

Effects of Stimulants and Depressants on Brine Shrimp

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Introduction

A whole-animal opioid potency test is being developed using a generated strain of *C. elegans*



The generated strain has the mu-opioid receptor, allowing them to respond to opioids with relevant sensitivity



Before working with *C. elegans*, brine shrimps are being used as a model for them



Brine shrimp development

Image from aquariumbreeder.com



C. elegans

Image from socmucimm.org

Objective

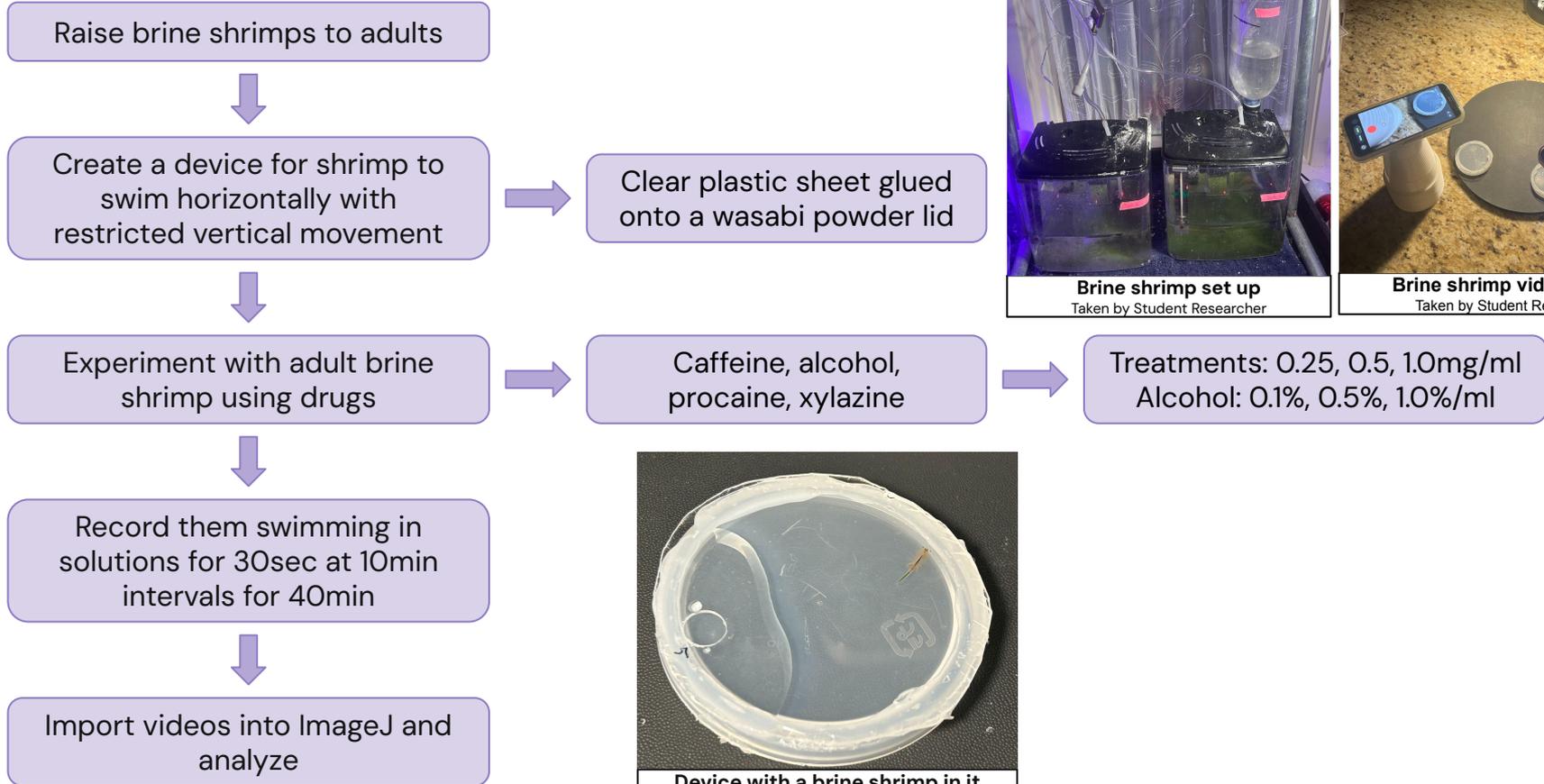
Develop a method to handle and image the brine shrimps in a controlled environment

