A Study of the Effects of Caffeine and Serotonin on the Rate of Neurotransmission of the Discoid Roach, *Blaberus discoidalis*

Abstract

According to the Food and Drug Administration, 90% of Americans consume caffeine daily. Caffeine is a drug and a central nervous stimulant that blocks the activity of certain neurotransmitters that are important parts of a functioning body. Serotonin is a neurotransmitter that helps provide healthy sleeping patterns, boosts mood, and is sometimes used as treatment for depression and hyperactivity disorders.

Discoid Roaches were injected with 150 mg/L of caffeine and 200 mg/L of serotonin. A glass stirring rod was dropped on the roach leg 5 times per run, with 4 runs per trial, and 3 total trails. The neurotransmission amplitude was recorded from SpikeRecorder and Matlab.

It was hypothesized that discoid roaches injected with 150 mg/L of caffeine would have a higher action potential amplitude neurotransmission compared to roaches injected with 0 mg/L of caffeine. It was also hypothesized that discoid roaches injected with 200 mg/L of serotonin would have a higher action potential amplitude neurotransmission compared to roaches injected with 0 mg/L of serotonin.

The data supported the first hypothesis, the discoid roach legs exposed to 150 mg/L of caffeine had a higher mean amplitude than roaches exposed to 0.0 mg/L of caffeine. The data did not support the second hypothesis, the discoid roaches exposed to 200 mg/L of serotonin did not have a lower mean amplitude than roaches exposed to 0.0 mg/L of serotonin. It was hypothesized that serotonin would decrease the action potential amplitude, however the legs exposed to serotonin had an increase in action potential amplitude.

Introduction

Caffeine is found in many common foods such as colas and coffee. Caffeine is said to improve speed, strength, self-confidence, reaction time and stop depression. It is also said to sharpen memory, verbal fluency, concentration, and decision making (World of

Caffeine). However, caffeine also blocks the activity of the neurotransmitter, adenosine. Adenosine is a neurotransmitter that makes you tired or sleepy. Caffeine also interferes with dopamine, acetylcholine, serotonin, and norepinephrine (Caffeine Informer).

Serotonin in commonly used in medications and pills to help with mood enhancement and stability. Serotonin (5-hydroxytrytryptamine) is a neurotransmitter that acts as a mood stabilizer. Serotonin is made with a very important part of protein- tryptophan. Serotonin helps in providing healthy sleeping patterns. (Medical News Today) It also helps boost your mood and is sometimes used as a treatment for depression. (Web MD)

The Discoid Roach (*Blaberus discoidalis)* will be used in this study. They are a good model because even though they have a less amount of neurons than humans, they are similar. The structure and function of individual neurons are similar. The Discoid roach (*Blaberus discoidalis)* has a decentralized nervous system. It has ganglia (small brains) running down its body and a main ganglia at the front of the roach. (Backyard Brains)

Variables

IV1: Amount of Caffeine in mg/L
IV2: Amount of Serotonin in mg/L

DV: Rate of neurotransmissions in Spike Amplitude

Research Hypothesis

H1: *Blaberus discoidalis* neurotransmissions will have a higher mean amplitude when exposed to 150 mg/L of caffeine compared to *Blaberus discoidalis* exposed to 0.0 mg/L of caffeine.

H2: *Blaberus discoidalis* neurotransmissions will have a higher mean amplitude when exposed to 3000 mg/L of serotonin compared to *Blaberus discoidalis* exposed to 0.0 mg/L of serotonin.

Null Hypothesis

H01: *Blaberus discoidalis* neurotransmissions will have no difference in the mean when exposed to 150 mg/L of caffeine compared to *Blaberus discoidalis* exposed to 0.0 mg/L of caffeine.

H02: *Blaberus discoidalis* neurotransmissions will have no difference in the mean when exposed to 200 mg/L of caffeine compared to *Blaberus discoidalis* exposed to 0.0 mg/L of caffeine.

Background

What is a Neurotransmitter?

There are certain requirements for something to be classified as a neurotransmitter. First, it must be produced inside a neuron, it must be found in a neuron's terminal button, and must be released into the synaptic gap when the action potential arrives . It must produce an effect on the postsynaptic neuron. After it has transmitted it's signal to this neuron, it must deactivate rapidly. It also must have the same effect on the postsynaptic neuron when applied experimentally as it does when released by a presynaptic neuron.

Types of Neurotransmitters

1. Acetylcholine:

    -excitatory

    -triggers muscle contraction

    -Stimulates the excretion of certain hormones

    -Involved with wakefulness, attentiveness, and thirst

    - Alzheimers disease is associated with a lack of acetylcholine in certain regions of the brain

2. Dopamine:

-controlling movement and posture

-modulates mood

-plays a central role in “positive reinforcement”

-Plays a loss of Dopamine causes muscle rigidity typical of Parkinson’s disease

3. Gamma- animobutric acid(GABA)

-Inhibitory

-widely distributed in the neurons of the cortex

-motor control, vision

-regulates anxiety

-High levels help treat epilepsy and calms people suffering form Huntington’s disease

4. Glutamate

-excitatory

-learning and memory

-associated with Alzheimers disease

5. Norepinephine

-attentiveness, mood, appetite, pain

-regulates body temperature

-plays a role in mood disorders such as manic depression

6. Serotonin

-regulating body temperature

-sleep, mood, appetite, pain

-imbalances causes depression, suicide, impulsive behavior, aggressiveness

Caffeine

Caffeine is a drug. It is found in many common foods such as coffee, soda, chocolate, and ice cream. Caffeine is also a fat soluble. Therefore, it passes though all cell membranes easily. Caffeine can easily pass through the blood-brain barrier. The blood-brain barrier is a defense mechanism for the central nervous system. Intaking caffeine can make you feel less tired or fatigued. The reason for this is because caffeine blocks adenosine receptors. Adenosine is a neurotransmitter that plays the role of making you feel tired or sleepy. Hence, when these receptors are blocked, your body feels less tired. Caffeine is also has good effects on your body. Caffeine increases the transmission of the neurotransmitter Dopamine. Dopamine is responsible for your mood and it protects your brain cells from disease. Caffeine also increases the transmission of Acetylcholine. Acetylcholine is a neurotransmitter that deals with muscular activity and long term memory. Caffeine adjusts serotonin levels which therefore, makes you feel more

relaxed, alert, energetic, relieves migraines and depression. Overall,caffeine is said to enhance your mood, increase relaxation, relieve boredom, boost self confidence, improve speed, improve endurance, improve energy output, improve strength and reaction time, increase thermogenesis (metabolic rate), protect body and brain cells from long term damage, pain relief, and protection.

Caffeine Tolerance

The first you use caffeine you have zero tolerance. The caffeine fully affects you. Caffeine makes you have feelings of euphoria, extreme alertness, positive feelings, increased motivation, and increased energy. Consuming the same amount of caffeine every day will cause caffeine to effect you less and less. Over a certain period of time, a person starts to feel tired and fatigued with the absence of caffeine. Usually, complete caffeine tolerance occurs after only 1-4 days. The reason caffeine tolerance occurs is beach the brain makes an excessive amount of adenosine to make up for the ones that the caffeine is blocking. Then, people intake more caffeine to make up for the excessive adenosine. This repeats into an ongoing circle.

The Discoid Roach

The Discoid Roach (Blaberus Discoidalis) is found in tropical areas such as Mexico, South America and Florida. They are white when born and become brown or black as they get older. Their average lifespan is about 16-24 months. Discoid roaches have vestigial wings which do not allow them to fly cannot fly or climb glass or plastic. They measure to about 12 millimeters when born and grow up to 50 millimeters not including

their antennae. They female Discoid roaches reproduce about 25-35 times a month. Discoid roaches are nocturnal animals. They are also decomposers; they eat ripe fruit and plant material. Roaches shed their exoskeleton or molt multiple times during their life. After a roach molts, it is white and can be easily injured. This occurs until a hormone called Bursicon causes the roach's exoskeleton to harden once again and darken to brown. If the roach is in the process of re-growing a limb, it can even put off molting until the regeneration is complete. Most of the nervous system activity of a roach takes place in nerve ganglia located throughout the roaches body. Therefore, a headless roach can live for more than a week and when it dies, it would die of thirst. A roach breathes through spiracles or holes in the sides of their body. Organs and tissue receive the oxygen from tubes called tracheae. Their eyes are made up of photoreceptor cells called Ommatidia. There is a hard ring called Ocular Sclerite that surrounds the Ommatidia. The movable antennae (antennal flagella) of a roach allow them to feel and smell. The antennae are made up of tiny hair segments. These segments are thicker next to the roaches head and thin out at the tips farthest from the roaches head.

