

Bio 397NP Neurobiology and Physiology Lab  
**Intro to Muscle Physiology**  
**Post Lab Assignment (Turn in 1 per group)**

Student Names: Taylor + Samira

1. What effect do you think the increasing stimulus voltages might have on the force of contraction of the muscle? State this as a hypothesis:

Increasing stimulus voltages results in an increase in force of ~~the~~ muscle contraction.

2. What effect do you think the increasing frequency of stimulation might have on the force of contraction of the muscle? State this as a hypothesis:

Increasing the frequency of stimulation results in a ~~several fold~~ increase in muscle contraction force through summation of each muscle contraction.

3. Did your results match your hypotheses?

Yes. As the amplitude increased the amount of force recorded by the transducer also increased.

Yes. When recording muscle force ~~was~~ with high frequency stimuli one could see a staircase line graph meaning a summation of forces.

4. Describe the experiment you will perform next week. Include: Hypothesis, Prediction, and Experimental Methods (use back as needed).

~~Hypothesis: Administering epinephrine to the Adductor Byssal ~~and~~ Retractor Muscles via a solution will cause the muscle to contract.~~

~~Prediction:~~



hypothesis: Epinephrine causes muscle contraction.

Prediction: Adding epinephrine to the adductor Byssal Retractor muscle via solution will produce a muscle contraction that has the same force as a muscle contraction induced by electrical stimuli. IS significant / recordable.

## Methods: Preliminary Data

What concentration of epinephrine do you need?

1.) set up muscle to transducer.

2.) create serial dilutions of epinephrine  
 $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$  molar

\* start with the highest one to make sure a high enough concentration is attained to induce contraction. Change muscle after

3.) find minimum concentration needed to create contraction

## Experiment

2.) continued solutions  
Make 3 ~~different~~ solutions  
for each concentration.

1.) set up muscle to transducer

2.) create serial dilutions. lowest concentration is found

3.) add each concentration (starting w/ lowest) to the muscle. Record muscle contraction force each time. different dilutions, starting w/ lowest concentration

4.) Does a greater concentration lead to greater muscle force? Record latency and relaxation periods. Record contraction time.

5.) add the lowest concentration needed to make contraction at different frequencies. Is summation observed? Does the sum of force equate to the greatest amount of force generated in step 4?

6.) The greatest force generated by epinephrine. What amplitude of electrical stimuli ~~does~~ is needed to produce the same amount of force.

3.) continued. Add all three solutions for each ~~concentration~~ concentration and obtain average force of muscle contraction for each concentration.

**Intro to Muscle Physiology**  
**Post Lab Assignment (Turn in 1 per group)**

Student Names: Michael Sheikhai, Zoë Dixon

1. What effect do you think the increasing stimulus voltages might have on the force of contraction of the muscle? State this as a hypothesis:

Increasing stimulus has a positive effect on force of contraction to a point of maximal contraction.

2. What effect do you think the increasing frequency of stimulation might have on the force of contraction of the muscle? State this as a hypothesis:

In an ordinary muscle contraction an action potential triggers a release of calcium. Increasing frequency of stimulation amounts to multiple muscle contractions. The force of each contraction is smaller than the last (a smaller action potential was triggered due to the lower amount of calcium available; the cell having not had time to reach equilibrium before the next action potential) and the contractions plateau ~~as~~ the stimulus ends just before.

3. Did your results match your hypotheses?

In exercise 1 the hypothesis formulated matched the results as there was a positive correlation of increasing stimulus to force.

In exercise 2 our hypothesis did not completely match our results.

The plateau in force did occur which made our hypothesis true, however with increasing frequency the maximum force did also increase in a positive correlation.

4. Describe the experiment you will perform next week. Include: Hypothesis, Prediction, and Experimental Methods (use back as needed).

Hypothesis: By testing the effects of Vasopressin and Lactic acid independently and in conjunction, it will be possible to observe the concentration of substances in correlation to forces of muscle contraction.

Prediction: Vasopressin would stimulate muscle contractions, while Lactic acid would reduce or prevent muscle contractions. Together they would cause irregular or unnatural contractions, Vasopressin is inhibited by Lactic acid.

Experimental Methods: In this comparative study we are going to utilize instant ocean serial dilution to create a range of concentrations for Vasopressin and Lactic acid. Individual use of each solution will be first to be tested and observed on Mussel Muscles. After diagnosing the effects it will be possible to see the most effective chemical starting concentrations for the forces and effects on the Mussel Muscles.

