Cockroaches on Caffeine: Behavioral analysis of the Madagascar Hissing

Cockroach(*Gromphadorhina portentosa)* after a long term Caffeine Supplemented Diet.

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Abstract:

Caffeine is a commonly consumed psychoactive drug in our society. As consumption increases, defining what counts as mild-moderate caffeine abuse is critical to understand. This project looked at whether the Madagascar Hissing Cockroach (*Gromphadorhina portentosa*) can be used as a model organism for future research into caffeine and caffeine's effect on behavior for *G. portentosa*. This was done by recording one caffeine gelatin fed group and one non caffeine gelatin group 16 hours over the course of 40 days. Data analysis was done by picking 8 points, 4 during the day and 4 during the night, and recording what each cockroach was doing during that time. Results showed that on a caffeine supplemented diet, levels of locomotion increased and evidence of circadian rhythm disruption occurred, which matches past studies of caffeine's effect on invertebrates and humans. Aggression levels also rose in the caffeine supplemented group, while burrowing rates decreased significantly. It's important to gain this information, as researching what constitutes caffeine use disorder and what organisms we can use to model behavior shifts can be very helpful in the future.

Acknowledgements:

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Introduction:

Caffeine is a commonly consumed psychoactive drug which produces its psychomotor stimulant and reinforcing effects through antagonism at adenosine receptors and indirect effects on dopaminergic neurotransmission(Ferre,2016) . Previous research has supported the idea that people who consume coffee daily have developed signs of dependency for caffeine (Ogawa and Ueki, 2007). In 2013, caffeine use disorder first appeared in the Diagnostic and Statistical Manual(DSM) by including the disorder in section III: conditions for further study (American Psychiatric Association, 2013), While caffeine use disorder(CUD) is listed in DSM , the lack of data regarding the clinical significance of it has resulted in this disorder being listed as needing further research(APA,2013)

Caffeine use has been shown to have a multitude of positive and negative effects depending on the dosage and usage in animals (Caravan, I., Sevastre Berghian, A., Moldovan, R., Decea, N., Orasan, R., & Filip, G. A. (2016). Expanding on how caffeine interacts with neurological systems is a growing area of interest, with research into how it affects the common fruit fly exists(Wu, Ho, Crocker, Yue, Koh,Sehgal, 2009) and establishing the behaviors associated with CUD in other species is a possible area of interest. The introduction of model organisms,or organisms that can serve as a study for how certain biological processes work,can be used for understanding the effects of caffeine on behavior. The Madagascar Hissing Cockroach(*Gromphadorhina portentosa)*,which can serve as a model organism due to its high reproductive rate and relatively easy care, is something that is increasingly appealing for study. *G. portentosa* also is increasingly an appealing option to observe the changes psychoactive substances can have on behavior,in part to *G. Portentosa*, like a majority of cockroaches, possessing similar neurotransmitters as mammals (Huber, 2020),

How does long term caffeine intake affect the general behavior of *G. portentosa*? Finding whether *G. portentosa's* behavior over long term caffeine intake matches past explorations into caffeine effect on invertebrates can serve to further support whether it is a model organism and whether new behavior changes can be observed that aren't fully documented before. This project will utilize a small colony of *G. portentosa*, split into two groups of 6, to test changes in behavior across a 40 day time period. Our independent variable will be caffeine intake, as one group will be fed a concentration of 1.9 G of caffeine in 100 ml water ,which will be made into gelatin.Each group will then be recorded and observed for 6 key behaviors: Aggression, Resting, Antenna Activity with no locomotion, Eating, Burrowing, and Moving. Our hypothesis will be that locomotion rates will increase, alongside a decrease in resting behavior. The thought process behind this is that the effects on *G. portentosa* will be in line with other invertebrates, solidifying it as a model organism.

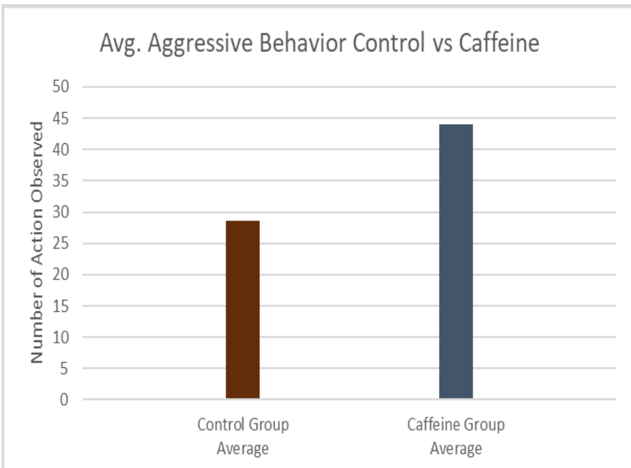
Methods:

This project was conducted entirely in a physical format, utilizing a sample group of *G. Portentosa* as the basis of our results. Two groups of 6, three female and 3 male, were sampled from a larger colony and placed into an enclosure(6" x 18" x 24") divided in the center. Substrate consisting of coconut fiber and wood chips was spread 2 inches thick along the bottom. Once in enclosure, each sample was marked with a marker, to provide identification. Over the course of experimentation, the two groups of *G. Portentosa* will be determined, with one having the caffeine supplemented diet while the other being fed a non supplemented diet. The caffeine group will be fed a mixture of collagen gel, containing 1.9 g of caffeine powder, 100 ml of water, creating 10 G of collagen gel, while the control group will be fed purely 10g of collagen gel.

As this project set out to observe the effects of caffeine intake, two methods of obtaining long term visual data were used in tandem. Using both a camcorder and a Note 8 cleared for storage propped on a tripod directly above the tank, we were able to record 16 hours of footage. This was obtained by filming 6 hours of footage daily with the camcorder from 8 a.m to 2 p.m, and by using the Auto Clicker app by True Development Studios, we were able to set to Note 8 to begin recording from 2-4 and then start recording 8 p.m to 4 a.m. Daily, the video from both recording devices would be extracted to our laptops, allowing us to analyze the data.

Data analysis was done by reviewing the 16 hours of footage, and recording what each sample was doing every 2 hours. By labeling each sample with a number, we could accurately keep track of which cockroach was which over the course of 40 days. Splitting all behaviors into 6 categories: Eating, Activity(Locomotion), Activity(Antenna Moving+No Locomotion), Aggressive behavior:such as Agnostic Hissing,Abdomen shoving or thrusting,and the butting/ramming of horns, No Activity(Resting), and being Burrowed(Resting), the footage recorded could be split among all 6 categories for a total of 320 points(8 per day across 40 days).Graphs were made using Excel, comparing the averages of each group with each behavior. By averaging each quantifiable behavior and determining the standard deviation of the Control and Experimental, an unpaired T- Test was used to quantify if there was a significant difference between our non caffeine group and our caffeine supplemented group. By using the T value obtained from each behavior, a P value was calculated to determine if our null hypothesis (Of no change between groups) could be rejected. Analysis of these p values was used to determine if the changes observed were significantly different and could be linked to change in diet.

Results:

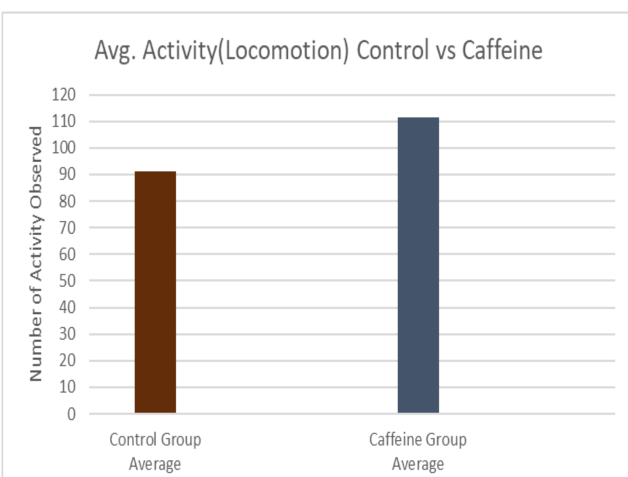


Agression			
Caffeine(Mean)	Pvalue	Tvalue	Std Dev
44	0.01	5.322	4.55

Figure 1: Depiction of average instances of aggression between control group and Caffeine group of *G. portentosa.*

Figure 1B: Table showing relation between caffeine and aggression, attached Mean, T and P value, and Standard deviation.

Analysis of average instances of aggressive behavior between control and caffeine group found significant positive relationship between caffeine and aggressive behavior in *G. portentosa.* Statistically Significant P value of 0.01 as the P value is below 0.05.