Cell communication

Neurotransmitters are stored in small, bubble-like compartments called vesicles. Each vesicle holds a single type of neurotransmitter. The vesicles travel to the end of the neuron, and fuse with the membrane. They dump their contents into the synaptic gap. After neurotransmission release, the neuron recycles the empty vesicles, refilling and reusing them several more times before they need to be replaced.

Serotonin

Serotonin (5-hydroxytrytryptamine) is a neurotransmitter that acts as a mood stabilizer. Serotonin is made with a very important part of protein- tryptophan. Serotonin helps in providing healthy sleeping patterns. It also helps boost your mood and is sometimes used as a treatment for depression. Serotonin is said to be linked to feeling better an living longer. Serotonin is not directly found in foods and cannot be taken in the brain because Serotonin cannot cross the blood-brain barrier. The blood-brain barrier is a defense barrier for the central nervous system. But tryptophan is found in many natural foods and eating foods with high tryptophan levels can effect your serotonin levels. Some common foods with high tryptophan levels are eggs, cheese, pineapples, tofu, salmon, nuts, seeds, and turkey. However, all the amino acids “fight” for entry into the brain, and the tryptophan has a low chance of effecting your serotonin levels. But, when a high amount of carbohydrates are consumed, insulin is produced and that helps other amino acids enter different body parts, letting tryptophan have an easy access into the brain.

Experimental Design

Title: A Study of Caffeine and Serotonin on the Rate of Neurotransmission

Independent variable (IV)1: Caffeine in mg/L
Independent variable (IV)2: Serotonin in mg/L

Dependent variable: Rate of Neurotransmissions in Action Potential Amplitude
Hypothesis:
H1: *Blaberus discoidalis* neurotransmissions will have a higher mean amplitude when exposed to 150 mg/L of caffeine compared to *Blaberus discoidalis* exposed to 0.0 mg/L of caffeine.

H2: *Blaberus discoidalis* neurotransmissions will have a higher mean amplitude when exposed to 3000 mg/L of serotonin compared to *Blaberus discoidalis* exposed to 0.0 mg/L of serotonin.

Constants: Lighting, food, living conditions for the roach, taps on roach leg, water to inestitize roach, scissor to cut roach leg, food for roach, diabetic syringe to inject into roach leg

Caffeine:

	Control	**E1**	**E2**
Treatment:	0.0 mg/L	150 mg/L	300 mg/L
Sample:	15	15	15
Trials:	3	3	3

Serotonin:

	Control	**E1**	**E2**
Treatment:	0.0 mg/L	200 mg/L	500 mg/L
Sample:	15	15	15
Trials:	3	3	3

Safety Considerations

The following hazard and precaution statements were taken from the Stone science research safety considerations resource.

Hazard: Electricity and water present a potential shock hazard
Precaution: Use a Ground Fault Interrupt (GFI) receptacle, grounded equipment and work under adult supervision

Hazard: Glassware presents a potential hazard when broken. Sharp edges or glass fragments can cause injury.
Precaution: Wear personal protective equipment including: goggles, gloves and aprons. Work under adult supervision.

Hazard: Hand and Power Tools are potentially harmful if used in an inappropriate fashion. Injury can result from the improper use of tools.

Precaution: The student will be instructed in the proper use of the appropriate hand and power tools and the related safety equipment. All individuals present will use appropriate personal safety equipment such as goggles, face shields, aprons and gloves. The student will be supervised by a adult while working with hand or power tools.

Organism Information

Common Name: Discoid Roach

Genus and Species Name: *Blaberus discoidalis*

Description: The Discoid Roach (*Blaberus discoidalis*) is found in Mexico, South America and Florida. Their average lifespan is 16-24 months. Discoid roaches cannot fly and cannot climb glass or plastic. Discoid roaches are decomposers, they eat ripe fruit and plant material. They mothers reproduce about 25-35 times a month. They are nocturnal roaches. They measure to about 1/4 inch when born and grow up to 2 inches as adults.

Source: Aaaron Pauling or Backyard Brains

Experimental Methods

Care for Roach

1. Split all the roaches into two containers
2. Feed the roaches twice every week with the primary meal of lettuce, and the occasional tomato and apple
3. Clean out the container once every two weeks
4. Place two halves of an egg carton in each container on the bedding to provide shade and darkness

Setting Up Recorder

1. Download SpikeRecorder from Backyard Brains
2. Click Settings
3. Turn on left microphone
4. Turn off all other settings

Creating Solutions

1. Take 4 caffeine tablets
2. Crush them up to a fine powder using a mortar and pistol
3. Fill up two beakers with 100 ml of water
4. Measure 15 mg of the powder and place into one beaker
5. Stir both beakers with a stirring rod
6. Repeat steps 1-6 with Serotonin tablets except with 20 mg in step 4

Making Stimulus

1. Take two dissecting pins, a straw, and a glass stirring rod
2. Measure 1.5 inches on the glass stirring rod and make a mark with black sharpie
3. Cut the straw to __ inches
4. Tape the dissecting pins o the bottom of the straw
5. Mark on the straw with black sharpie where the top of the dissecting pins touch
6. Place the glass stirring rod inside the straw
7. Place contraption on spikerbox mat right above the cockroach leg

Leg Detachment

1. Fill a beaker with ice water
2. Take one roach out of either container with your hand
3. Place roach into ice water beaker and wait for 1 minute for roach to inistetize
4. Take roach out of cup with tweezers and place the roach on a napkin
5. Place one finger on the underbelly of the roach and hold out leg
6. Take small, dissecting scissors and cut the bottom of the leg, detaching it from the body
7. Take diabetic syringe and inject into Caffeine solution an fill up to 1 ml
8. Take diabetic syringe and inject substance into the coxa of the roach
9. Attach leg onto spiker box by puncturing the the needles into the coxa and the femur of the roach
10. Move on to the "Recording Spikes" section, and once done return back and complete steps.
11. Repeat steps 5-9 for two legs of the roach
12. Place roach back in container with hand

13. Repeat steps 1-12 for 2 roaches
13. Repeat steps 1-13 twice out of the Serotonin solution and no solution in step

Recording Spikes

1. Connect laptop to spikerbox by plugging in blue cord into microphone slot
2. Click the record button on the Spike Recorder App
3. Pull the glass stirring rod up to 1.5 inches
4. Drop the rod on the roach leg from 1.5 inches every 5 seconds for 25 seconds
5. Click the stop recording button after 25 seconds
6. Save recording

Analyzing Spikes

1. Download Matlab
2. Open up Matlab
3. Run pointSpike code
4. Input recordings from “Recording Spikes” section
5. Click Run
6. Select the data cursor and place it on the 5 spikes on the ouput graph
7. Record the Y: Voltage (V) in the logbook
8. Convert all the data into millivolts (mV)

Materials

Quantity	Product
	Discoid Roaches
Unlimited	Ice water
1	Diabetic Syringe
1	Spiker Box
	Tablet

Quantity	Product
	Cords related to Spiker Box
1	Roach Container and Living Needs
Unlimited	Roach Food and Water
	Caffeine
	Serotonin
1	Matlab or Analyzing resource
1	Pencil
Unlimited	Lettuce and Tomato
2	Dissecting Pins
1	Straw
1	Glass Stirring Rod

Data Analysis Procedure

The following data analysis procedure was taken from the Stone science research data analysis resource.

