Animal Lab Modules 1 and 2

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***Abstract*— Rats and other small animals are often used in laboratory settings as a way to test the effectiveness of therapeutic drugs prior to clinical trials. Rats specifically are frequently used because of their anatomical, physiological, and genetic similarities to humans. These lab modules introduced our team to the process of anesthetizing and prepping a lab animal as well as performing both an ECG and a twitch stimulation test. This laboratory succeeded in introducing students to working with live animals and gathering data from a live lab subject.**

I. Introduction

Small animals such as rats are commonly used in preliminary trials for many experimental drugs. This is due to their physiological likeness to humans as well as being more affordable than other animals and easy to maintain.

The first step in performing a laboratory test on a rat is to anesthetize the rat. For this lab a combination of ketamine and xylazine were used as anesthesia and the dosage was determined by the weight of the rat. Once the rat is no longer moving and it's reflexes have been checked, the next step is to shave the areas of the rat that will be important for testing.

For the ECG test, three areas on the underside of the rat were shaven, inside the two front legs and inside the left hind leg. The ECG displays normal heart activity through the use of electronic readings. The normal waveform displayed by the ECG contains three major parts, the P-wave, the QRS complex, and the T-wave. Being able to clearly see all parts of the waveform ensures that the ECG leads were properly placed and that an accurate reading is being displayed.

Action potential is a change in membrane potential due to the diffusion of Na^+ ions moving into an axon as K+ ions exit in order to return to resting membrane potential (rmp) [1]. Cells are negatively charged due to the selective permeability of the plasma membrane limiting diffusion of positively charged ions like potassium and the nucleus containing negatively charged molecules of DNA [1]. Action potentials are produced in response to stimulations.

The P-wave is produced by the "spread of atrial depolarization" within the heart [1]. Depolarization creates a potential difference resulting in the upward bump of the ECG line. Similarly, the QRS complex results from the "spread of the depolarization into the ventricles" [1]. This represents a larger spike on the ECG line. Following this potential difference, the "repolarization of the ventricles" induces the T-wave, which is the upward bump in similar size to the P-wave [1]. The depolarization and repolarization both move the ECG line upwards, because they are opposing in both potential changes and spread directions.

These action potentials "briefly open voltage-gated Ca^{2+} channels in the plasma membrane" [1]. This diffusion of ions incites calcium ions to exit the sarcoplasmic reticulum. Calcium ions bind with troponin on actin filaments displacing tropomyosin and exposing myosin binding sites. Myosin heads bind with actin and release inorganic phosphate. The Myosin heads direct the actin filaments in one direction by releasing ADP molecules. An ATP molecule binds to the myosin head, releasing it from the actin filament due to a conformational change. Myosin ATPase breaks down the newly binded ATP into ADP and inorganic phosphate, beginning the cycle of muscle contraction with additional calcium ions [1].

For the twitch stimulus test, the outside of the rat's hind leg was shaven in order to extract the sciatic nerve which would receive an electrical stimulus in order to trigger the twitching motion. The force stimulus test displays the magnitude of force created by the natural twitch reaction of the rat. This test can also show how the rat's muscle fatigues after multiple repeated stimuli. A suture was placed in between the tibia and the achilles tendon and was tied to a force transducer that would measure the force created by the twitch.

II. Materials and Methods

A. *Anesthesia Administration and Experimental ECG of Module 1*

Before administering anesthesia, a rat (Sprauge-Dawley, retired breeder, Animal number: BME20-120, Charles River Laboratories) was obtained and weighed. The recorded weight was substituted into this equation calculate the correct amount of ketamine and xylazine necessary to anesthetize the rat:

$$(.39(kg)) * \left(\frac{\left(\frac{100mg}{kg}\right)}{\left(\frac{39mg}{100ml}\right)}\right) = .39mL\ ketamine$$

$$(.39(kg)) * \left(\frac{\left(\frac{10mg}{kg}\right)}{\left(\frac{3.9mg}{20ml}\right)}\right) = .195mL\ xylazine$$

Ketamine and xylazine were dosed at .39mL and .195mL respectively for a total dose of .585mL of anesthesia. The injection occurred in the rat's left caudal area. After 3 minutes, the rat had become still and its reflexes were tested. The rat did not flinch after pinching in between it's toes, touching around the eye, and releasing a drop of saline into its eye. Due to the lack of responsiveness, the rat was brought to the shaving area and prepared for the procedure. The rat was

shaved on its dorsal left hind leg between the hip and knee, the ventral right caudal area, and the ventral left and right arms between the shoulder and elbow.