In the next step the most effective chemical concentrations will be tested in a compound solution to test the adverse, or favorable outcomes of muscle contractions. This will be the data for the comparative study.

D Finding concentration values in solution that are useable.

$$1 \text{ Da} = 1 \text{ g/mol}$$

$$\text{Vasopressin} = \text{C}_{46} \text{H}_{65} \text{N}_{13} \text{O}_{12} \text{S}_2$$
$$1056.218 \text{ Da} \times \frac{1 \text{ g/mol}}{1 \text{ Da}} = 1056.218 \text{ g/mol}$$

DL Lactic Acid / L-Lactic Acid / D-Lactic Acid =

$$\frac{\text{Da}}{\text{Da}} \times \frac{1 \text{ g/mol}}{1 \text{ Da}} = \text{g/mol}$$

We're measuring the muscle contractions under different concentrations of vasopressin and under different concentrations of lactic acid in the cell over time. We're going to be doing this with different frequencies, observing the force that can be produced under each change in frequency with the addition of vasopressin and lactic acid.

**Intro to Muscle Physiology**  
**Post Lab Assignment (Turn in 1 per group)**

Student Names: Katlyn Gabriel, Shelby Mitchell

1. What effect do you think the increasing stimulus voltages might have on the force of contraction of the muscle? State this as a hypothesis:

We think that increasing the stimulus voltages will result in a stronger contraction of the muscle.

2. What effect do you think the increasing frequency of stimulation might have on the force of contraction of the muscle? State this as a hypothesis:

When the frequency of the stimulus is faster than the amount of time the muscle takes to relax, the contractions become additive. The muscle contracts the same amount per stimuli, but added ~~to~~ to the point of the last contraction rather than a resting point.

3. Did your results match your hypotheses?

Yes, refer to graphs & lab notebooks

4. Describe the experiment you will perform next week. Include: Hypothesis, Prediction, and Experimental Methods (use back as needed).

For next week, we will be relating how serotonin affects peak muscle contraction ~~time~~ and relaxation time. We think that increasing serotonin concentration with serial dilution will result in a ~~stronger~~ weaker force of contraction and a shorter relaxation time. We ~~predicted~~ know that ~~the~~ serotonin plays an important role in muscle relaxation and contraction, and we plan to test that with the experiment as follows: we will add serotonin to our muscle starting with our lowest serial dilution after running a trial with no serotonin. We will do 5 trials at each concentration:

none,  $10^{-6}$ ,  $10^{-5}$ ,  $10^{-4}$ , etc. and will repeat this with a second mussel. By raising the concentrations from the serial dilutions we performed this week and running more trials (each trial lasts until the muscle relaxes back to its pre-stimulated state), we hope to assess the ~~long term~~ effects of serotonin on muscle contraction<sup>relaxation</sup> in the anterior byssal ~~mus~~<sup>retractor</sup> muscle of a mussel.

**Intro to Muscle Physiology**  
**Post Lab Assignment (Turn in 1 per group)**

2/8/17

Student Names: Erina Taradei, Nick Welch

1. What effect do you think the increasing stimulus voltages might have on the force of contraction of the muscle? State this as a hypothesis:

We hypothesize that increasing stimulus voltage will increase the force of contraction of the muscle.

2. What effect do you think the increasing frequency of stimulation might have on the force of contraction of the muscle? State this as a hypothesis:

We hypothesize that increasing the stimulus on the muscle will make the muscle ~~fast~~ continue to contract until it reaches a threshold and hyperpolarizes.

3. Did your results match your hypotheses?

yes

4. Describe the experiment you will perform next week. Include: Hypothesis, Prediction, and Experimental Methods (use back as needed).

We hypothesize that exposing the mussel to a more basic environment will induce relaxation of the muscle, while a more acidic environment will induce muscle contraction. We will expose the mussel ~~with~~ to a range of pH's from 4 to 9, (realistic physiological pHs). ~~and keep the other~~ we will prepare various solutions + add them to instant ocean. We will first spray the mussel and look for an acute

result, and if that doesn't produce immediate results we may incubate the mussels in the solution for ~~pro~~ testing.

### What we will Assay

- time from baseline to return to baseline following contraction; length of contraction
- ~~for activity~~



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Student Names: Melissa Santos, Stephanie Aboody  
Bethany Hryniewich

1. What effect do you think the increasing stimulus voltages might have on the force of contraction of the muscle? State this as a hypothesis:

By increasing stimulus voltage, the force of the contraction will be stronger.

2. What effect do you think the increasing frequency of stimulation might have on the force of contraction of the muscle? State this as a hypothesis:

By increasing the frequency of stimulation, the force of the contraction will be stronger.

3. Did your results match your hypotheses?

Only for acetylcholine that increased the response, while serotonin did the opposite and decreased response to stimuli.

4. Describe the experiment you will perform next week. Include: Hypothesis, Prediction, and Experimental Methods (use back as needed).

We are going to measure the influence (if any) of ATP on the strength of the muscle contractions.

Experimental Methods would include a similar set up, with dilutions and different concentrations of ATP.

Hypothesis: If the [ATP] is higher, then <sup>the</sup> stronger the muscle contraction and the more the muscle will be able to withstand pulses.

Prediction: We predict that as we increase the [ATP] the muscle will become stronger and be able to withstand more pulses as well as.

1-10 mmol of ATP

Starting: 50mg/mL of inst. ocean

molar mass: 507.18 g/mol

highest:  $\frac{10 \text{ mmol}}{1 \text{ L inst. ocean}} \times \frac{0.5072 \text{ mg}}{1 \text{ mol}} = 5.072 \text{ mg}$

10.144 mg in 2 mL instant ocean = 10 mmol

4 serial dilutions:

$10^{-3}$  - starting

$10^{-4}$  - 20ul of  $10^{-3}$  + 200ul inst. ocean

$10^{-5}$  - 20ul of  $10^{-4}$  + 200ul i.o.

$10^{-6}$  - 20ul of  $10^{-5}$  + 200ul i.o.

$10^{-7}$  - 20ul of  $10^{-6}$  + 200ul i.o.

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Student Names: Alex Alexander Justine Guarn

1. What effect do you think the increasing stimulus voltages might have on the force of contraction of the muscle? State this as a hypothesis:

2. What effect do you think the increasing frequency of stimulation might have on the force of contraction of the muscle? State this as a hypothesis:

If  $CaCl_2$  is 1 Molal  
~~increase~~

Instant Ocean

$Ca^{2+} = 9.4 \text{ mmol/kg} = 0.0094 \frac{\text{mol}}{\text{kg}}$

$Cl^- = 521 \text{ mmol/kg} = 0.521 \frac{\text{mol}}{\text{kg}}$

$Na^+ = 462 \text{ mmol/kg} = 0.462 \frac{\text{mol}}{\text{kg}}$

3. Did your results match your hypotheses?

EDTA 1 to 1 mole ratio.

so = to  $[Ca^{2+}]$  of Inst. Ocean.

$\frac{1 \text{ mol Calcium}}{1 \text{ mol EDTA}}$

~~If EDTA reacts~~

4. Describe the experiment you will perform next week. Include: Hypothesis, Prediction, and Experimental Methods (use back as needed).

Hypothesis: Addition of ~~EDTA~~ <sup>EDTA</sup> ~~Ca<sup>2+</sup>~~ <sup>Ca<sup>2+</sup></sup> will result in a ~~decrease~~ <sup>decrease</sup> in muscle tension while an addition of ~~EDTA~~ <sup>Ca<sup>2+</sup></sup> will increase muscle tension. ~~an addition of Ca<sup>2+</sup> ions will result in an increase muscle tension. due to differences in concentration of Ca<sup>2+</sup>~~ <sup>to a less effective extent</sup>

Prediction: Addition of ~~K<sup>+</sup>~~ <sup>K<sup>+</sup></sup> as concentration of ~~K<sup>+</sup>~~ <sup>K<sup>+</sup></sup> increases the muscle relaxation will increase

Addition of ~~Na<sup>+</sup>~~ <sup>Na<sup>+</sup></sup> as concentration of ~~Na<sup>+</sup>~~ <sup>Na<sup>+</sup></sup> increases the muscle tension will increase

Addition of ~~Ca<sup>2+</sup>~~ <sup>Ca<sup>2+</sup></sup> as  $[Ca^{2+}] \uparrow$  the muscle tension will increase.

Add EDTA: muscle tension will decrease. the effects of EDTA will be greater than the effects of  $Ca^{2+}$ .

## Experimental Methods:

We will use the same setup similar to when using neurotransmitters as drawn in our lab notebooks.

We will begin with ~~Ca<sup>2+</sup>~~ EDTA and add 5 drops of the most diluted solution to the muscle. Three trials will be run and the change in voltage will be measured. This will be repeated for each serial dilution and for the ~~Ca<sup>2+</sup>~~ EDTA Ca<sup>2+</sup>. Between each trial, ~~we~~ it is important to rinse with ~~distilled water~~ saltwater. ~~to~~ to eliminate the effects ~~of the ions~~ the ions from the previous trials. ~~&~~ keep the mussel alive.

## Reagent List:

~~Ca<sup>2+</sup>~~, distilled water.  
we will perform a serial dilution.

EDTA & Ca<sup>2+</sup>

Ex { Pt 1: +EDTA 1M  
Pt 2: +Ca<sup>2+</sup> 1M  
Pt 3: +Ca<sup>2+</sup> then +EDTA

1M +	0.25M
1M +	0.50M
1M +	0.75M
1M +	1.0M

Data we'll record → Assay Muscle Contraction focusing on the relationship of Ca<sup>2+</sup> to EDTA in mussel's muscle.

Ca <sup>2+</sup> <del>[Ca<sup>2+</sup>]</del>	Trial #	Muscle Contraction Δ Voltage
a	1	
	2	
	3	
b	1	
	2	
	3	
c	1	
	2	
	3	

EDTA <del>[EDTA]</del>	Trial #	Muscle Contraction Δ Voltage
a	1	
	2	
	3	
b	1	
	2	
	3	
c	1	
	2	
	3	

**Intro to Muscle Physiology**  
**Post Lab Assignment (Turn in 1 per group)**

Student Names: Anna Wang, Rachel Fredericks

1. What effect do you think the increasing stimulus voltages might have on the force of contraction of the muscle? State this as a hypothesis:
2. What effect do you think the increasing frequency of stimulation might have on the force of contraction of the muscle? State this as a hypothesis:
3. Did your results match your hypotheses?
4. Describe the experiment you will perform next week. Include: Hypothesis, Prediction, and Experimental Methods (use back as needed).

- The next experiment will test the effect of decreasing temperature on the force of contraction.
- Hypothesis: it is more difficult for a muscle to contract in cold temperatures than in warm temperatures
- Prediction: decreasing temperature will decrease the force of contraction

→ methods

## Methods

- we will heat water and record its temp. with a thermometer. There will be a gradual decrease in temperatures until they reach room temperature.
- will also have water refrigerated and water with (more or less) ice. temperatures recorded

↳ gradient of water temperatures.

- dunk different mussel in the different temperatures for set time
- remove mussels, stimulate at certain voltage (same throughout), record contraction

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Student Names: Yenhely Gomez, Roderick Towner

1. What effect do you think the increasing stimulus voltages might have on the force of contraction of the muscle? State this as a hypothesis:

- If we increase stimulus voltage then the force of muscle contraction will also increase.

2. What effect do you think the increasing frequency of stimulation might have on the force of contraction of the muscle? State this as a hypothesis:

- If we incre the frequency then the force of muscle contraction will also increase due to summation of several contraction.

3. Did your results match your hypotheses?

- For the increase of voltage, it was not tested because the mussel only seemed to react to 3V. so we only used 3V.
- For the increase in frequency, our results matched the hypothesis. (see graphs on journal)

4. Describe the experiment you will perform next week. Include: Hypothesis, Prediction, and Experimental Methods (use back as needed).

See Back

Hypothesis: Administering nicotine will reduce muscle resulting in a lower the maximum force output.

- Nicotine is an antagonist to acetylcholine

Prediction: increasing concentration of nicotine will inhibit muscle contraction.

Experimental method:

- set up parameters similar to exercise 2 to induce summation of tetanus

- use different concentrations of nicotine

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Start with: 9 mL of salt H<sub>2</sub>O  
1 mL of nicotine  
↓  
Dilute 1 mL of }  
to 9 mL of salt H<sub>2</sub>O  
and so on

- find primary literature for a starting concentration
- Dose response experiment.

- use concentration of  $10^{-6}$  M and work our way up from there.  $\rightarrow$  Dilute in salt water solution

$\hookrightarrow 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}$  M

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- Run the experiment and shock the mussel without nicotine as a control.

- Then add lowest dilution and add concentration in a increasing order

- We will looking for the time it takes to get to tetanus and the force at tetanus.