Observe their behavior in a controlled environment and how they react when exposed to drugs

Hypothesis

If brine shrimp are dosed with controlled substances, then their speed and behavior will change in response to it

Procedure Overview



Brine shrimp set up
Taken by Student Researcher

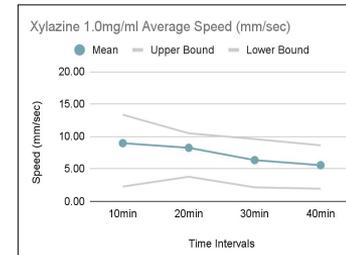
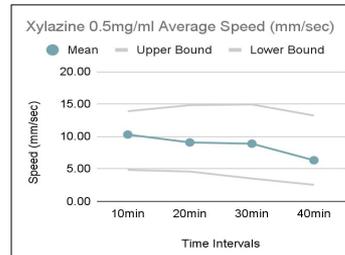
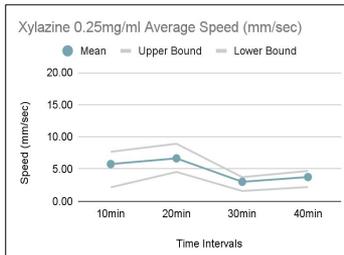
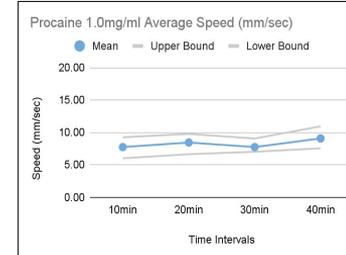
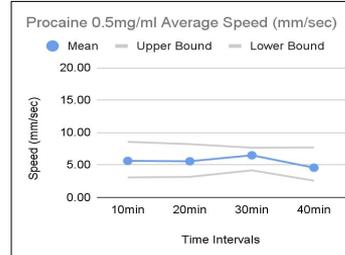
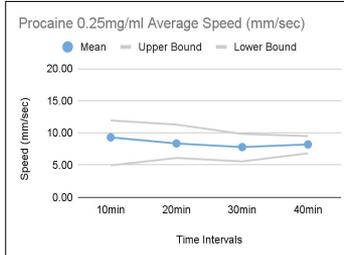
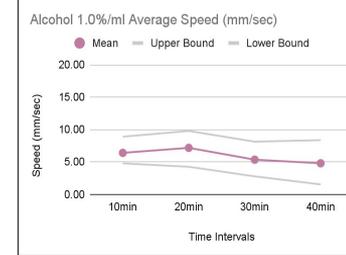
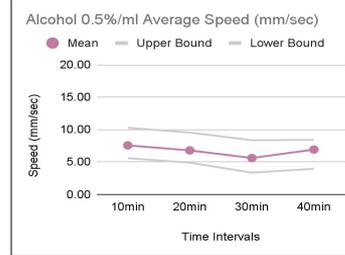
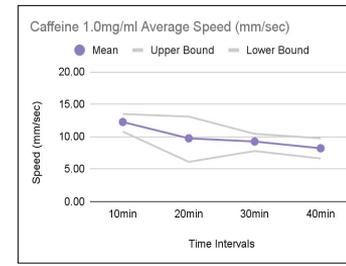
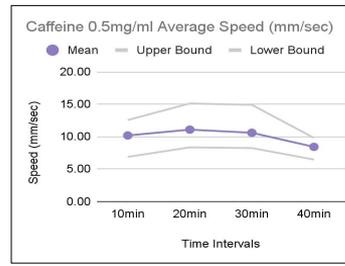
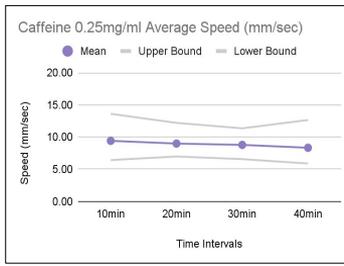
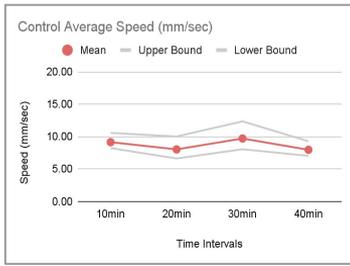


