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The Absence of Effects of Caffeine and Nicotine on *Daphnia magna* Heart Rate

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A higher concentration of a stimulant will produce more action potentials in the cardiac cells. In this experiment, we studied the effects of the concentration of two stimulants, caffeine and nicotine, on the heart rate in *Daphnia magna*. In order to test this, we added four drops of each solution on the *Daphnia magna* and allowed them to sit for seven minutes. After the seven minutes, we recorded a fifteen second video in slow motion to accurately measure the heart rate. We performed three trials per solution to correctly test our hypothesis. According to statistical tests, our hypothesis was not supported. There was no significant difference between the *Daphnia magna* heart rate with or without stimulants. We expect this approach to interest those curious about the effects of certain drugs or beverages on heart rate.

Introduction

In order for any individual to stay alive, the heart must constantly beat. Because of this beating, action potentials must constantly be produced. An action potential is a change in the electrical potential across a membrane. Specifically, cardiac action potential is the change in voltage across the membrane of cardiac cells. These action potentials are produced by the movement of ions across the membrane of the cell through ion channels. Cardiac action potentials are produced through special cells known as sinoatrial nodes, which creates an action potential that passes along the cell membrane, causing the cardiac cells to contract (Carvalho, 1969). The heart is a key organ in maintaining any type of life. In order to increase medical knowledge, individuals study the effects of certain activities or solutions on heart rate.

In our experiment, we used the *Daphnia magna* to measure heart rate because of their translucent bodies, making it easy to see through a microscope with the human eye. Similar to humans and other vertebrates, *Daphnia magna* have myogenic hearts. With their translucent bodies and myogenic hearts, *Daphnia magna* are useful species in studies on the effects of certain drugs on heart rate.

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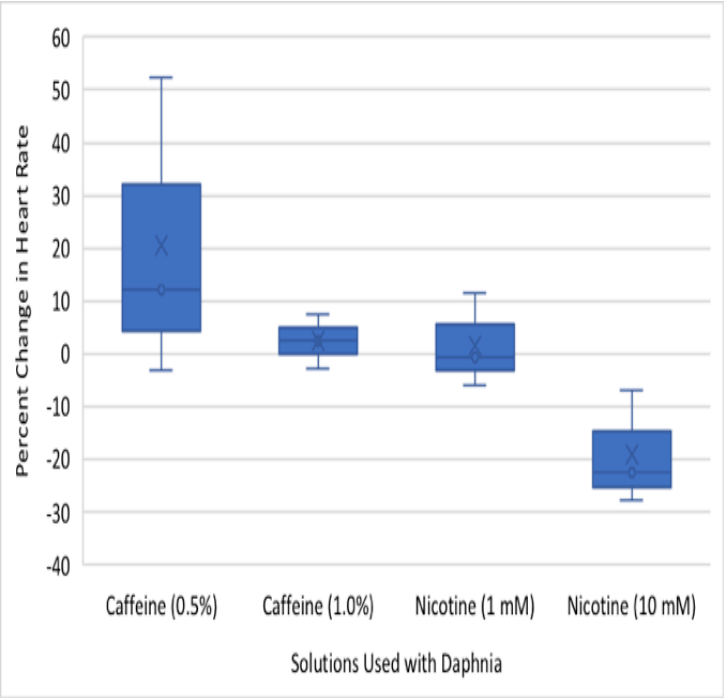

Figure 1. Shows the percentage change in heart rate of the Daphnia magna while submerged in the different concentrations of each solution. There are no apparent trends.