Raw data will by summarized by calculating means for each group and the resulting means will be graphed. An ANOVA statistical analysis will be performed to determine if differences in the means of the groups are statistically significant. The hypothesis will be accepted or rejected based on the results of the ANOVA test.

Data Tables

Trial 1-

Control- 0.0 mg/L	Caffeine- 150 mg/L	Serotonin- 200 mg/L
0.03771 mV	0.07372 mV	0.06679 mV
0.03355 mV	0.1126 mV	0.02643 mV
0.02615 mV	01119 mV	0.01694 mV
0.01471 mV	0.1113 mV	003857 mV
0.04109 mV	01112 mV	0.06633 mV
0.042 mV	00.03299 mV	0.068804 mV
0.03285 mV	0.1131 mV	0.06038 mV
0.03068 mV	0.1124 mV	0.04427 mV
0.04903 mV	0.1136 mV	0.05148 mV
0.04204 mV	0.0421 mV	0.04237 mV
0.04055 mV	0.1135 mV	0.1136 mV
0.03756 mV	0.05668 mV	0.7782 mV
0.03534 mV	0.08117 mV	0.06032 mV
0.0435 mV	0.06294 mV	0.06097 mV
0.03176 mV	0.06191 mV	009169 mV
0.05603 mV	0.1126 mV	0.05097 mV
008255 mV	0.113 mV	0.07865 mV
0.06462 mV	0.1131 mV	0.02825 mV
0.04796 mV	0.04847 mV	0.1085 mV
0.07207 mV	0.1123 mV	0.1002 mV

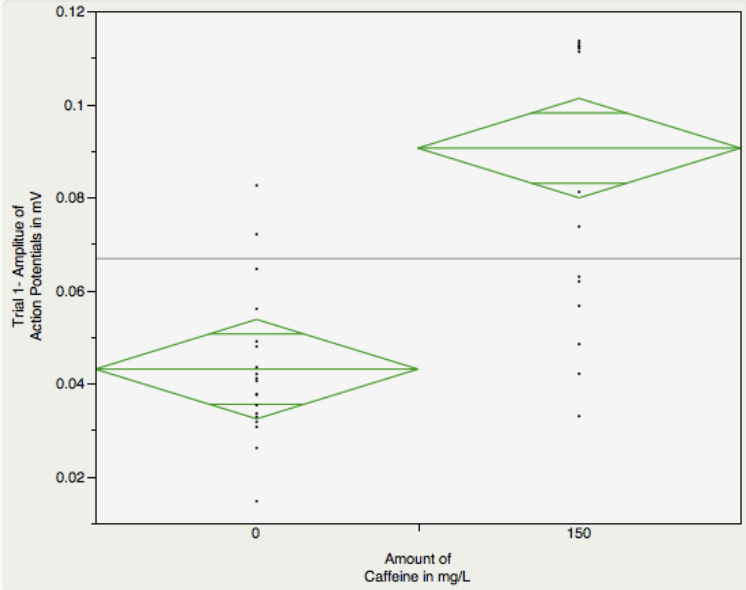
Trial 2-

Control- 0.0 mg/L	Caffeine- 150 mg/L	Serotonin- 200 mg/L
0.07714 mV	0.1128 mV	0.1135 mV
0.06355 mV	0.1136 mV	0.1136 mV
0.1136 mV	0.08779 mV	0.1136 mV
0.03869 mV	0.08607 mV	0.1124 mV
0.08441 mV	0.08445 mV	0.1131 mV
0.07766 mV	0.1135 mV	0.1136 mV
0.1136 mV	0.0762 mV	0.1132 mV
0.1136 mV	0.05144 mV	0.1131 mV
0.1136 mV	0.03587 mV	0.1136 mV
0.02656 mV	0.1102 mV	0.1136 mV
0.09572 mV	0.07355 mV	0.05603 mV
0.09553 mV	0.1136 mV	0.03797 mV
0.1104 mV	0.1099 mV	0.05517 mV
0.06231 mV	0.1136 mV	0.03097 mV
0.08723 mV	0.1134 mV	0.03495 mV
0.03627 mV	0.1136 mV	0.06477 mV
0.06044 mV	0.1136 mV	0.06631 mV
0.07117 mV	0.1136 mV	0.1136 mV
0.04556 mV	0.1081 mV	0.06645 mV
0.1136 mV	0.1136 mV	0.06015 mV

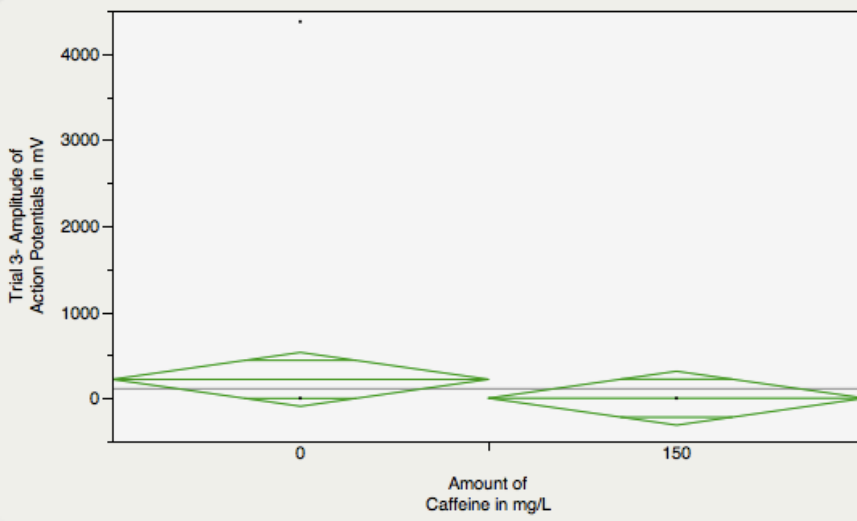
Trial 3-

Control- 0.0 mg/L	Caffeine- 150 mg/L	Serotonin- 200 mg/L
0.1136	0.1136	0.1135
0.1136	0.03528	0.1026
0.03929	0.03546	0.1136
0.08203	0.03317	0.1136
0.04676	0.03116	0.1136
0.05355	0.1135	0.09637
0.04563	0.03841	0.1136
0.0356	0.04001	0.1136
0.03427	0.04312	0.09227
0.05268	0.08725	0.04108
0.05405	0.1135	0.06527
0.04374	0.1136	0.02109
0.0554	0.1109	0.1136
0.06637	0.1136	0.02456
0.05829	0.03261	0.02018
0.095	0.1132	0.04101
0.1136	0.1132	0.02269
0.1136	0.1133	0.01646
0.1015	0.07992	0.02471
0.06631	0.1131	0.01382

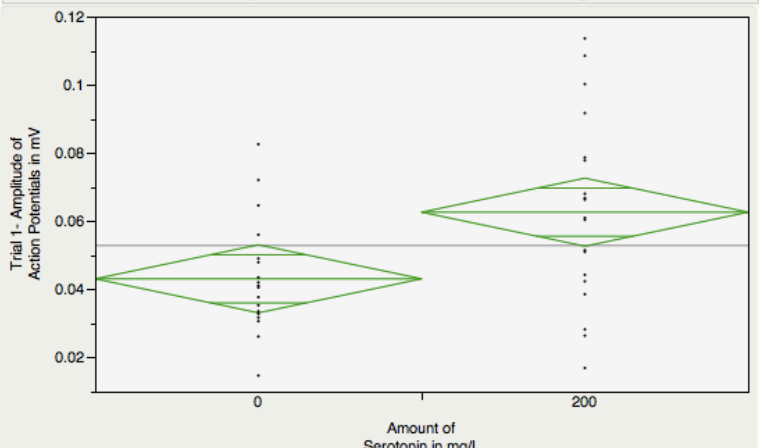
Oneway Analysis of Trial 1- Amplitue of Action Potentials in mV By Amount of Caffeine in mg/L