The rat's limbs were taped down securely to the table as ECG leads were placed in the skin of the shaved ventral areas (fig 1). The ECG of the rat was recorded for a minute and a half resulting in more than 13,000 data points.

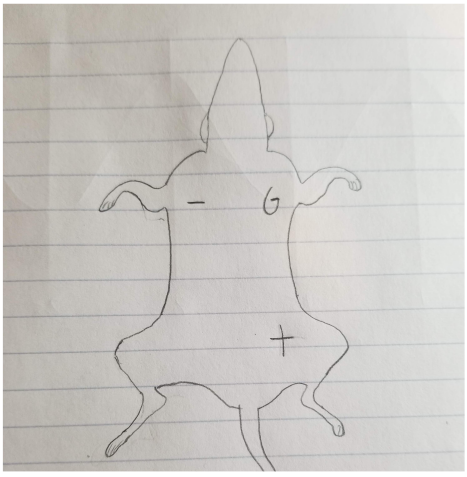

Fig 1: Visual of ECG Lead Placement

B. Surgical Procedures of Module 2

After removing the ECG leads, the rat was placed dorsal up on the table. A quarter-sized piece of skin was cut off of the shaved hind leg using operating scissors. The curved hemostats were used to break through a lining of collagen as serrated forceps kept the skin taut. Separating the muscle through blunt dissection minimized bleeding. The sciatic nerve was isolated and captured with the stimulating electrode. The nerve was kept hydrated with saline solution every two minutes to promote conductance.

A surgical suture needle was passed through between the achilles tendon and bone of the same hind leg. The needle was cut from the suture with operating scissors and disposed of in the sharps bin. The suture was tied down to the achilles tendon through a surgical knot. A loop was tied on the other end of the suture to attach to the force transducer. The rat was positioned in a way that the hind leg was taut with the force transducer.

The electrical stimulator was set up for three distinct measurements. A single twitch was recorded with a stimulator set up of an electrical stimulus every 500 ms through the sciatic nerve. Force and stimulus were recorded for 30 seconds. A twitch summation was recorded with a stimulus sent twice in short succession. The second stimulus was produced before the muscles in the rat could fully recover. Force and stimulus were recorded for 30 seconds. Muscle fatigue was recorded with multiple different stimuli timing. The first set of stimuli was produced every 100 ms. Each following test was done with 20 ms less, for example the next set of stimuli were produced every 80 ms and so on. Two seconds of each period were recorded for force and stimulus.

After all data was saved and recorded, the electrode was removed from the sciatic nerve. A lethal dose of 0.5 ml sodium pentobarbital was injected directly into the heart of the rat. After euthanasia, the lab space was properly cleaned and sanitized.

III. Results

The rat's weight was recorded as 0.39kg and was administered .585mL of anesthesia made up of .39ml of ketamine and .195mL of xylazine. The rat took 3 minutes to become immobile within its cage and did not respond to any of the reflex tests but could be seen visibly breathing.