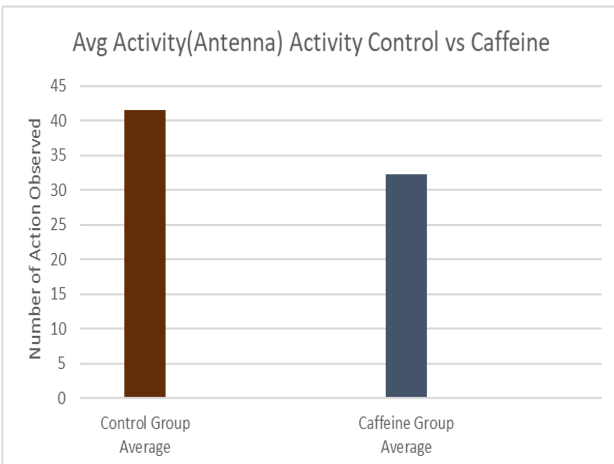


Activity(Locomotion)			
Caffeine(Mean)	Pvalue	Tvalue	Std Dev
111.5	0.004	4.84	10.36

Figure 2: Depiction of average instances of locomotion between control group and Caffeine group of *G. portentosa.*

Figure 2B: Table showing relation between caffeine and Locomotion, with attached Mean, T and P value, and Standard Deviation.

Analysis of average instances of Locomotion between control and caffeine group found significant positive relationship between caffeine and Locomotion in *G. portentosa.* Statistically Significant P value of 0.004 as the P value is below 0.05.

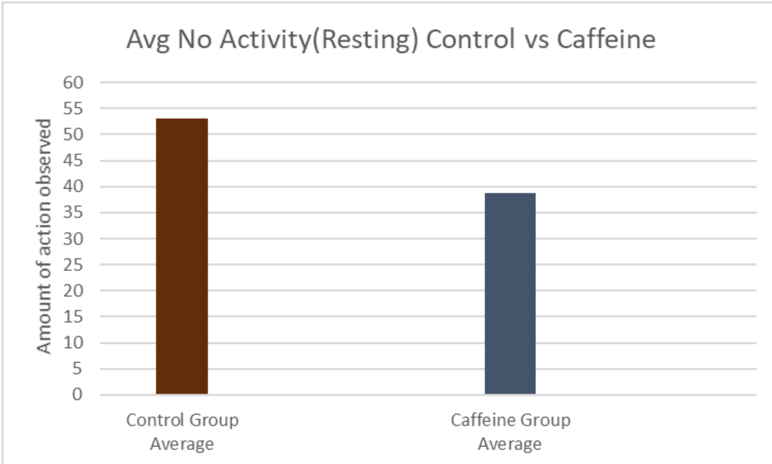


Antenna Activity(No Locomotion)			
Caffeine(Mean)	Pvalue	Tvalue	Std Dev
32.33	0.004	-5.01	4.49

Figure 3: Depiction of average instances of Antenna motion+No locomotion between control group and Caffeine group of *G. portentosa.*

Figure 3B: Table showing relation between caffeine and Antenna motion+No Locomotion, attached Mean, T and P value, and Standard deviation.

Analysis of average instances of antenna motion+no locomotion behavior between control and caffeine group found significant positive relationship between caffeine and antenna motion+no locomotion behavior in *G. portentosa.* Statistically Significant P value of 0.004 as the P value is below 0.05.

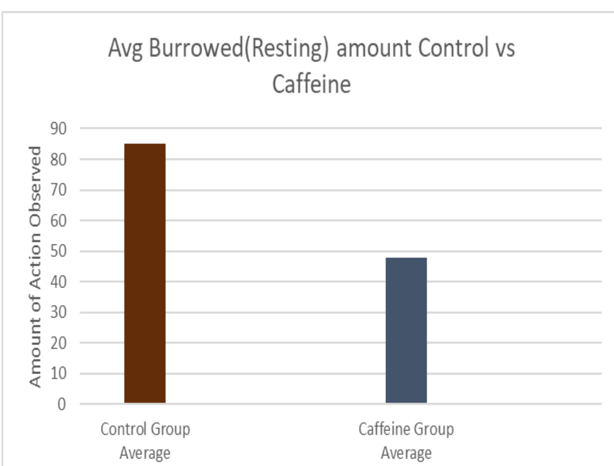


No Activity(Resting)			
Caffeine(Mean)	Pvalue	Tvalue	Std Dev
38.667	0.01	-3.75	9.48

Figure 4: Depiction of average instances of No Activity(Resting) behavior between Control group and Caffeine group of *G. portentosa.*

Figure 4B: Table showing relation between caffeine and No Activity(Resting), attached Mean, T and P value, and Standard deviation.

Analysis of average instances of No Activity(Resting) behavior between control and caffeine group found significant negative relationship between caffeine and antenna motion+no locomotion behavior in *G. portentosa.* Statistically Significant P value of 0.01 as the P value is below 0.05.