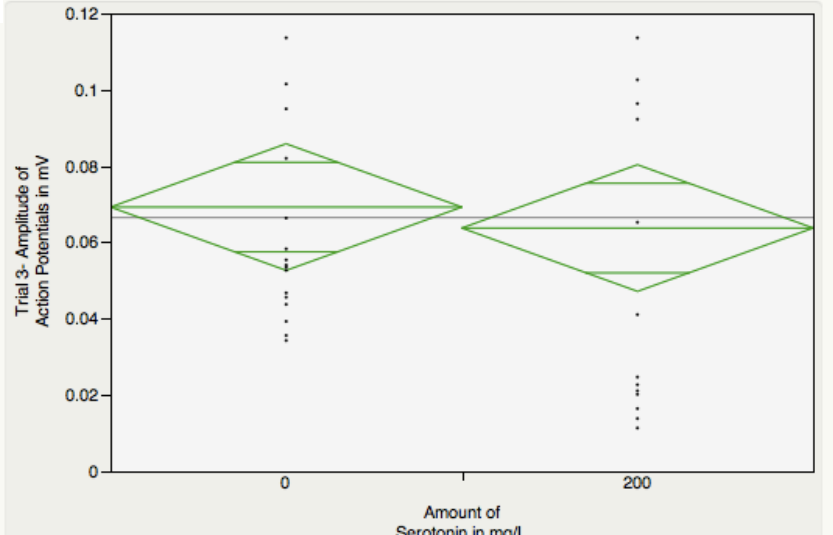
Oneway Analysis of Trial 3- Amplitude of Action Potentials in mV By Amount of Caffeine in mg/L

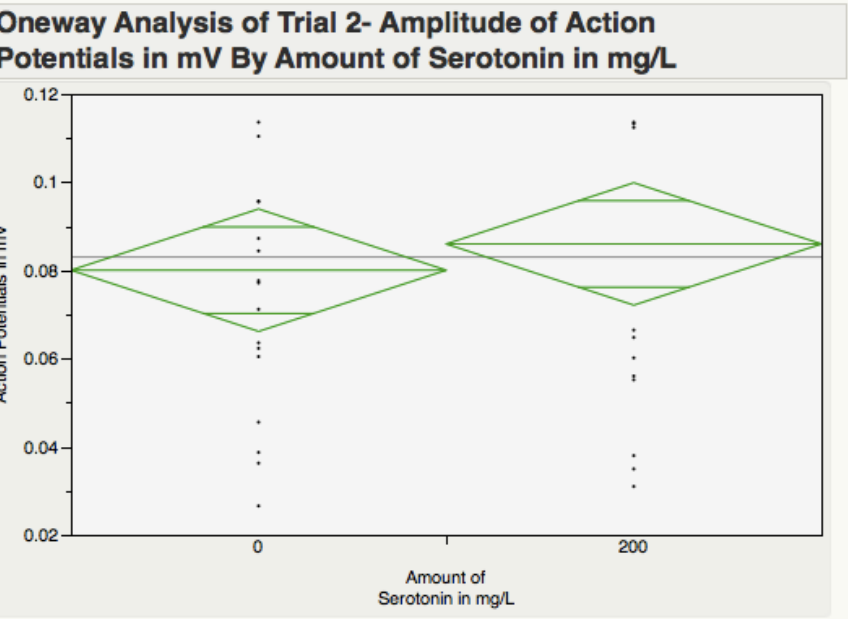


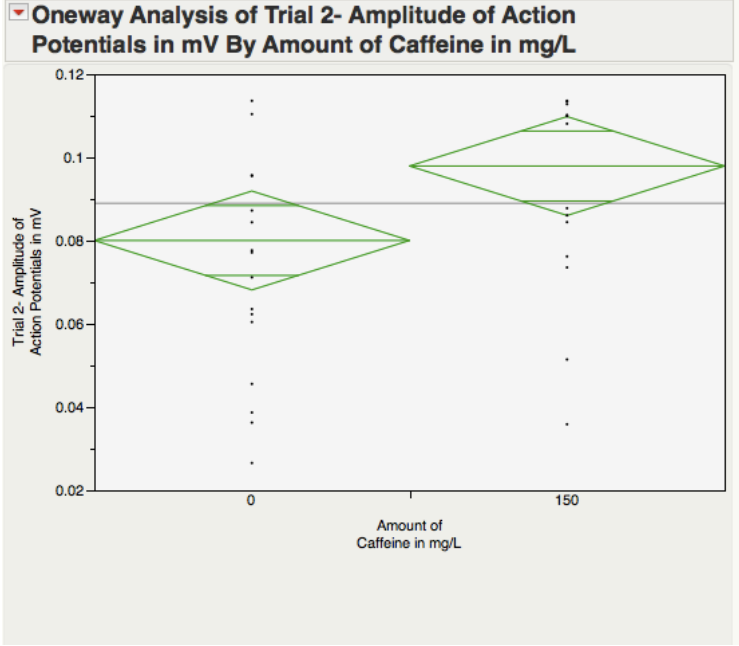
Oneway Analysis of Trial 1- Amplitude of Action Potentials in mV By Amount of Serotonin in mg/L

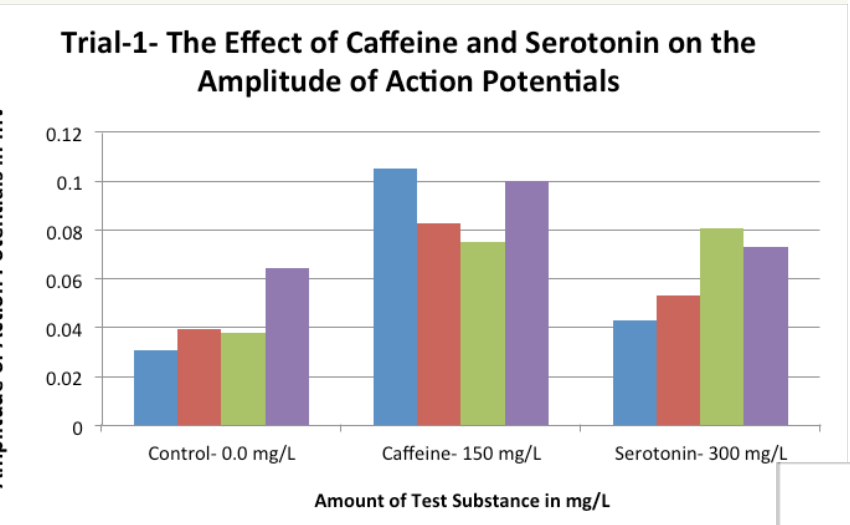


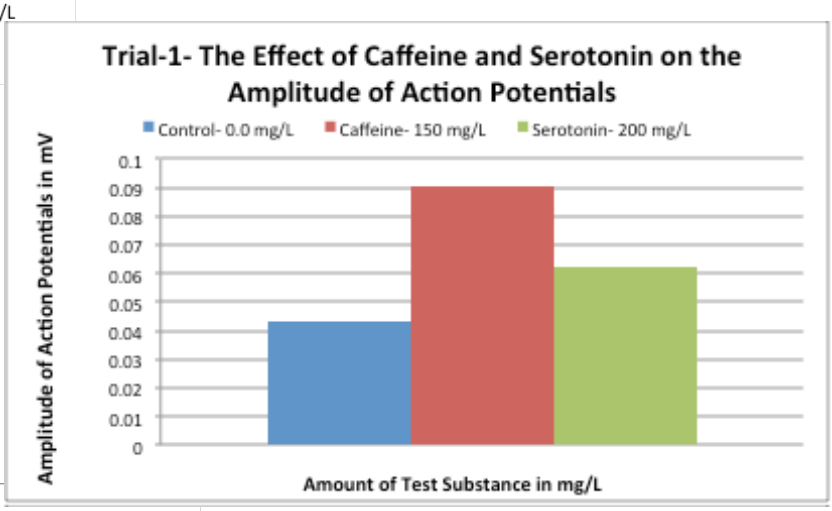
Oneway Analysis of Trial 3- Amplitude of Action Potentials in mV By Amount of Serotonin in mg/L