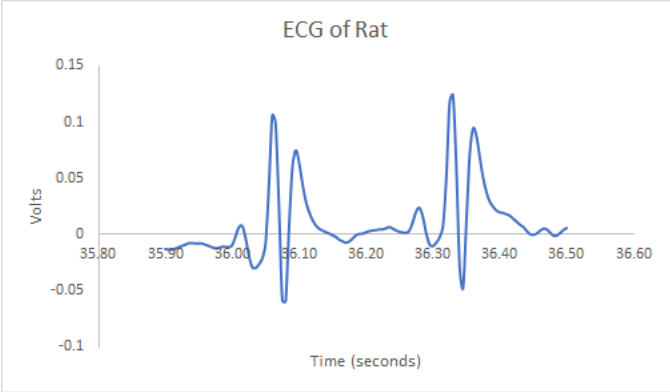

Fig 2: ECG of anesthetized rat

Figure 2 shows the ECG of the rat with the leads placed as shown in figure 1. This ECG allowed us to calculate the beats per minute (BPM) of the rat which was approximately 240 BPM. There was not much noise in the ECG which indicates that the rat was properly shaven and that the leads were placed accurately. Looking at the ECG you can see the P-wave which is the initial small spike and the QRS complex which immediately follows the P-wave. However, it is hard to see where the T-wave begins as the peak immediately following the QRS complex is quite large. Additionally, the T-wave normally occurs further from the QRS complex but in this case, if it is the large peak, it is occurring much sooner than expected. This discrepancy could be due to slight error in placing the leads.

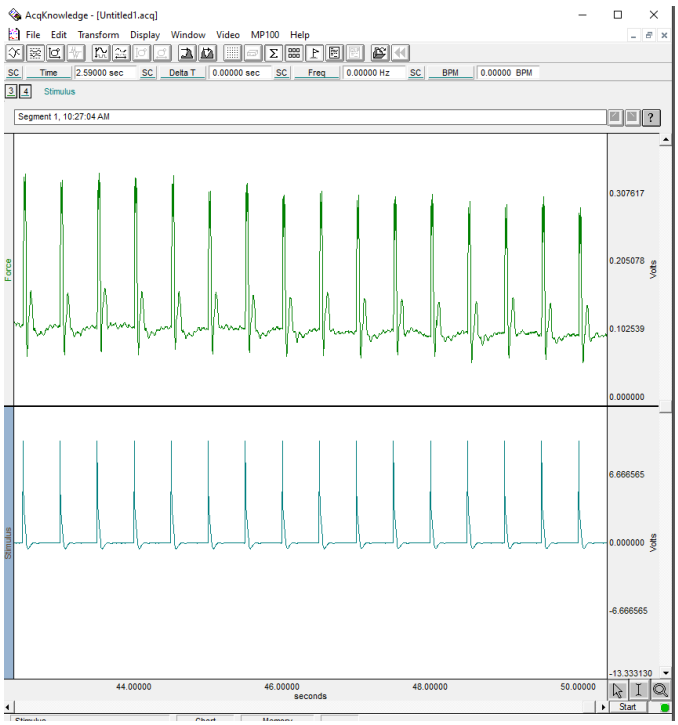

Fig 3: Force and stimulus of a simple twitch

Figure 3 shows the results of the simple twitch testing during which one electrical stimulus was sent to the sciatic nerve every 500ms. The graph on the top shows the force created by the twitch and the graph on bottom shows the voltage and frequency of the electrical stimulus. From this figure we can see that the rat's muscles were able to twitch regularly at this interval while showing little fatigue.