A stimulant is a substance that raises the levels of physiological and nervous activity in the body (Anstie, 1865). These substances will increase vital movements, such as muscle contraction, brain activity, and heart rate. Caffeine and nicotine are both commonly used stimulants, used by most of the population in today's world. Caffeine is found in popular drinks, such as sodas, energy drinks, and coffee. Used everyday, caffeine is found to produce headaches when consumed in large quantities. The effects of caffeine also include jitteriness, anxiety, insomnia, rapid heart rate, and irritability. Caffeine has a high performance-enhancing effect, which is accomplished through the antagonism of the adenosine receptors (Meeusen, 2013). Typically when adenosine binds with its receptors, neural activity slows down. However, caffeine acts as an adenosine receptor antagonist, meaning that it binds to the same receptors, but increases the neural activity. Adenosine is a neurotransmitter that is dependant on the levels of calcium (Ca) in the extracellular environment (Chen, 2001). Like other muscles, cardiac muscles use calcium to contract. Calcium flows into the cells through channels and allows the muscle fibers to slide past each other and contract. Once calcium enters the sodium (Na) channel, this creates the inactivation of the channel, which then causes the membrane potential to stop rising. Adenosine decreases calcium entry through sodium channels opening in the axon (Fredholm, 1999).

Nicotine is a key ingredient in tobacco, which is used in many drugs such as cigarettes, chewing tobacco, or cigars. Studies have shown that those who inhale larger doses of nicotine show a reduction in anxiety and an increased heart rate (Gilbert, 1989). Nicotine increases heart rate with the use of the neurotransmitter, acetylcholine. In the case of nicotine, acetylcholine binds to the nicotinic acetylcholine receptors (Karlin, 2004). This receptor is primary in muscle contraction and can be found to increase heart rate. This neurotransmitter

binds to the sodium and potassium (K) channels in the axon, increasing the flow of both sodium and potassium into the channels. This increases the voltage of the axon, which then intensifies the action potential, therefore increasing heart rate.

To answer the question, will higher concentrations of stimulants increase heart rate, we hypothesized that the use of a higher concentration of a stimulant will increase heart rate because higher concentrations of both nicotine and caffeine will open the sodium channels at a faster rate, increasing action potentials in the heart. If our hypothesis is supported, then higher concentrations of nicotine and caffeine will increase heart rate at a faster rate, compared to the lower concentrations. If our hypothesis is unsupported, then our control, the *Daphnia magna* placed in tank water, will have a higher heart rate than both stimulants, or there will be no effect on the *Daphnia magna.*

Methods

In our experiment, we examined the effects of two stimulants, caffeine and nicotine, on *Daphnia magna* heart rate.We measured how different concentrations of both stimulants affect the production of action potentials in cardiac cells. In order to test the effects of caffeine and nicotine on heart rate, we manipulated the concentration of each solution and measured the heart rate that resulted. We chose to manipulate these variables in order to accurately test the way in which each solution affected the action potentials produced in the cardiac cells. To perform this experiment consistently, we had a constant trial time of seven minutes to allow enough time for the *Daphnia magna* to sit in their solution. We established a control, the *Daphnia magna* placed in tank water, to determine the base heart rate when only exposed to water from its natural habitat. Our experimental group included the different solutions and concentrations: Nicotine with concentrations of 0.62% and 6.2%, as well as Caffeine with concentrations of 0.5% and 1.0%. The individual *Daphnia magna* was extracted from their tank and placed on a slide under a microscope in order to measure its initial heart rate. In order to keep the *Daphnia magna* from swimming around while measuring the heart rate, we had to soak up a small amount of the tank water by use of a paper towel. Refer to Canvas protocol for *Daphnia magna* procedures and tips (Shaw and French, 2018). After the initial heart rate was collected, we added four drops of the given solution into the water by a dropper and allowed it to sit for 7 minutes with the *Daphnia magna*. After seven minutes elapsed, we once again had to soak up a small amount of the solution to keep the *Daphnia magna* from moving around too much. We then measured the heart rate of the *Daphnia magna* in the solution and compared it to the heart rate of the same *Daphnia magna* when in the tank water. In order to collect the heart rate, we made a slow motion video that lasted 15 seconds and used a hand held counter to accurately measure the heart rate. Our data analysis includes the calculations of the percent change between pre-exposure and post-exposure of each Daphnia magna in each solution ((Change / pre-exposure BPM) x 100). We performed a Paired T-Test to observe the statistical difference between the *Daphnia magna* placed in tank water (pre-exposure), compared to the same *Daphnia magna* placed in the different solutions (post-exposure). We also ran an Unpaired T-Test using the percent change equation above to find the statistical difference between the concentrations of the solutions. All statistical tests were run using Past3 (Hammer & Harper, 2013). We used a box and whisker plot graph to show the range of our data easily, along with our averages.

Results

The box and whisker plot graph illustrates the percentage change in heart rate of the *Daphnia magna* submerged in different concentrations of each solution (Refer to Figure 1). We found no

apparent data trends. The *Daphnia magna* immersed in caffeine with a concentration of 0.5% appeared to be an outlier with a percent change of 52.3%, whereas the other percentages were substantially lower in the caffeine 0.5% concentration. The average percent change of the Caffeine (0.5%) was higher than that of the Nicotine (10 mM). In addition, the Caffeine (1.0%) and the Nicotine (1 mM) showed a similar percent change throughout the duration of the experiment.

The Unpaired T-Test was conducted to compare the effect of caffeine concentration on the heart rate of *Daphnia magna* in 0.5% and 1.0% concentrations. There was not a significant difference between the two concentrations; t (2) =2.7764, p=0.34471. The Unpaired T-Test was conducted to compare the effect of nicotine concentration on the heart rate of *Daphnia magna* with 1mM and 10mM concentrations. There was not a significant difference between the two concentrations; t (2) =2.7764; p=0.063235.

A Paired T-Test was conducted to compare the effect of caffeine (0.5%) on the heart rate of *Daphnia magna*. There was not a significant difference between the two conditions; t (2) =-1.1738, p=0.36132. A Paired T-Test was conducted to compare the effect of caffeine (1.0%) on the heart rate of *Daphnia magna*. There was not a significant difference between the two conditions; t (2) =0.88736, p=0.46851. A Paired T-Test was conducted to compare the effect of nicotine (1 mM) on the heart rate of *Daphnia magna*. There was not a significant difference between the two conditions; t (2) =-0.83524, p=0.83524. A Paired T-Test was conducted to compare the effect of nicotine (10 mM) on the heart rate of *Daphnia magna*. There was not a significant difference between the two conditions; t (2) =3.1858, p=0.086007.

Discussion

We hypothesized the use of a higher concentration of a stimulant will increase heart rate because higher concentrations of both nicotine and caffeine will open the sodium channels at a faster rate, increasing action potentials in the heart. We concluded that our data did not support our hypothesis, because our statistical test showed that there was not a significant statistical difference in stimulant concentration and *Daphnia magna* heart rate. We were able to conclude that nicotine and caffeine did not have a significant effect on the heart rate of the *Daphnia magna.* To answer the question in the introduction, we concluded that stimulants do not affect heart rate in *Daphnia magna*. It was unexpected that there would be an outlier in the *Daphnia magna* submerged in caffeine with a concentration of 5% because the other percent changes were much lower.

Humans have a much smaller surface area to volume ratio, while the *Daphnia magna* have a substantially larger surface area to volume ratio. This lead us to believe that size could play a part in action potential production. A smaller surface area to volume ratio would need more action potentials to perform daily activities. Our experiment had several limitations, one being that we only used small percentages of each stimulant. With larger concentrations, there is a possibility we would have seen more effect (Villegas-Navarro, 2003). We performed some trials on pregnant *Daphnia magna*, which could have had an effect on our data. The concentrations would have affected the offspring, either rapidly increasing or decreasing the mother's heart rate. Despite our alternate explanations, there was no actual experimental error that influenced our data collection.

In future experiments, the experimenter could focus specifically on using larger *Daphnia magna* subjects, because their hearts are much easier to spot, therefore making it easier to measure their heart rate. The experimenter could also use higher concentrations of the solutions that would be more realistic as to what humans intake on a daily basis. This might give us more accurate data to compare to a human's heart rate. Also having a

shorter exposure time might lead to a more accurate result, because unlike humans, *Daphnia magna* express effects of exterior influences faster (Anonymous, 2015). Those interested in studies regarding stimulant effect on heart rate should use caution when directly relating *Daphnia magna* heart rate to humans due to the results found in this experiment. Despite their similarities, we suggest testing new drugs on other mammals such as rats or mice before moving to humans.

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