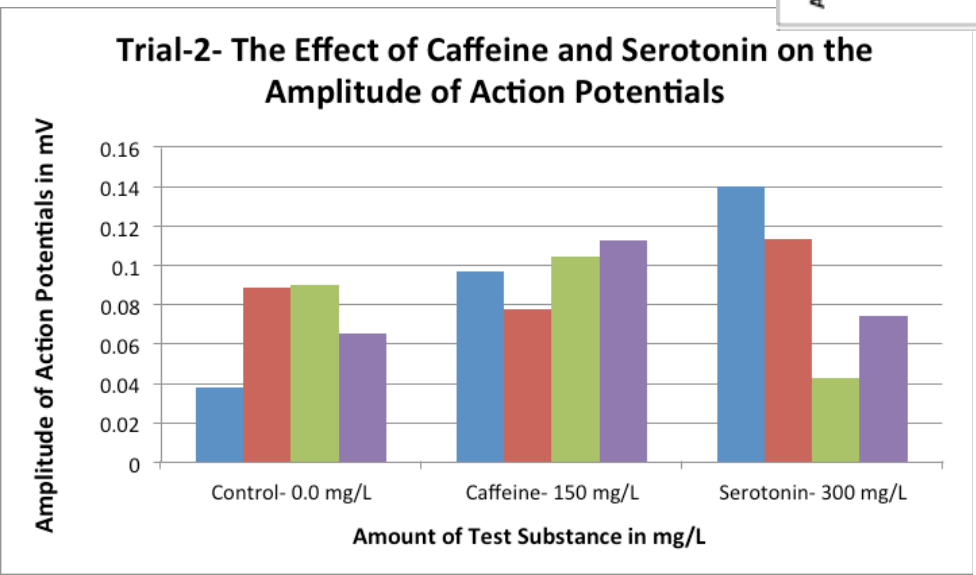


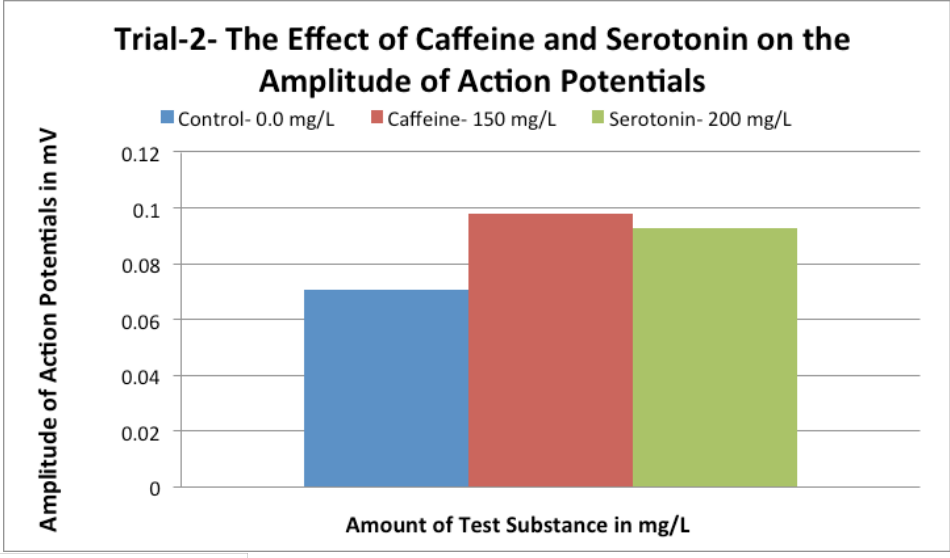

Oneway Analysis of Trial 2- Amplitude of Action Potentials in mV By Amount of Serotonin in mg/L
0.12
0.1
0.08
0.06
0.04
0.02
0
200
Amount of Serotonin in mg/L

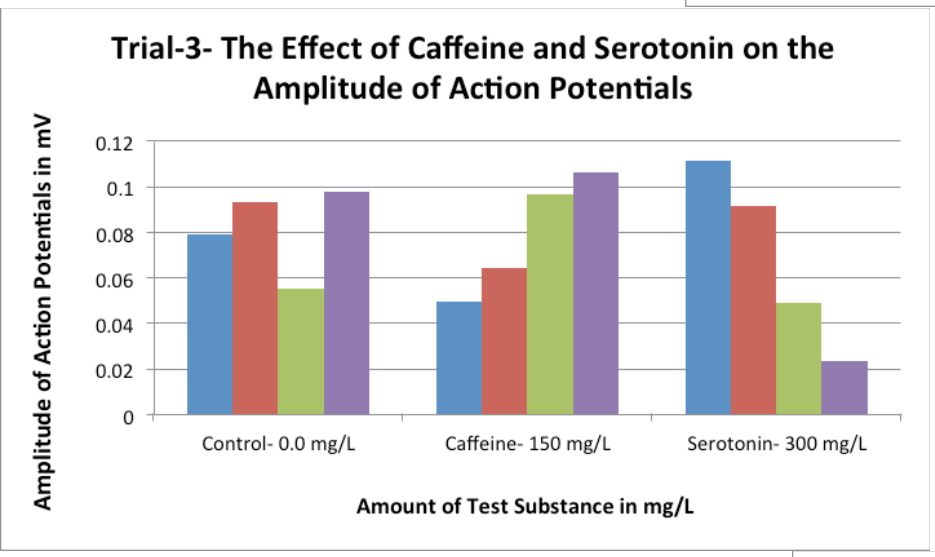

Oneway Analysis of Trial 2- Amplitude of Action Potentials in mV By Amount of Caffeine in mg/L
0.12
0.1
0.08
0.06
0.04
0.02
Trial 2- Amplitude of Action Potentials in mV
0
150
Amount of Caffeine in mg/L

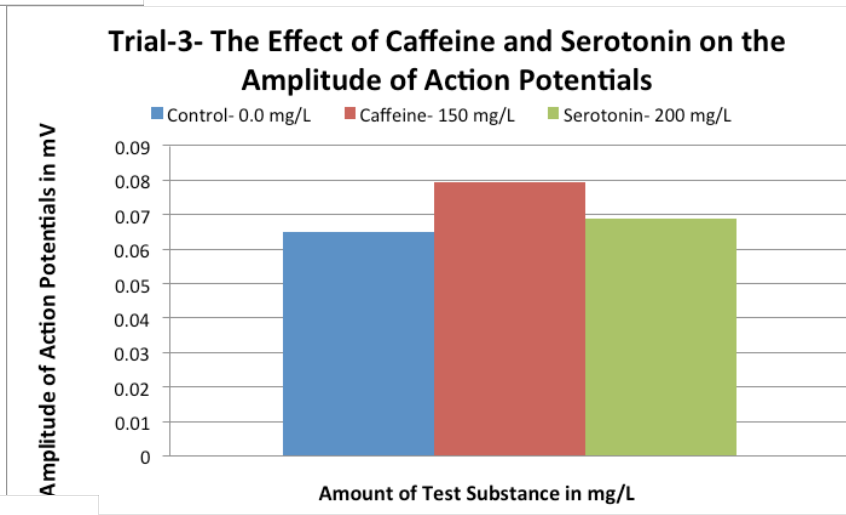

Trial-1- The Effect of Caffeine and Serotonin on the Amplitude of Action Potentials
0.12
0.1
0.08
0.06
0.04
0.02
0
Control- 0.0 mg/L
Caffeine- 150 mg/L
Serotonin- 300 mg/L
Amount of Test Substance in mg/L