Brine shrimp video set up
Taken by Student Researcher



Device with a brine shrimp in it
Taken by Student Researcher

Data



All graphs made by Student Researcher

- For each drug, the trial values at each time interval were calculated by adding the total speed from the 30sec video and dividing by the number of frames for an average speed
- The trials were averaged together to result in an average speed over time with upper and lower bounds to show variability between trials



Student Researcher recording set up
Taken by Marya Lieberman

Statistical Analysis

Each trial with its time intervals, was averaged. The Mann-Whitney U test was used to compare each drug to the control to show its individual significance and effect. The same values were used to create box plots to visualize and compare the spread of data. The p-values were insignificant due to small sample size ($p > 0.05$), but drugs were shown to have an effect compared to the control (small=0.1; medium=0.3; large=0.5).

All tables and graphs made by Student Researcher

0.25mg/ml Average Speed (mm/sec)					
Trial #	Control	Caffeine	Alcohol	Procaine	Xylazine
Trial 1	7.85	7.07	6.52	8.82	2.62
Trial 2	10.56	7.11	9.41	6.93	5.56
Trial 3	7.72	12.46	9.94	9.51	6.21

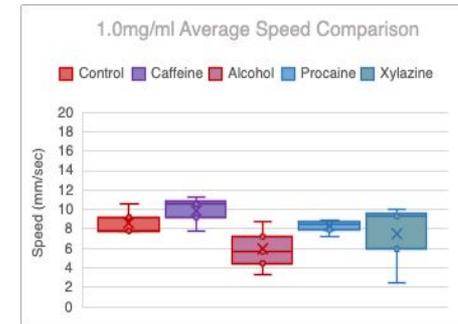
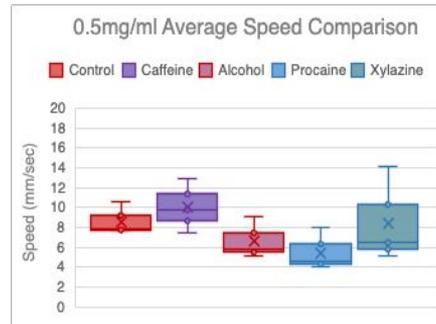
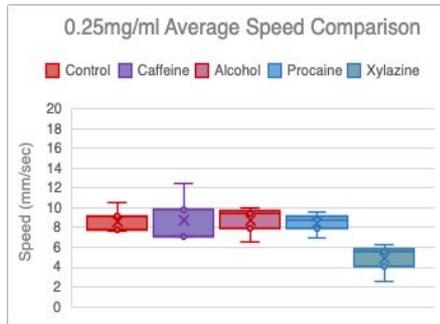
0.5mg/ml Average Speed (mm/sec)					
Trial #	Control	Caffeine	Alcohol	Procaine	Xylazine
Trial 1	7.85	9.83	9.13	3.99	14.22
Trial 2	10.56	7.47	5.81	8.02	5.20
Trial 3	7.72	12.89	5.19	4.64	6.49

1.0mg/ml Average Speed (mm/sec)					
Trial #	Control	Caffeine	Alcohol	Procaine	Xylazine
Trial 1	7.85	10.53	5.66	8.95	9.31
Trial 2	10.56	7.82	3.33	7.29	2.52
Trial 3	7.72	11.21	8.79	8.51	9.98

0.25mg/ml Mann-Whitney U Test Results: Control vs. Drug				
Drug	U-Stat	z-score	p-value	r (effect size)
Caffeine	3	-0.65	0.51	0.27
Alcohol	4	-0.22	0.83	0.09
Procaine	4	-0.22	0.83	0.09
Xylazine	0	-1.96	0.05	0.80

0.5mg/ml Mann-Whitney U Test Results: Control vs. Drug				
Drug	U-Stat	z-score	p-value	r (effect size)
Caffeine	5	0.22	0.83	0.09
Alcohol	2	-1.09	0.28	0.45
Procaine	2	-1.09	0.28	0.45
Xylazine	3	-0.65	0.51	0.27

1.0mg/ml Mann-Whitney U Test Results: Control vs. Drug				
Drug	U-Stat	z-score	p-value	r (effect size)
Caffeine	6	0.65	0.51	0.27
Alcohol	2	-1.09	0.28	0.45
Procaine	4	-0.22	0.83	0.09
Xylazine	4	-0.22	0.83	0.09



