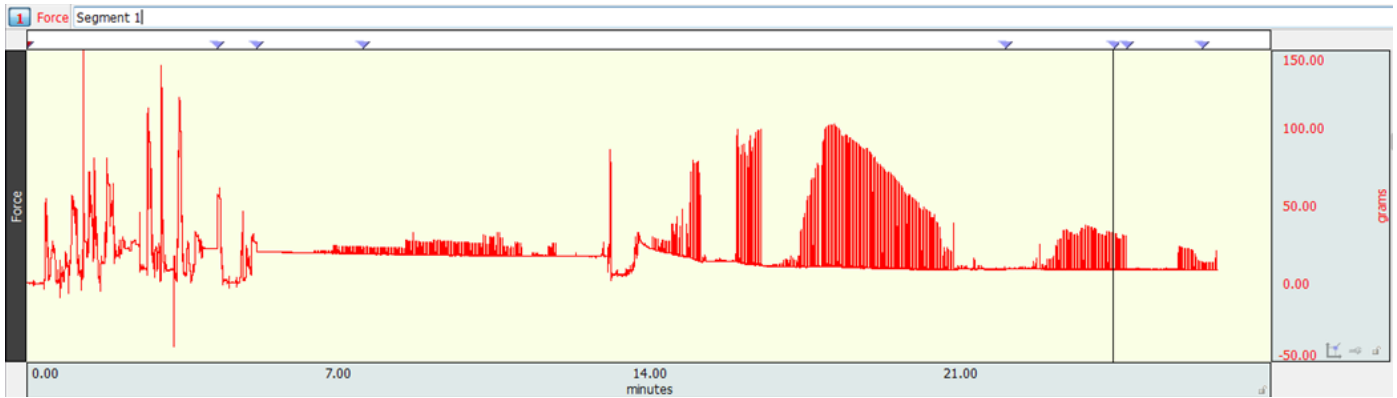
Overall, there are numerous components that affect the tension caused by a muscle twitch. By altering different variables, such as stimulus and frequency, it can be observed how muscles can act as single fibers and as motor units. If one aspect involved with neuron firing to signal a muscle contraction is changed, the entire twitch response process changes. Each step of the process has varying yet important roles on how the muscle can respond.

**References:**

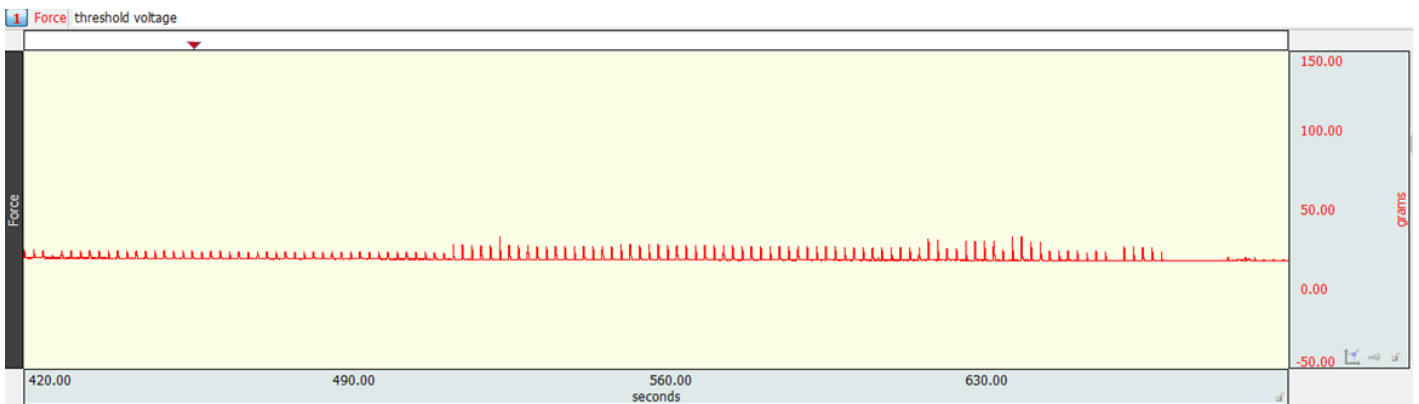
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**Raw Data:**