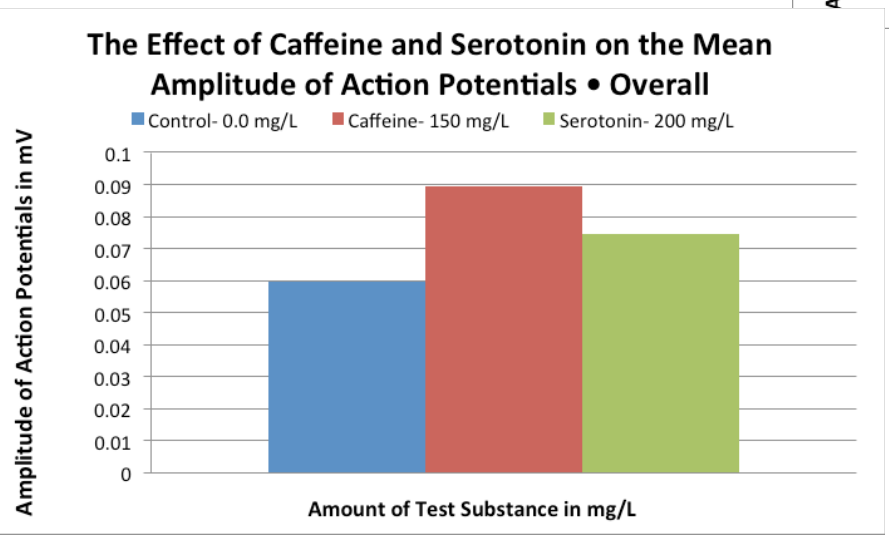

Trial-1- The Effect of Caffeine and Serotonin on the Amplitude of Action Potentials
Control- 0.0 mg/L
Caffeine- 150 mg/L
Serotonin- 200 mg/L
Amplitude of Action Potentials in mV
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Amount of Test Substance in mg/L

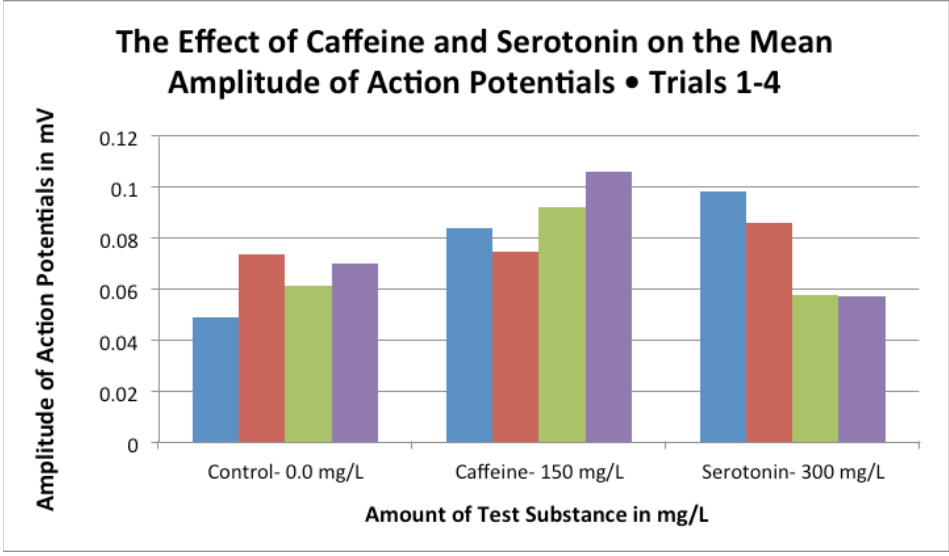

Trial-2- The Effect of Caffeine and Serotonin on the Amplitude of Action Potentials
Amplitude of Action Potentials in mV
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Control- 0.0 mg/L
Caffeine- 150 mg/L
Serotonin- 300 mg/L
Amount of Test Substance in mg/L

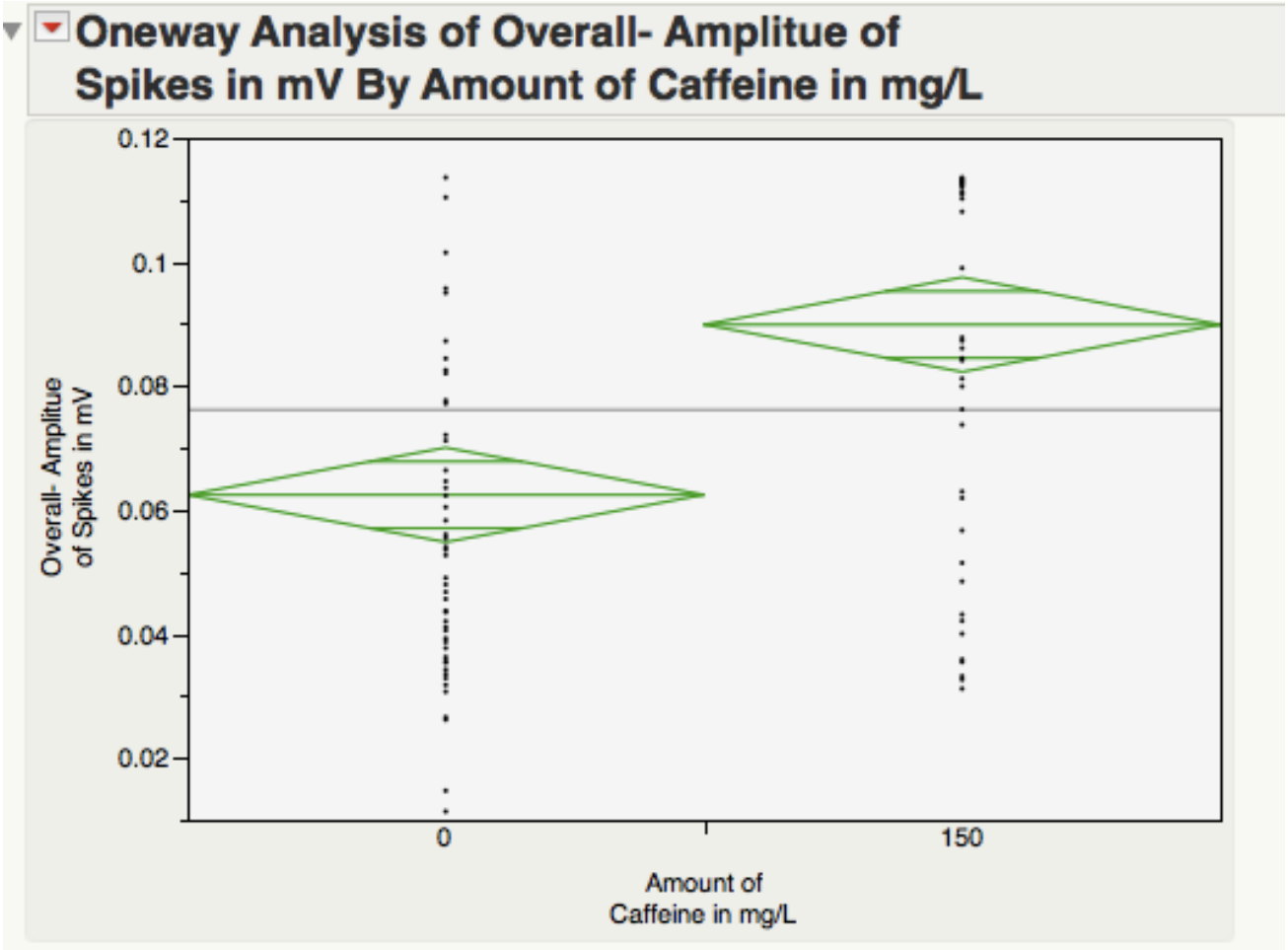

Trial-2- The Effect of Caffeine and Serotonin on the Amplitude of Action Potentials
Control- 0.0 mg/L
Caffeine- 150 mg/L
Serotonin- 200 mg/L
Amplitude of Action Potentials in mV
0.12
0.1
0.08
0.06
0.04
0.02
0
Amount of Test Substance in mg/L

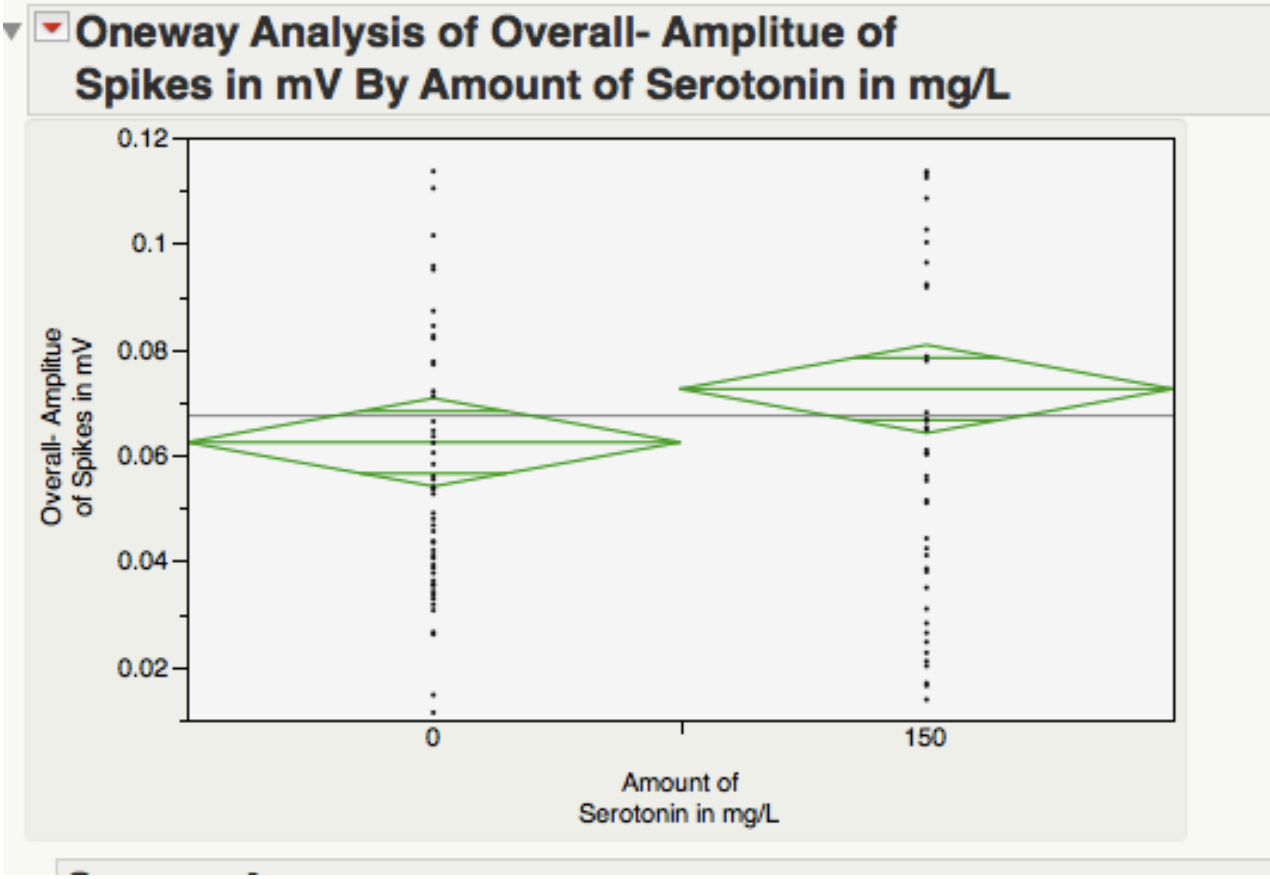

Trial-3- The Effect of Caffeine and Serotonin on the Amplitude of Action Potentials
Amplitude of Action Potentials in mV
0.12
0.1
0.08
0.06
0.04
0.02
0
Control- 0.0 mg/L
Caffeine- 150 mg/L
Serotonin- 300 mg/L
Amount of Test Substance in mg/L


Trial-3- The Effect of Caffeine and Serotonin on the Amplitude of Action Potentials
Control- 0.0 mg/L
Caffeine- 150 mg/L
Serotonin- 200 mg/L
Amplitude of Action Potentials in mV
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Amount of Test Substance in mg/L


The Effect of Caffeine and Serotonin on the Mean Amplitude of Action Potentials • Overall
Control- 0.0 mg/L
Caffeine- 150 mg/L
Serotonin- 200 mg/L
Amplitude of Action Potentials in mV
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Amount of Test Substance in mg/L


The Effect of Caffeine and Serotonin on the Mean Amplitude of Action Potentials • Trials 1-4
Amplitude of Action Potentials in mV
0.12
0.1
0.08
0.06
0.04
0.02
0
Control- 0.0 mg/L
Caffeine- 150 mg/L
Serotonin- 300 mg/L
Amount of Test Substance in mg/L


Oneway Analysis of Overall- Amplitue of Spikes in mV By Amount of Caffeine in mg/L
Overall- Amplitue of Spikes in mV
0.12
0.1
0.08
0.06
0.04
0.02
0
150
Amount of Caffeine in mg/L



Conclusion

C1- The data supported the hypothesis, the discoid roach legs exposed to 150 mg/L of caffeine had a higher mean amplitude than the roaches exposed to 0.0 mg/L of caffeine.

C2- The data did not support the hypothesis, the discoid roaches exposed to 200 mg/L of serotonin did not have a lower mean amplitude than the roaches exposed to 0.0 mg/L of serotonin. It was hypothesized that serotonin would decrease the amplitude of action potentials, however the legs exposed to serotonin had an increase in action potential amplitude.

Bibliography

Aaron Pauling. (2016) *Discoid Roach Care Sheet*. Available: http://www.aaronpauling.com/pages/discoid-roach-care-sheet (accessed: 2015, Oct. 10)

Backyard Brains. (N.D.) Why Roaches?. Available: https://backyardbrains.com/experiments/roboRoachSurgery (accessed: 2015,Oct 27)

Backyard Brains. (N.D.) *Experiment:Record From Live Nuerons!* Available: https://backyardbrains.com/experiments/spikerbox (accessed: 2015, Oct. 27)

Backyard Brains. (N.D.) *Data Analysis*. Available: http://wiki.backyardbrains.com/Data_Analysis (accessed: 2015, Oct. 27)

Bouchez, Colette. (2016). *Serotonin: 9 Questions and Answers*. Available: http://www.webmd.com/depression/features/serotonin (accessed: 2015, Oct. 19)

Brain Basics. (2011) *The Fundamentals of Neuroscience*. Available: http://www.bris.ac.uk/synaptic/basics/basics-4.html (accessed: 2015, Sep. 9)

Brain From Bottom To Top (N.D.) *Synapses.* Available: http:// *thebrain.mcgill.ca/* flash/i/i_01/i_01_m/i_01_m_ana/ i_01_m_ana.html (accessed: 2015, Sep 25)

Brain Facts. (2011). *Neurotransmitters:How Brain Cells Use Chemicals to Communicate* Available: http://www.brainfacts.org/brain-basics/cell-communication/articles2 011/neurotransmitters-how-brain-cells-use-chemicals-to-communicate/ (accessed: 2015, Sep 25)

Boeree, George. (2009). General Psychology. *Neurotransmitters.* Available: http://webspace.ship.edu/cgboer/genps yneurotransmitters.html (accessed: 2015, Sep 29)

Bouchez, Colette (2011). *Serotonin.* Available: http://www.webmd.com/depression/features/se

Cafasso, J. (2015). *Serotonin syndrome*. Available: http://www.healthline.com/health/seratonin-syndrome#Overview1 (accessed: 2016, Feb. 3)

Caffeine Informer (N.D.) *Deaths By Caffeine*. Available: http://www.caffeineinformer.com/a-real-life-death-by-caffeine (accessed: 2015, Nov. 3)

Caffeine Informer (N.D.) *Caffeine Metabolism*. Available: http://www.caffeineinformer.com/caffeine-tolerance (accessed: 2015, Nov. 3)

Caffeine Informer (N.D.) *Caffeine Tolerance*. Available: http://www.caffeineinformer.com/caffeine-metabolism (accessed: 2015, Nov. 3)

Carver, J. (N.D.) *The Chemical Imbalance in Mental Health Problems.* Available: http://www.drjoecarver.com/clients/49355/File/Chemical%20Imbalance.html. (accessed: 2016, Jan. 9)

Chudler, E. (2016) *Lights, Camera, Action Potential*. Available: https://faculty.washington.edu/chudler/ap.html (accessed: 2016, Jan. 20)

Council for Responsible Nutrition. (N.D.) *Consumer Q&A Caffeine Containing Dietary Supplements.* Available: http://www.crnusa.org/pdfs/CRNCaffeineConsumerQ+A.pdf (accessed: 2016, Jan. 1)

Drugs (N.D.). *Overtime Side Effects.* Available: http://www.drugs.com/sfx/overtime-side-effects.html (accessed: 2016, Jan. 3)

Eifling, S. (2012). *How Much Caffeine Would It Take to Kill You?* Available: http://www.popsci.com/science/article/2012-10/fyi-how-much-caffeine-would-it-take-kill-you (accessed: 2016, Feb. 3)

Everyday Health. (2014). *Serotonin Syndrome: 7 Things You Need to Know.* Available: http://www.everydayhealth.com/depression-pictures/serotonin-syndrome-things-you-need-to-know.aspx (accessed: 2016, Feb. 3)

Gage, G. (2103). *Using crickets to introduce neurophysiology to early undergraduate students*. Available: http://www.ncbi.nlm.nih.gov/pubmed/24319394 (accessed: 2016, Feb. 2)

Gavin, M. (2014). Moderation is Key. *Caffeine.* Available: http://kidshealth.org/teen/drug_alcohol/drugs/caffeine.html# (accessed: 2016, Dec. 6)

Gavin, M. (2014). What is Caffeine. *Caffeine.* Available: http://kidshealth.org/teen/drug_alcohol/drugs/caffeine.html# (accessed: 2016, Dec. 6)

Gavin, M. (2014). Caffeine Sensitivity. *Caffeine.* Available: http://kidshealth.org/teen/drug_alcohol/drugs/caffeine.html# (accessed: 2016, Dec. 6)

Gavin, M. (2014). Cutting Back. *Caffeine.* Available: http://kidshealth.org/teen/drug_alcohol/drugs/caffeine.html# (accessed: 2016, Dec. 6)

Healthline. (2015). *What is Serotonin?* Available: http://www.healthline.com/health/healthy-sleep/foods-that-could-boost-your-serotonin#Overview1 (accessed: 2015, Oct 10)

Healthy Lifestyle. (2016) *Caffeine: How much is too much*?. Available: http://www.mayoclinic.org/healthy-lifestyle/nutrition-and-healthy-eating/in-depth/caffeine/art-20045678 (accessed: 2016, Feb. 3)

Heffner, C. (2016) *Neurotransmitters.* Available: http://allpsych.com/psychology101/neurotransmitters/ (accessed: 2015, Sep. 29)

Hotel Intestine. (2012). *Gregarines From Blaberus Discoidalis.* Available: http://science.peru.edu/gregarina/gregs_bdisc.html (accessed: 2106, Jan. 2)

International Rules for Pre-college Science Research: Guidelines for Science and Engineering Fairs - 2015-2016. Washington, D.C: Society for Science and the Public

Konnikova, M. (2013). *How Caffeine Can Cramp Creativity*. Available: http://www.newyorker.com/tech/elements/how-caffeine-can-cramp-creativity (accessed: 2016, Feb. 3)

Kellevision. (2008). *Neurotransmitters, Depression and Anxiety.* Available: http://www.kellevision.com/kellevision/2008/05/neurotransmitte.html (accessed: 2016, Jan. 9)

Mayo Clinic. (2015). *Serotonin syndrome*. Available: http://www.mayoclinic.org/diseases-conditions/serotonin-syndrome/basics/definition/con-20028946 (accessed: 2016, Feb. 1)

Medical News Today (2015). *What is Sertonin?* Available: *http://w*ww.medicalnewstoday.com/articles/232248.php (accessed: 2015, Nov 8)

Medical News Today (2015). *Functions of Serotonin* Available: *http://www.medicalnewstoday.com/articles/*232248.php (accessed: 2015, Nov 8)

Medicines in my Home. (2007) *Caffeine and Your Body.* Available: http://www.fda.gov/downloads/UCM200805.pdf (accessed: 2016, Feb. 2)

Medline Plus. (N.D.). *Serotonin Syndrome*. Available: https://www.nlm.nih.gov/medlineplus/ency/article/007272.htm (accessed: 2016, Feb. 3)

Morris,M. (2016) *35 Things You Didn't Know About Caffeine*. Available: http://www.eatthis.com/caffeine (accessed: 2016, Feb. 5)

My Bearded Dragon. (2011) *Discoid Roach (Blaberus Discoidalis) Care Sheet.* Available: http://mybeardie.com/bearded-dragon-blog/bearded-dragons-for-sale/discoid-roach-bl aberus-discoidalis-care-sheet (accessed: 2015, Oct. 14)

Nawrot, P. (2003). *Caffeine-Containing Dietary Supplements*.Available: *http://crnusa.org/caffeine/Q+A.html* (accessed: 2016, Jan. 3)

Neurogistics. (2015) *What are Neurotransmitters*? Availablehttps://www.neurogistics.com/TheScience/WhatareNeurotransmi09CE.asp (accessed: 2015, Sep. 29)

Pietrangelo, A. (2014). *The Effects of Caffeine on the Body*. Available: http://www.healthline.com/health/caffeine-effects-on-body (accessed: 2016. Feb. 3)

Perry, S. (2011). Neurotransmitters: How brain cells use chemicals to communicate. *Society for Neuroscience.*Available: *http://www.brainfacts.org/brain-basics/cell-communication/articles/2011/*neurotransmitters-how-brain-cells-use-chemicals-to-communicate/ (accessed:2015, Sep 25)

Professionable Reptiles. (2011) *Discoid Roach*. Available:http://www.progeckos.com/caresheets/discoid.htm (accessed: 2015, Oct. 23)

Psychologist World. (2016). *Serotonin*. Available: http://www.psychologistworld.com/biological/neurotransmitters/serotonin.php (accessed: 2016, Feb. 1)

Rosling, C. (N.D.). *Serotonin as a neurotransmitter*. Available: http://www.chm.bris.ac.uk/motm/serotonin/serotonin%20as %20a %20neurotransmitter.htm (accessed: 2015, Oct. 14)

U.S. National Library of Medicine. (2016). *Caffeine*. Available: https://www.nlm.nih.gov/medlineplus/caffeine.html (accessed: 2016, Feb. 3)

WebMD. (2016) *Caffeine Myths and Facts.* Available:http://www.webmd.com/balance/caffeine-myths-and-facts (accessed: 2016, Feb. 3)

WebMD. (2016) *Caffeine Myths and Facts1*. Available: http:// www.webmd.com/balance/caffeine-myths-and-facts?page=1 (accessed: 2016, Feb. 3)

WebMD. (2016) *Caffeine Myths and Facts2*. Available: http:// www.webmd.com/balance/caffeine-myths-and-facts?page=2 (accessed: 2016, Feb. 3)

WebMD. (2016) *Caffeine Myths and Facts3*. Available: http://www.webmd.com/balance/caffeine-myths-and-facts?page=3 (accessed: 2016, Feb. 3)

WebMD. (2016). *Caffeine*. Available: http://www.webmd.com/vitamins-supplements/ingredientmono-979-caffeine.aspx?activeingredientid=979 (accessed: 2016, Feb. 3)

WebMD. (2016). *What is Serotonin Syndrome?* Avalaible: http://www.webmd.com/depression/guide/serotonin-syndrome-causes-symptoms-treatments (accessed: 2016, Feb. 3)

Wilson, T (N.D.). How Cockroaches Work. *Cockroach Anatomy and Physiology.* Available: http://animals.howstuffworks.com/insects/cockroach2.htm (accessed: 2015, Nov 15

Wilson, T (N.D.). How Cockroaches Work. *The Cockroach Lifestyle and Behavior.* Available: http:// *animals.howstuffworks.com/insects/* cockroach2.htm (accessed: 2015, Nov 15)

Wise Geek (2003) *What are Neurotransmitters*? Available:http://www.wisegeek.org/what-are-neurotransmitters.htm#comments(accessed: 2015, Sep.25)

World of Caffeine. (N.D.). Caffeine and Neurotransmitters. *The Science and Culture of the World's Most Popular Drug*. Available: *http:// worldofcaffeine.com/caffeine-and*-neurotransmitters/ (accessed: 2015, Oct 9)

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