Results

0.25mg/ml

- Caffeine generally showed similar speeds to the control, but had a small increase along with an approaching moderate effect ($r=0.27$)
- Alcohol and procaine showed similar speeds and both had a negligible to small effect ($r=0.09$)
- Xylazine showed a significant decrease in speed and the largest effect ($r=0.80$), with a p-value approaching significance ($p=0.05$)

0.5mg/ml

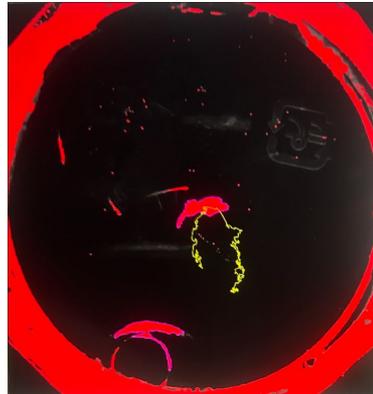
- Caffeine showed similar to higher speeds with a negligible effect due to overlap
- Alcohol and procaine both showed decreased speeds and substantial moderate effect ($r=0.45$)
- Xylazine showed mixed speeds being lower or higher to the control and showed high variability but having an approaching moderate effect

1.0mg/ml

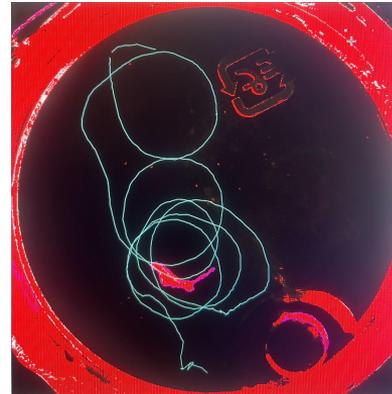
- Caffeine showed similar to higher speeds, having an approaching moderate effect
- Alcohol showed lower speeds with a substantial moderate effect
- Procaine showed similar speeds and had a negligible to small effect
- Xylazine showed mixed speeds being lower or higher with noticeable variability, but had a negligible to small effect



Unedited image of brine shrimp
Taken by Student Researcher



Xylazine: 10min 1.0mg/ml
Taken by Student Researcher



Procaine: 30min 1.0mg/ml
Taken by Student Researcher



Xylazine: 30min 0.5mg/ml
Taken by Student Researcher

Discussion

Variability between trials likely reflects differences in behavior including: spasms, bursts of speed, twitchiness, lethargy, and circular locomotion

These abnormal behaviors may result from physiological stress or drug-induced effects on neural receptors and motor control pathways

0.25mg/ml

- No noticeable behavioral differences were observed, except for a decrease in speed for xylazine.
 - This may indicate have a stronger or more sensitive impact on brine shrimp activity

- Caffeine data aligns with their stimulant properties
- Alcohol data aligns with their depressant properties
- Procaine data aligns with their anesthetic properties
- Xylazine aligns with their anesthetic properties

0.5mg/ml

- Caffeine showed similar to higher speeds, spasms, bursts of speed, and twitchiness
- Alcohol showed similar to lower speeds, spasms, twitchiness, and lethargy
- Procaine showed lower speeds, spasms, twitchiness, lethargy, and circular locomotion
- Xylazine showed both higher and lower speeds, spasms, bursts of speeds, twitchiness, and lethargy

1.0mg/ml

- Caffeine showed similar to higher speeds, spasms, bursts of speed, and twitchiness
- Alcohol showed similar to lower speeds, spasms, twitchiness, and lethargy
- Procaine showed similar speeds, spasms, twitchiness, and circular locomotion
- Xylazine showed similar to lower speeds, spasms, twitchiness, and lethargy

Conclusion

Caffeine generally maintained or increased in speed

Alcohol and procaine generally showed decreased speed

Xylazine showed a mixed response and significant variability, but generally decreased in speed

Data suggests drugs induced a behavioral difference in the brine shrimps, supporting my hypothesis

Improvements

Increase trials to improve reliability, refine video tracking methods, and quantify behavior

Further steps

Construct better device, potentially find a more efficient tracking software, and eventually work with *C. elegans*

Application

Provides a relevant method for testing drug potency and foundation for developing the *C. elegans* potency test

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