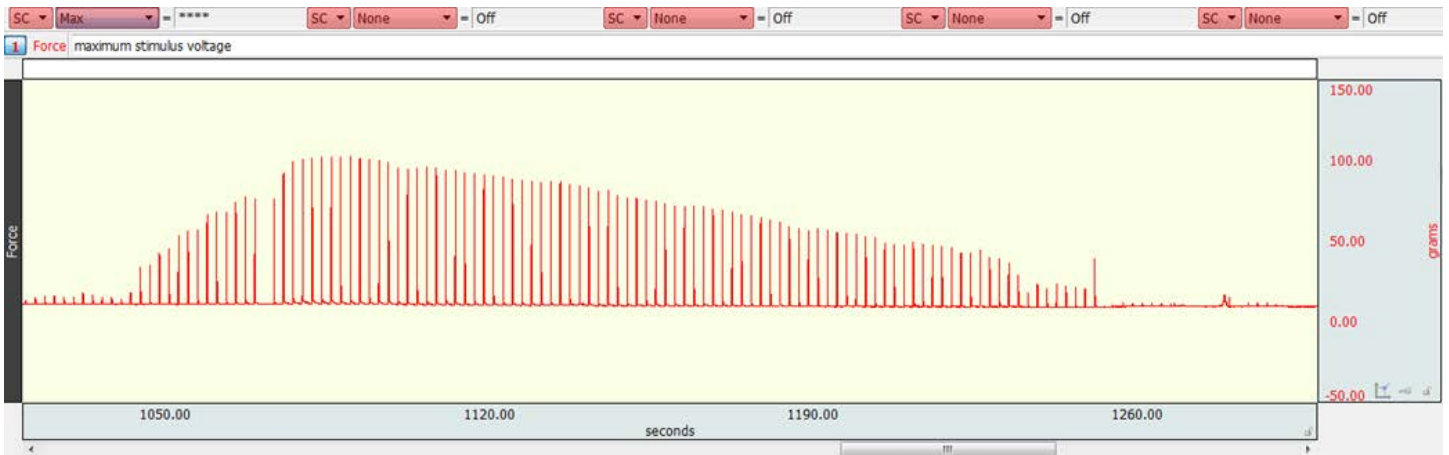
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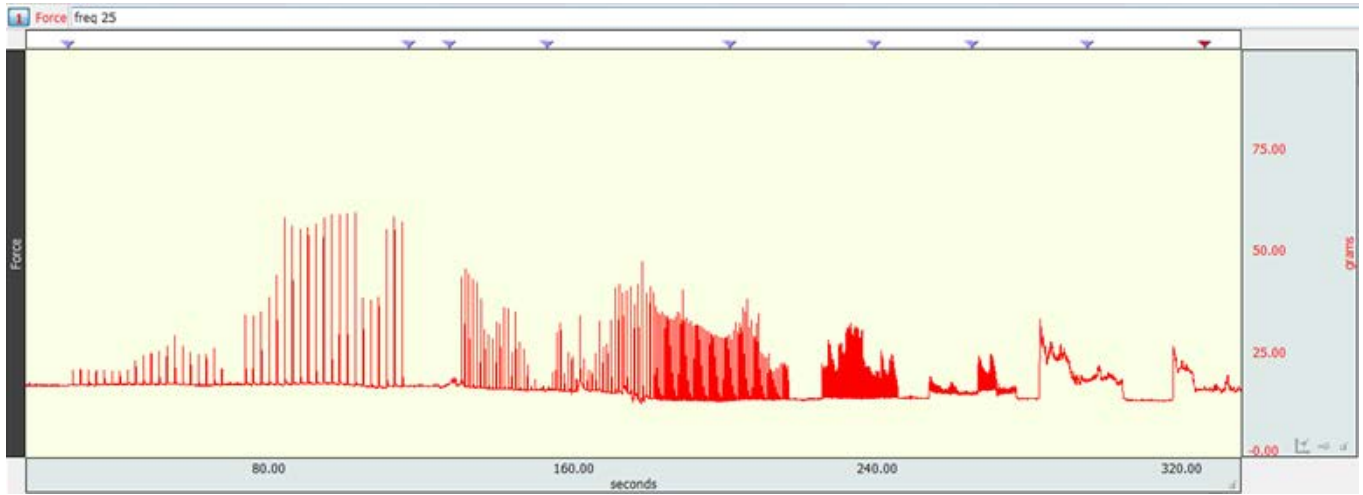
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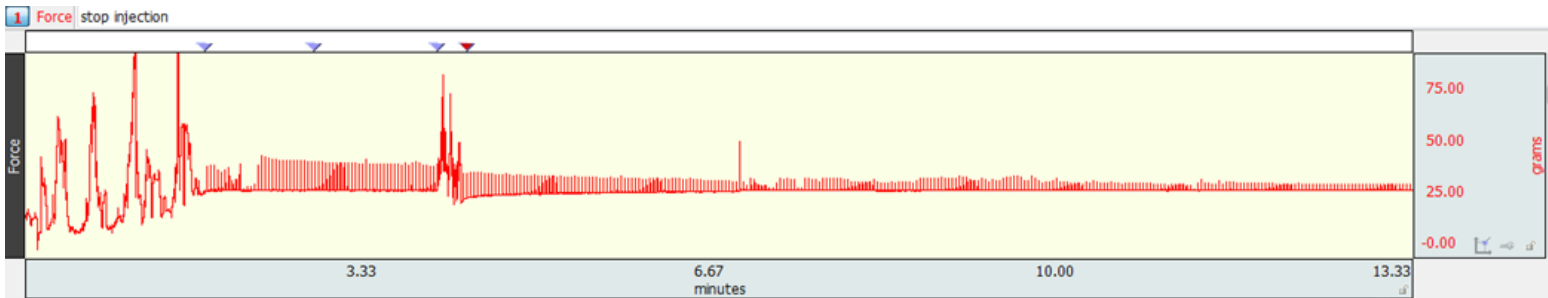
**Zoomed data used for maximum voltage:**



Frequency data:



Tubocurare:



Direct Stimulation:

