Analysis of Frog Heart Physiology

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Methods:

This laboratory examined the effects of different stretch levels and drugs on the heart rate, function and contraction of frog heart. First, a bullfrog was decapitated and pithed to expose the heart. Then, a force transducer was calibrated by hanging several weights on it. To examine the effects of different levels of stretch on heart muscles, a suture was tied to the apex of the heart, and that suture was attached to the transducer. Then, force of contractions was measured with each stretch. Second part of the laboratory consisted of examining the electrogram signals/ECG of the frog heart in response to different drugs. A bipolar electrode was utilized to measure those electrogram signals in response to each drug and at baseline before rerecording for each drug. It was placed along the midline of the heart's ventricle.

Results:

i. Photograph of Frog Thorax: *See Appendix*

ii. Representative Electrogram Recording: *See Appendix*

iii. Contractile Force Vs. Stretch:

The calibration plot shown in Figure 3 was used to convert the transducer values in voltage to units of force in Newtons. The equation that was used to do the conversion is shown in Figure 3.

The goal of this laboratory was to examine the effects of increasing stretch levels on the force of contraction of the frog heart. There was an increase in force of contraction with increasing levels of stretch, as shown in Figure 4. Quantified data plots of using the transducer force values were used to represent the Frank-Starling Curve shown in Figure 5, in which the results shown an increase in tension with increasing pretension.

iv. Physiological Effects of Drugs:

The ECG and force data from Figure 6-13 show the effects of each drug, representing the heart rate, shape of ECG and contraction force. The results show that the heart rate in response to acetylcholine, atropine, CRS, caffeine and cadmium chloride were reduced by 28%, 18.75%, 13%, 4% and 7.6% (percent change shown in Figure 14), respectively, compared to the baseline. The force of contraction was also reduced for acetylcholine and cadmium chloride (78% and 3.4%, respectively). On the other hand, the force of contraction increased in response to atropine, caffeine, CRS and epinephrine by 58%, 18%, 11% and 3%, respectively. Finally, increased heart rate was observed in response to epinephrine about 4%. Furthermore, there is a noticeable change in the shapes of each ECG waves and intervals in response to each drug compared to the baseline ECG. For example, the ECG for acetylcholine (ACh) shows longer PR interval, and almost diminished ECG waves, specifically, the QRS complex

and the P and T wave for 10 mM ACh and cadmium chloride as shown in Figure 8 and Figure 10, respectively. The PR interval also increased for atropine, caffeine and CRS, indicating slower heart rate. On the other hand, the PR interval reduced in response to epinephrine and the S wave in the QRS complex appeared larger compared to baseline ECG as shown in Figure 12.

Conclusion:

i. Electrogram Implications:

One of the most noticeable changes in the electrogram/ECG was the PR interval. PR interval is defined as the depolarization of the sinus node in the atria to the beginning of ventricular contraction. The longer PR interval that resulted in response to the few drugs may indicate a delayed conduction of sinoatrial nodal impulse to the ventricles, implying that the drugs had a negative chronotropic effect on the heart. The short PR interval that was also observed indicated a positive chronotropic effect on the heart. The diminished QRS complex that was observed with some drugs could indicate the drugs' negative inotropic effect on the heart, therefore, the reduced contractility.

ii. Contractile Force Vs. Stretch:

The experiment simulated increased preload effect on the heart by applying different stretch levels to measure the force of contraction. The simulation resulted in a linear increase of tension as a function of pretension (Frank-Starling Curve). Like skeletal muscles, the cardiomyocytes contract via actin-myosin cross-bridge attachments. The number of cross-bridge attachments indicates the amount of force generated. Therefore, physiological implications behind the linear increase seen in the Frank-Starling curve would be that with each increasing stretch level, the actin-myosin cross-bridge attachments increased optimally, therefore, producing optimal and increased force of contraction with each higher stretch level.

iii. Physiological Effects of Drugs:

a. Acetylcholine: This drug is known to show negative chronotropy and inotropy, and the same was observed in this experiment. This is due to the increase in hyperpolarization of pacemaker cells of the SA node by altering the I_f channels that delays Ca^{2+} through its channel reach threshold, therefore slowing depolarization. That may have been the case for the frog heart.

b. Atropine: The response to this drug resulted in negative chronotropic effect, which did not meet the expectation. This may be due to the physiology of frog heart or amounts of atropine did not have enough time to block the muscarinic acetylcholine receptors to increase the heart rate before recording the ECG.

c. Cadmium Chloride: This drug is known to show negative chronotropic and inotropic effect on the heart, and this was also observed in this experiment. This effect may have been observed due to Cd^{2+} divalent nature, therefore, competing with Ca^{2+} to enter into the myocardial cell. However, Cd^{2+} ions do

not have the same structure as Ca^{2+} ion. As result, the Cd^{2+} do not bind with troponin in the thin filaments inside the heart muscle fiber, as a result, affecting the acto-myosin attachments and reducing the contraction.

d. Caffeine: This is known for its positive chronotropic and inotropic effects. However, this was somewhat observed in this experiment. The positive inotropic effect that was observed may be due to an increase in heart muscle sensitivity to Ca^{2+}, therefore, increasing the force of contraction. The caffeine in frog heart may have an opposite effect than seen in human hearts or this may also be due to human error, which resulted in negative chronotropy.

e. Epinephrine: Positive chronotropic and inotropic effects were observed in response to this drug, which was expected. This may be due to epinephrine increasing the Ca^{2+} conductance in the pacemaker cells and quick removal of it by the SR, therefore, increasing the heart rate.

f. Cold Ringer Solution (CRS): This drug also somewhat met the expectations in this experiment as it showed negative chronotropic effect but positive inotropic effect. The negative chronotropic effect maybe due to reduced energy of motion in the molecules involved, therefore, causing overall delay in the process of muscle contraction.

iv. Limitations in Experiment:

The knowledge of the drugs' effects in frog physiology is limited. Therefore, the results not meeting the expectation may be the reason. For example, the effect of caffeine or KCL (Figure included but effect not discussed due to insufficient data) is somewhat limited. There is a chance of damaging or altering the heart muscle's physiology when placing the bipolar electrode or when pulling the suture through the apex of the heart.

Appendix

Photograph of Frog Thorax:

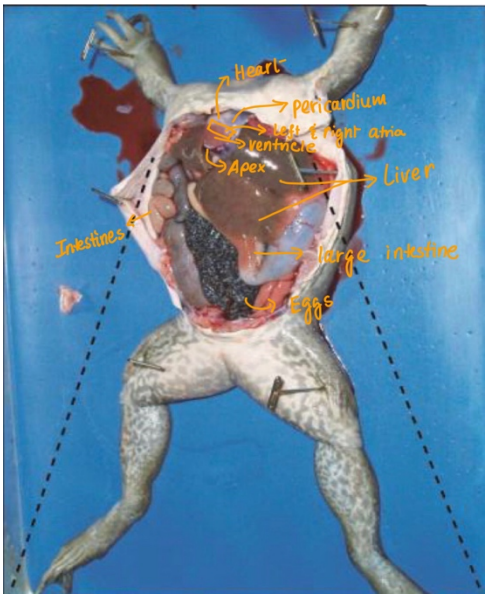

Figure 1. Labelling of organs in frog thoracic cavity and labels of the parts of the frog heart.

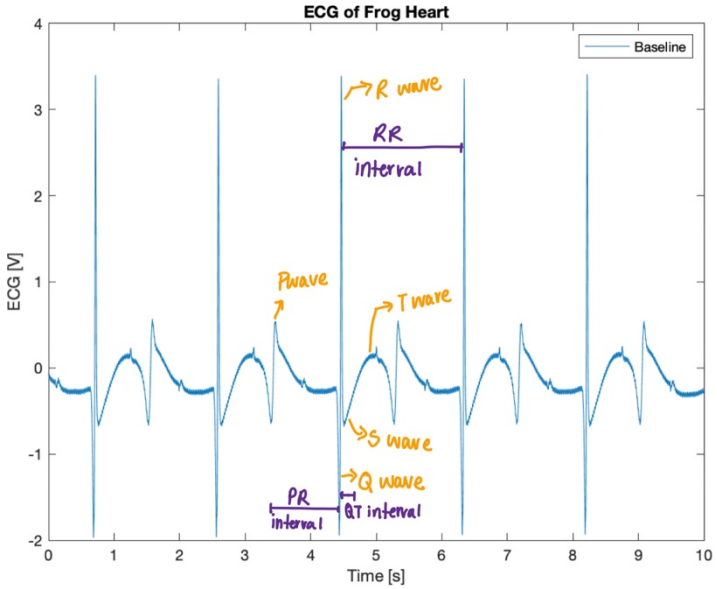

Figure 2. Representation of baseline electrogram signal recorded using the bipolar electrode. The P wave is the atrial depolarization and contraction phase of the frog heart, QRS complex consisting of the Q, R and S wave, represents the ventricular depolarization and beginning of contraction phase, and T wave represents the ventricular repolarization phase.

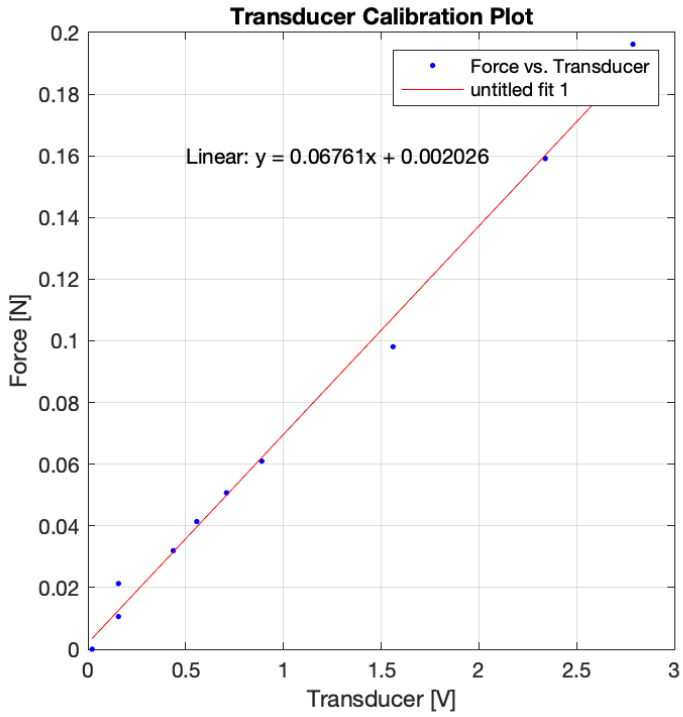

Figure 3. Calibration plot of the force transducer using different known weights. A linear fit was used to obtained the equation shown in the graph. This was used to convert the transducer values from Volts to Newtons.

S

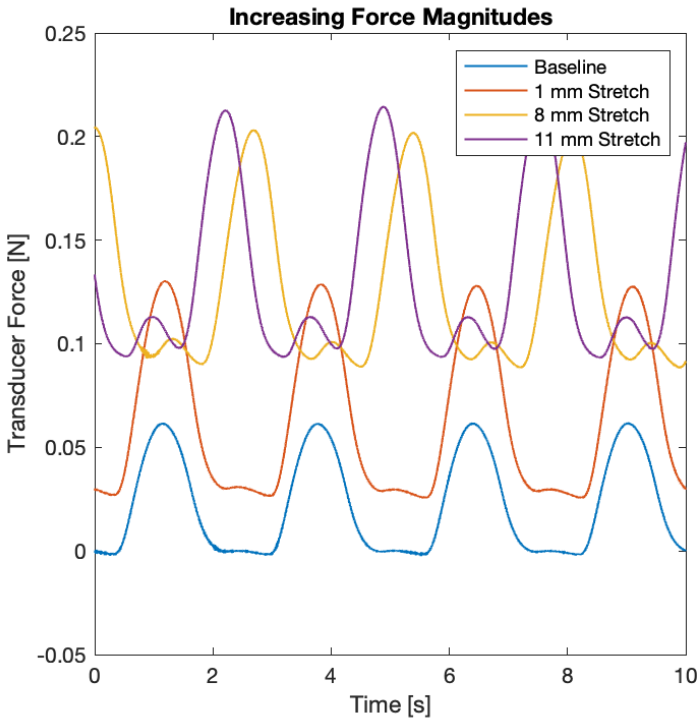

Figure 4. The figure shows an increase in force magnitudes with increased stretch levels.

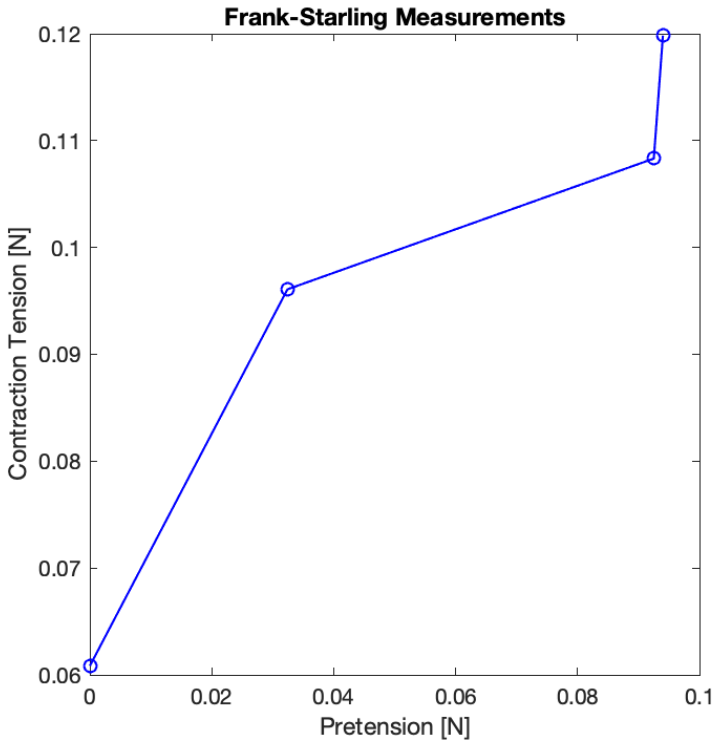

Figure 5. Quantified data of baseline, 1mm, 8 mm, 11 mm stretch from left to right, representing contraction tension as a function of pretention.

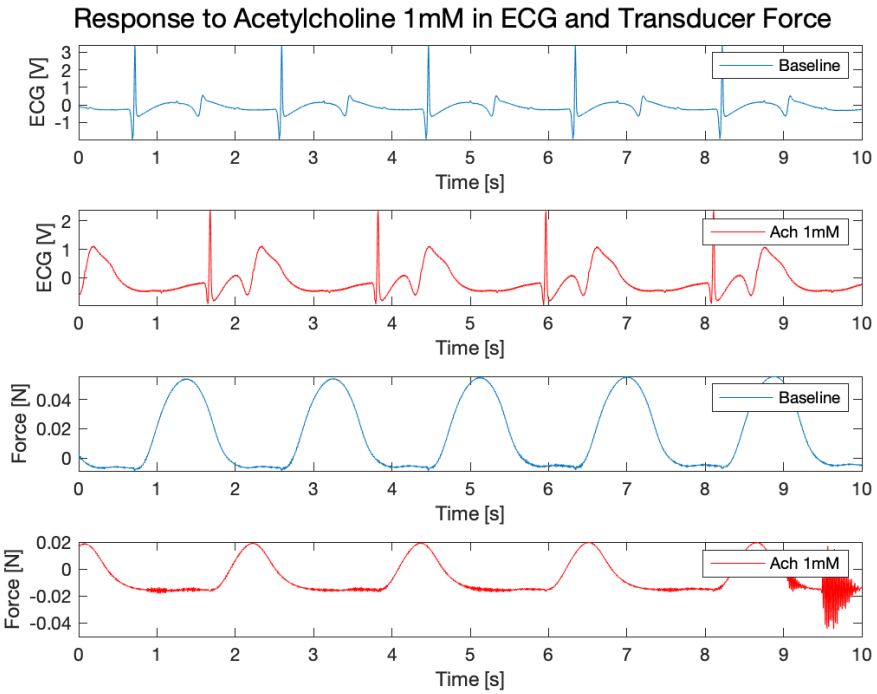

Figure 6. Comparison of baseline ECG and force data with frog heart's response to Acetylcholine 1 mM shown in ECG and force data.

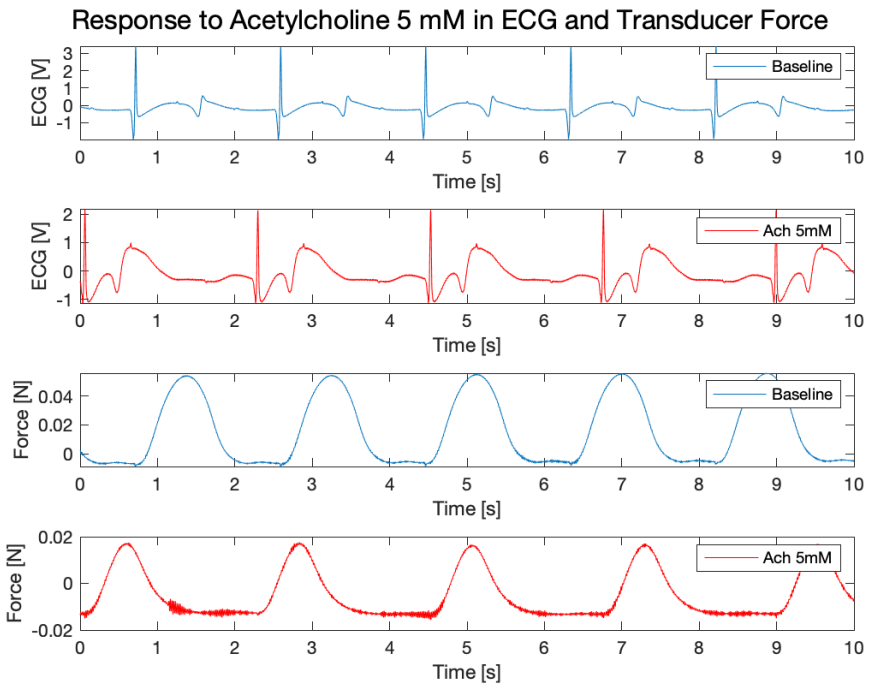

Figure 7. Comparison of baseline ECG and force data with frog heart's response to Acetylcholine 5 mM shown in ECG and force data.

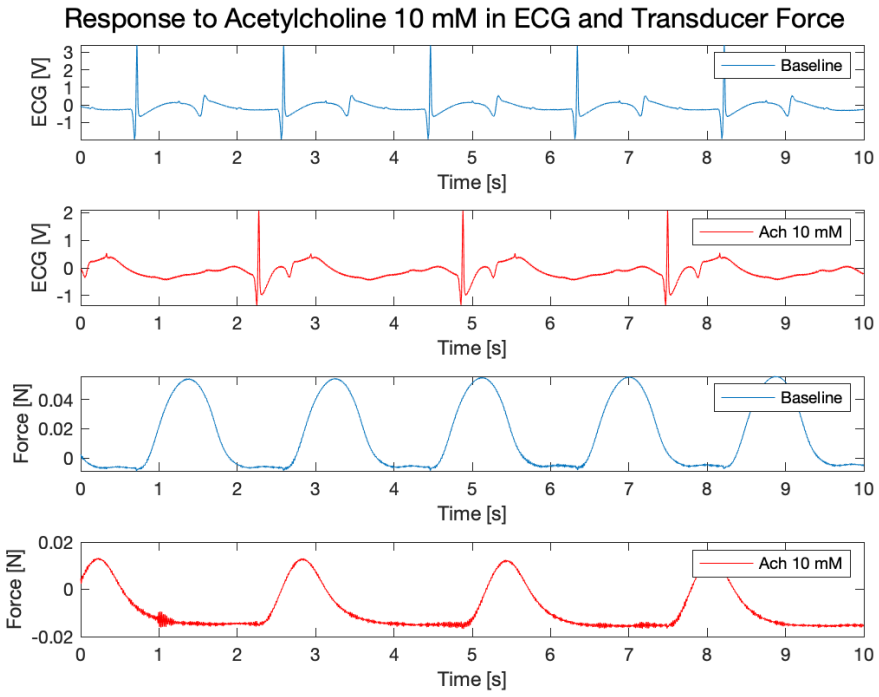

Figure 8. Comparison of baseline ECG and force data with frog heart's response to Acetylcholine 10 mM shown in ECG and force data.

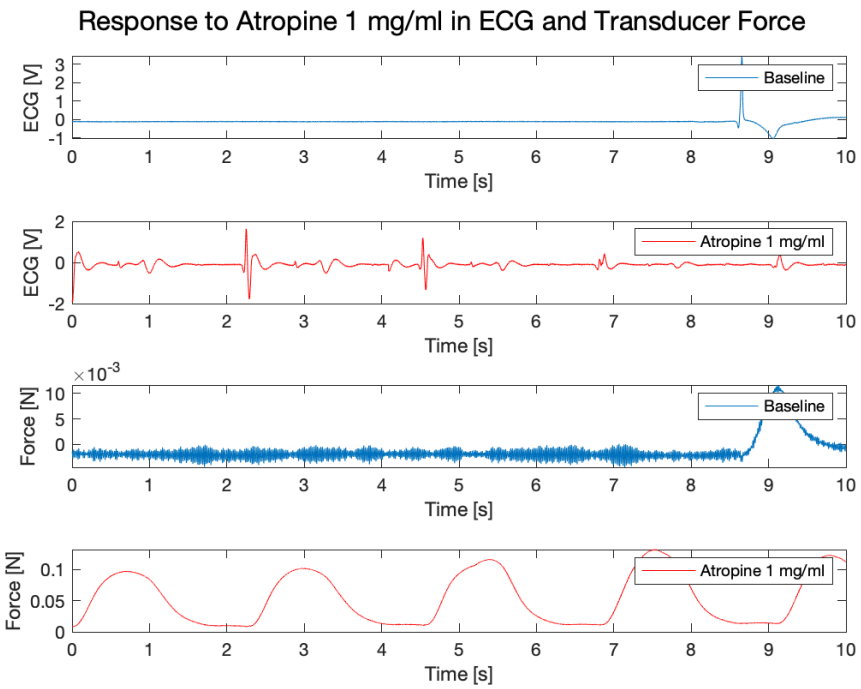

Figure 9. Comparison of baseline ECG and force data with frog heart's response to Atropine 1 mg/ml shown in ECG and force data.

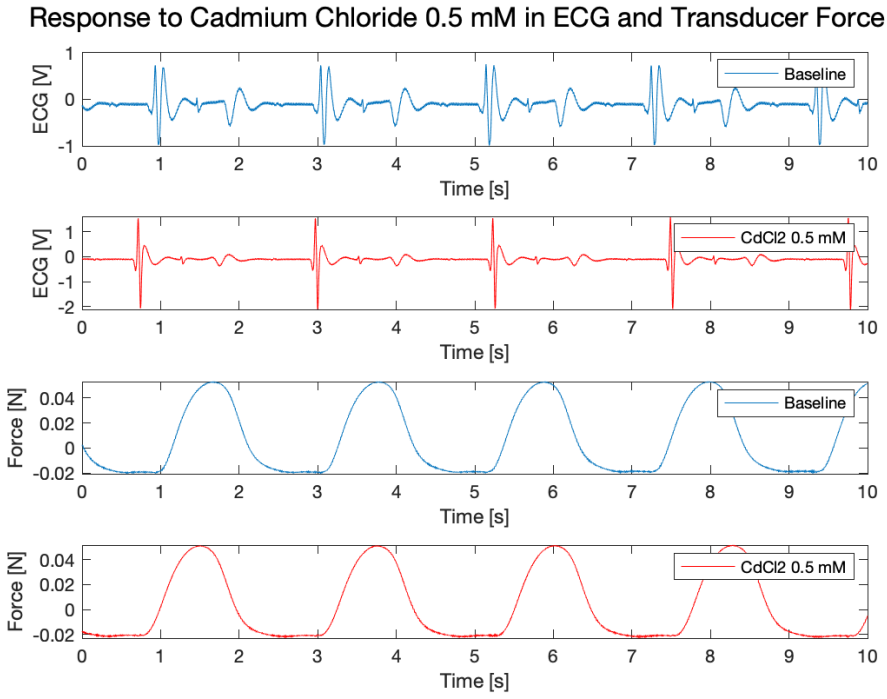

Figure 10. Comparison of baseline ECG and force data with frog heart's response to Cadmium Chloride 0.5 mM shown in ECG and force data.

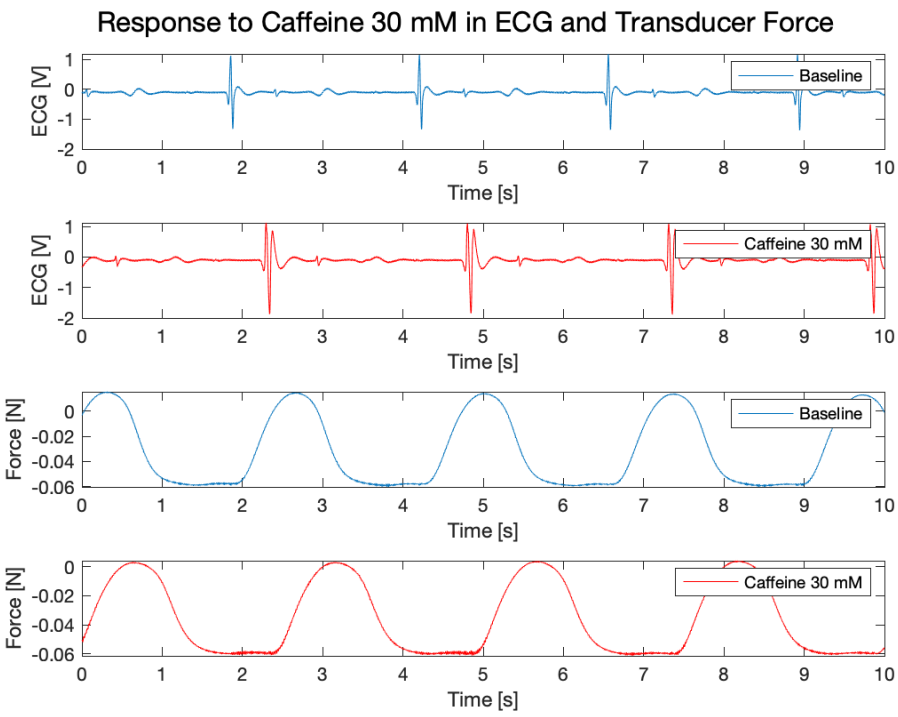

Figure 11. Comparison of baseline ECG and force data with frog heart's response to Caffeine 30 mM shown in ECG and force data.

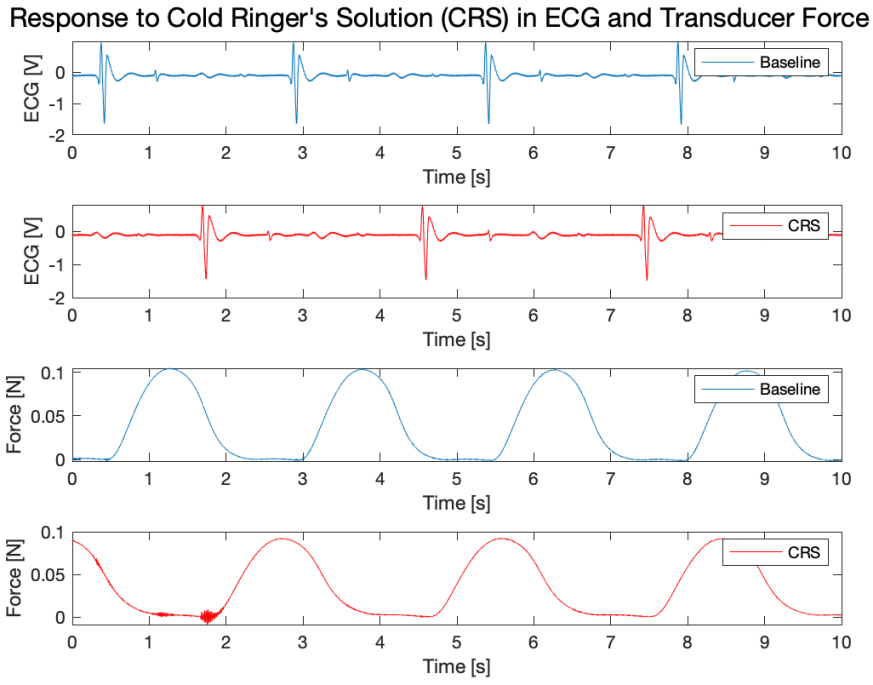

Figure 12. Comparison of baseline ECG and force data with frog heart's response to CRS shown in ECG and force data.

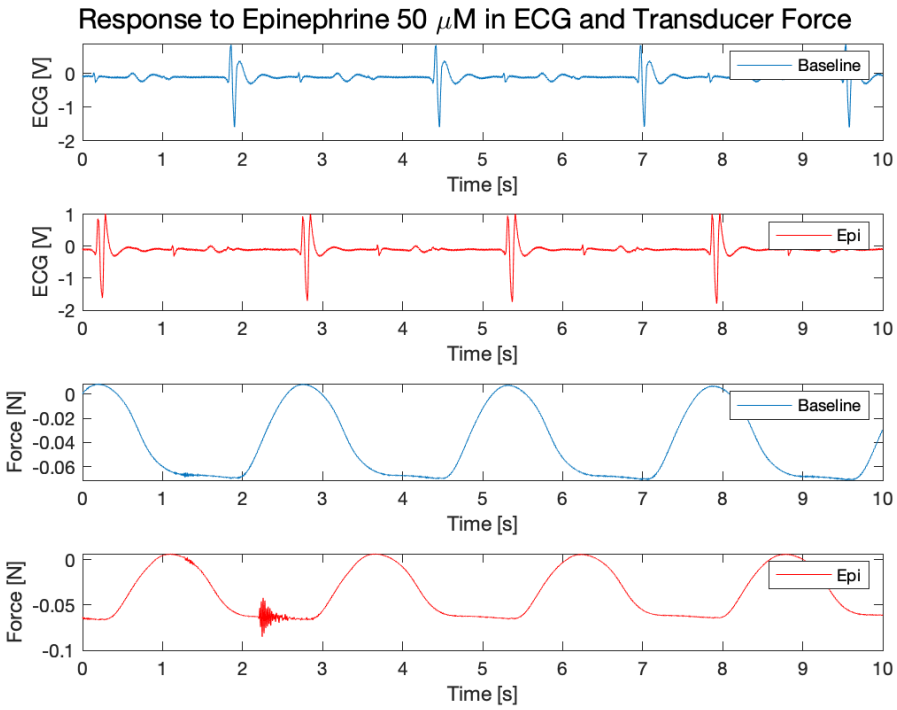

Figure 12. Comparison of baseline ECG and force data with frog heart's response to Epinephrine microM shown in ECG and force data.

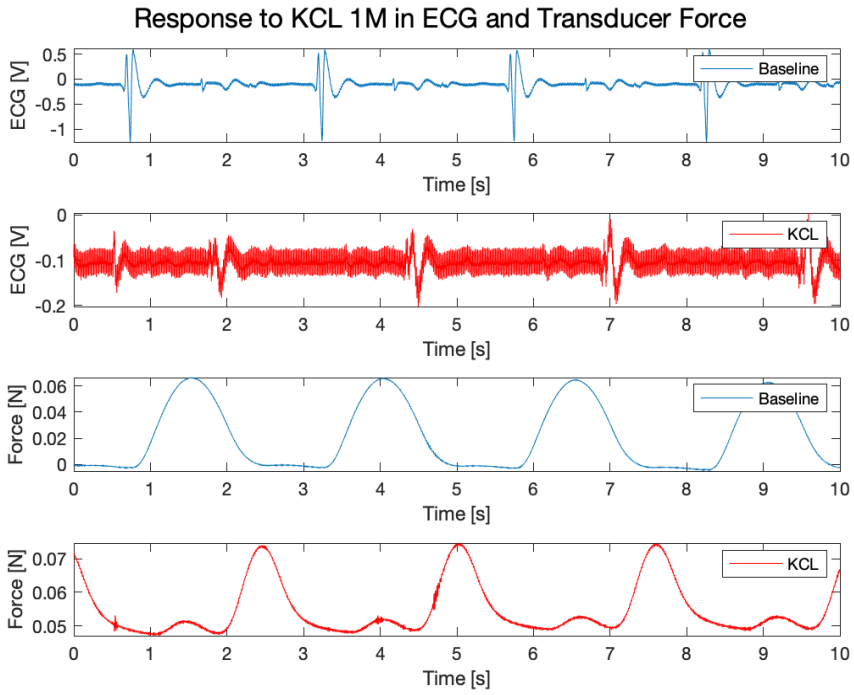

Figure 13. Comparison of baseline ECG and force data with frog heart's response to KCL 1M shown in ECG and force data.

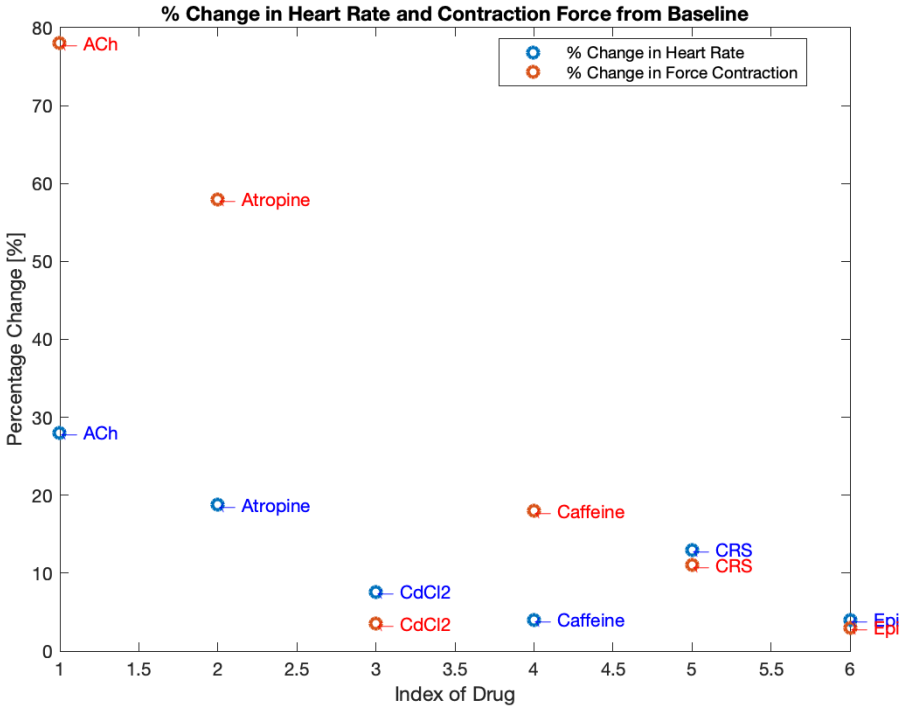

Figure 14. Representation of % change in heart rate and force contraction for each drug.