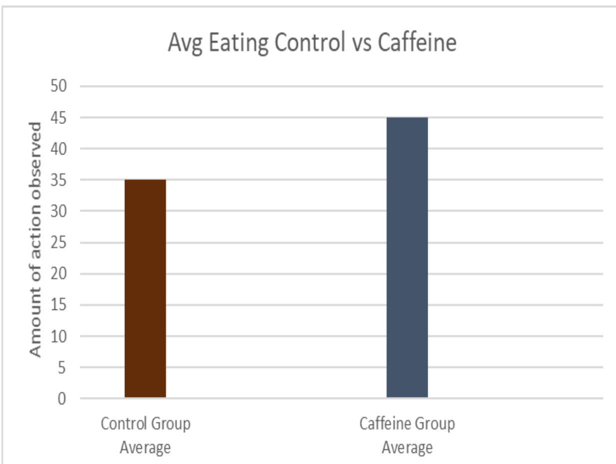


Burrowed(Resting)			
Caffeine(Mean)	Pvalue	Tvalue	Std Dev
47.83	0.001	-6.45	14.12

Figure 5: Depiction of average instances of burrowed behavior observed between Control group and Caffeine group of *G. portentosa.*

Figure 5B: Table showing relation between caffeine and burrowing, attached Mean, T and P value, and Standard deviation.

Analysis of average instances observed of Burrowed motion between control and caffeine group found significant negative relationship between caffeine and Burrowed motion in *G. portentosa.* Statistically Significant P value of 0.001 as the P value is below 0.05.



Eating			
Caffeine(Mean)	Pvalue	Tvalue	Std Dev
45	0.03	2.95	8.3

Figure 6: Depiction of average instances of eating behavior observed between Control group and Caffeine group of *G. portentosa.*

Figure 6B: Table showing relation between caffeine and eating, attached Mean, T and P value, and Standard deviation.

Analysis of average instances observed of eating motion between control and caffeine group found significant positive relationship between caffeine and eating in *G. portentosa.* Statistically Significant P value of 0.03 as the P value is below 0.05.

Discussion;

We hypothesized that long term Caffeine intake would have a significant positive relationship with aggressive behavior and the results of this project would support the idea that *G. Ponderosa* being a suitable model organism for testing behavioral changes with caffeine. We found that long term caffeine consumption did have a significant result on aggression levels in *G. portentosa.* Unexpectedly, long term caffeine intake had other unexpected effects on burrowing and rates of eating.

Our analysis of rates of aggressive behavior in *G. portentosa* after long term caffeine intake showed that there was a significant positive relationship between a caffeine diet and instances of aggressive behavior. This is new data concerning how caffeine can change the behavior of *G. portentosa* in an observational setting. This could relate to investigations into whether caffeine increases aggression, and whether the role it plays is substantial or not. The data we have suggests it has a significant correlation with aggression, but this could be expanded upon in further research on this subject.

We also found that rates of locomotion increased and resting decreased, which means the caffeine supplemented diet group were awake longer times and acted with more movement during observation. This is consistent with past studies supporting that chronic consumption of caffeine lead to a lengthening of the circadian period in both humans(Roehrs and Roth,2008), and other invertebrates like the fruit fly(Wu, et al, 2009). This is vastly important in supporting the idea that *G. portentosa* can be used as a model organism for behavioral tests involving psychoactive substances, like caffeine.

If this research were to continue, we would like to have a larger sample size and time period of observation, in order to better understand how each dependent variable with longer intake. This could possibly support or weaken the results and conclusions already drawn. Involving more substances and comparing whether larger colonies would interact with behavior is something we would be interested in, as being limited to 6 in each group is not entirely normal for colonies of *G. portentosa*. Expanding the capabilities of recording equipment could also drastically improve precision of results, as we were limited to not being able to review 16 hours of footage daily,and as such limiting data collection to set time periods throughout the day, which is not fully representative of all behaviors. Autonomous programs like Anymaze and DeepLabCut offer autonomous ways to catalog behavioral data, yet are very pricey,ranging upwards of 10,000, compared to our cost effective method of gathering data.

Conclusion:

Across all dependent variables, there was a correlation between long term caffeine intake and either a positive correlation or negative on the behavior of *G. portentosa*. The effects of caffeine on locomotion and circadian rhythm were consistent with other studies on invertebrates. Rates of aggression increased significantly, while rates of burrowing decreased with caffeine consumption, which is a future area of study on exactly why that happened. In all, *G. portentosa* and its behavioral changes under long term caffeine consumption support the idea that it is a model organism for caffeine behavioral changes, while opening new areas of interest, focusing on the increase in aggression or decrease in burrowing. With caffeine consumption becoming more prevalent and dosages increasing , mild symptoms of CUD and how it is diagnosed is of importance, and by supporting the use of *G. portentosa* as a model organism, there is hope that gateways to new research can be unlocked.

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