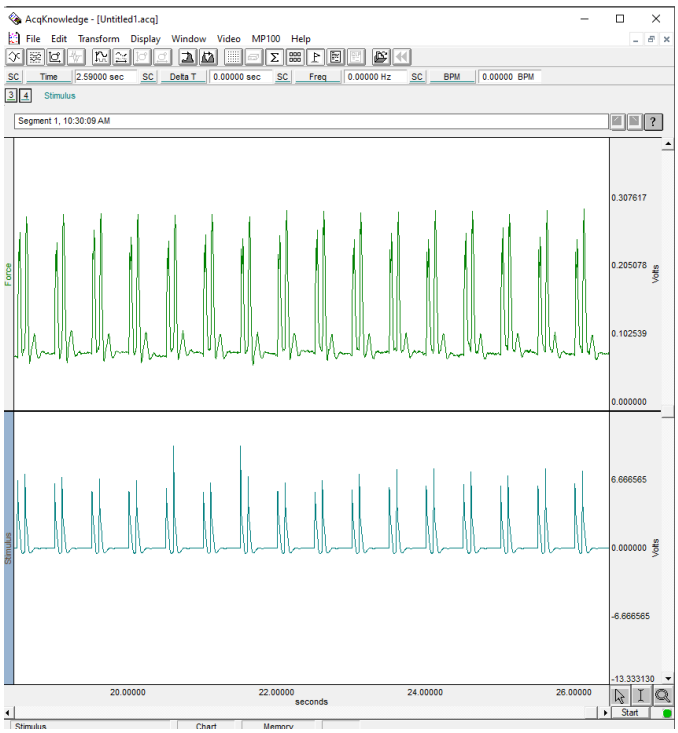

Fig 4: Force and stimulus of twitch summation

Figure 4 shown above, displays the force summation test in which the rat was stimulated twice in short succession. The top graph shows the force readings and the bottom graph shows the intervals of the stimulus. As expected, when stimulated twice in a short interval of time, the overall force produced by the twitching increased. This is shown by the second peaks in the top graph being noticeably greater in amplitude than the first peaks.

Figures 5 and 6 show the test of stimulating the twitch reflex every 40ms and 20ms respectively until fatigue. The top graph shows the force readings and the bottom graph shows the intervals of the stimulus. As the graph shows, the rat had a strong initial reflex but as it was subjected to repeated stimulus the muscles that were contracting to cause the twitch quickly tired and could no longer output the same amount of force. In both tests it only took about two seconds for the graph to show the fatigue and when this was noticed the rat seemed to no longer be twitching when stimulated. In figure 5 the decline in force is much steeper whereas the decline in figure 6 is much more gradual and parabolic in shape. This could be due to the more frequent stimuli making the graph appear smoother because of more frequent twitching. Tests were done before this at intervals of 100ms, 80ms, and 60ms and this type of fatigue phenomena was not observed. Instead, the rat was able to maintain the twitching motion with as little as 60ms rest time for more than 10 seconds.

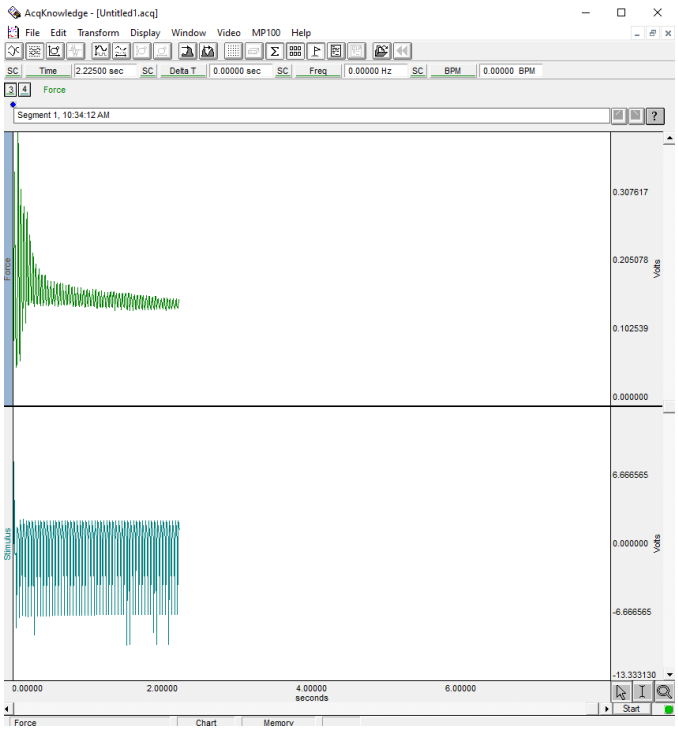

Fig 5: Force and stimulus of fatigue with 40 ms intervals

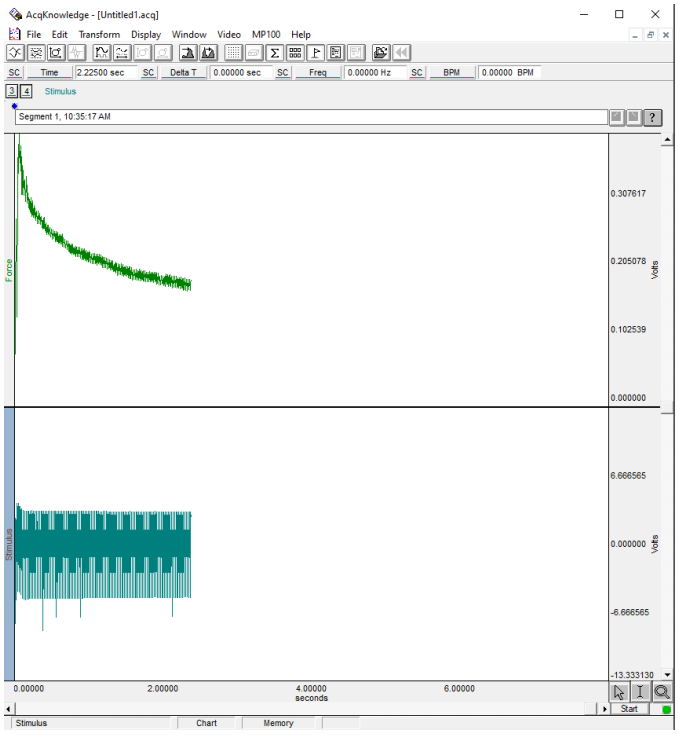

Fig 6: Force and stimulus of fatigue with 20 ms intervals

IV. Discussion

The goals of this experiment were to properly anesthetize a rat to record its heart voltage using an ECG test, and the other goal was to attach a stimulus to the sciatic nerve of the rat and a suture between the tibia and achilles tendon to record the force asserted by the rat's leg based on a recorded stimulus applied.

The first step of the experiment was to properly anesthetize the rat, which was done by the TA. To ensure that the rat was properly anesthetized, we first pinched between the rats toes to identify any reflexes that the rat may have. Since the rat had no reflexes, we then touched around the rats eye to observe any blinking reflexes, then placed drops of saline in the eyes to observe anymore blinking. Both tests showed no reflexes so we knew the rat was properly anesthetized.

We then shaved the rat inside of both shoulders and the right hip to subcutaneously insert the ECG leads by pulling the skin to tension with tweezers and pushing the pointed lead through the skin. The ECG reading showed a clear reading with little noise on the graph due to the close shave that was given in the areas that the leads were inserted.

The next step of the lab stimulated the sciatic nerve to observe the force created when the leg was held as a free body. To begin the lab, a large section of the right thigh was shaved. After this, tweezers were used to pull skin to tension, approximately the size of a quarter. Once the skin was pulled to tension, a cut was made to remove a section of skin over the thigh of the rat. A blunt dissection was made through the muscle to isolate the sciatic nerve and a transmitter was attached. A suture was put behind the achilles tendon and attached to a force transducer.

The data presented in the graphs above from this experiment were clear and were displayed as expected. The simple twitch test showed a spike in force immediately following a stimulation because of the small amount of time needed to create the force in the muscle. The summation twitch test had an initial force that was slightly lower than the second force because the muscle did not reach full relaxation during each interval. When using 20 ms intervals, the forces displayed started high and reduced to 0 after a few seconds because the muscle became fatigued.

V. Conclusion

Overall, the lab shows how to take an ECG reading on a rat using subcutaneous leads and the force resulting from the electrical stimulation of a nerve. The graphs for both the ECG reading and the twitch tests showed little noise and had results that were expected from the previous knowledge of the lab. The ECG test was run using a positive, negative, and ground lead to measure to voltage potential across the anesthetized rat. The twitch tests that were run included a simple twitch, summation twitch, and tetanus and fatigue test. The experiments performed allowed the student groups to gain experience with calculating the proper doses to anesthetize a rat based on its weight, properly obtaining an ECG reading,

and how to stimulate a nerve to measure the force of muscle contraction.

VI. References

[1] Fox, S. (2016). *Human Physiology* (14th ed.). McGraw-Hill Education.