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Nicotine Decreases and Caffeine Increases Short-Term Heart Rate in Daphnia Magna

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Caffeine and nicotine are both stimulates that elevate heart rate in humans. We tested the effects of nicotine and caffeine on Daphnia to see if they would have similar reactions as humans. We choose to test both nicotine and caffeine to ensure that daphnia are a viable comparison for testing drugs that will be used by humans. We recorded the initial and final heart rate of the Daphnia in order to see the percent change after each of the chemicals. We expect these findings to set a precedent for future drug testing on Daphnia that can be used to predict result in humans.

Introduction

Nicotine and caffeine are often utilized by humans as stimulants. Stimulants are substances that increase neuro activity and thus increase activity interest, alertness, and elevate mood. In a test in which sixteen males were given varying amounts of nicotine, the participants that were exposed to the highest amount of nicotine experienced the greatest increase in heart rate (Parrott & Winder, 1989). Similarly, a test was conducted in which forty healthy men were randomly exposed to caffeine. Those men who received caffeine had an increase in heart rate while those in the control group experienced no change (Geethavani, Rameswarudu, & Reddy, 2014).

It is necessary to test the effects of drugs on Daphnia rather than on humans due to ethical standards. We chose Daphnia because they are easily observed by human eye with the assistance of a microscope. Daphnia (*Daphnia magna*) are small aquatic crustaceans with hearts that function similarly to humans (Villegas-Navarro, Rosas-L, & Reyes, 2003). The heart wall in Daphnia is approximately one cell thick, due to this, it has an increased sensitivity to change. This makes the Daphnia a good specimen to serve as a model for the effects of different substances on a human's heart (Stein, Richter, Zussman, & Brynjolfsson). We used Daphnia to observe the effects of nicotine and caffeine because these substances will quickly affect their heart function and allow us to predict what effects similar substances will have on humans. In order to address the question of whether Daphnia are good model organisms in place of humans, we hypothesized that nicotine and caffeine

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will cause an increase in heart rate of Daphnia because these substances both increase heart rate in humans.

In order to test our hypothesis, we exposed Daphnia to small doses of nicotine and caffeine and observed their change in heart rate. If our hypothesis is supported, there will be an increase in heart rate of both the nicotine and caffeine Daphnia. If our hypothesis is not supported, we will see no change or a decrease in the heart rate of Daphnia when exposed to caffeine and nicotine.

Methods

We measured the effects of nicotine and caffeine on the heart rate of Daphnia in beats per minute. For comparison, we tested the heart rate of Daphnia in the aquarium water in order to determine a resting heart rate. Nicotine and caffeine were chosen as the solutions to add to the aquarium water because they are commonly used stimulants by humans.

We first had to fill a beaker with Daphnia by scooping them out of the tank. After this, we used a modified dropper to pick up one Daphnia and place it onto our depression slide. The initial heart rate was recorded for 20 seconds. We then allowed the Daphnia to sit for 10 minutes in order to incubate. We placed the slide with the Daphnia onto the microscope and video recorded its heart rate for 20 seconds. We repeated this procedure 4 times for the aquarium water trial. For the first experimental group, we placed another Daphnia on the slide under the microscope and recorded its initial heart rate for twenty seconds. We then added 3 drops of 1mM nicotine to the depression slide containing the Daphnia. The Daphnia was allowed to sit for 10 minutes to give it time to absorb the nicotine. We then placed the slide back onto the microscope and recorded the heart rate (beats per minute) of the Daphnia for 20 seconds. This was repeated 4 times. For the final experimental group, we placed another Daphnia on the slide and recorded its initial heart rate for 20 seconds. After that we added 3 drops of 0.5% caffeine. The Daphnia was allowed to sit for 10 minutes to give it time to absorb the caffeine. We then placed the slide onto the microscope and recorded the Daphnia's heart rate for 20 seconds. This was also repeated 4 times.

Since the Daphnia's heart beats at such a fast rate, we utilized a smart phone adaptor to record the multiple 20 second trials. We re-watched the recording in slow motion in order to accurately record the Daphnia's heart rate in beats per minute. Using the hand counter, we then counted every beat within the 20 second video of Daphnia heart rate. After determining the amount of heart beats for 20 seconds, we multiplied that number by three in order to determine the amount of heart beats per minute for Daphnia.

After calculating the percent change $\left(\frac{post-exposure\ BPM-pre-exposure\ BPM}{preexposure\ BPM}\times 100\right)$ of the heart rate for the control group and the two experimental groups, we placed our data into a bar graph. Due to the fact that we had normally distributed, nominal, and measurement data we chose to run a One-Way ANOVA statistical test.

Results

We utilized the aquarium water to set a baseline in order to compare the effects of the types of solutions on Daphnia heart rate. Compared to the aquarium water, nicotine caused the heart rate in Daphnia to decrease, while caffeine caused the heart rate to increase (See Figure 1.1).

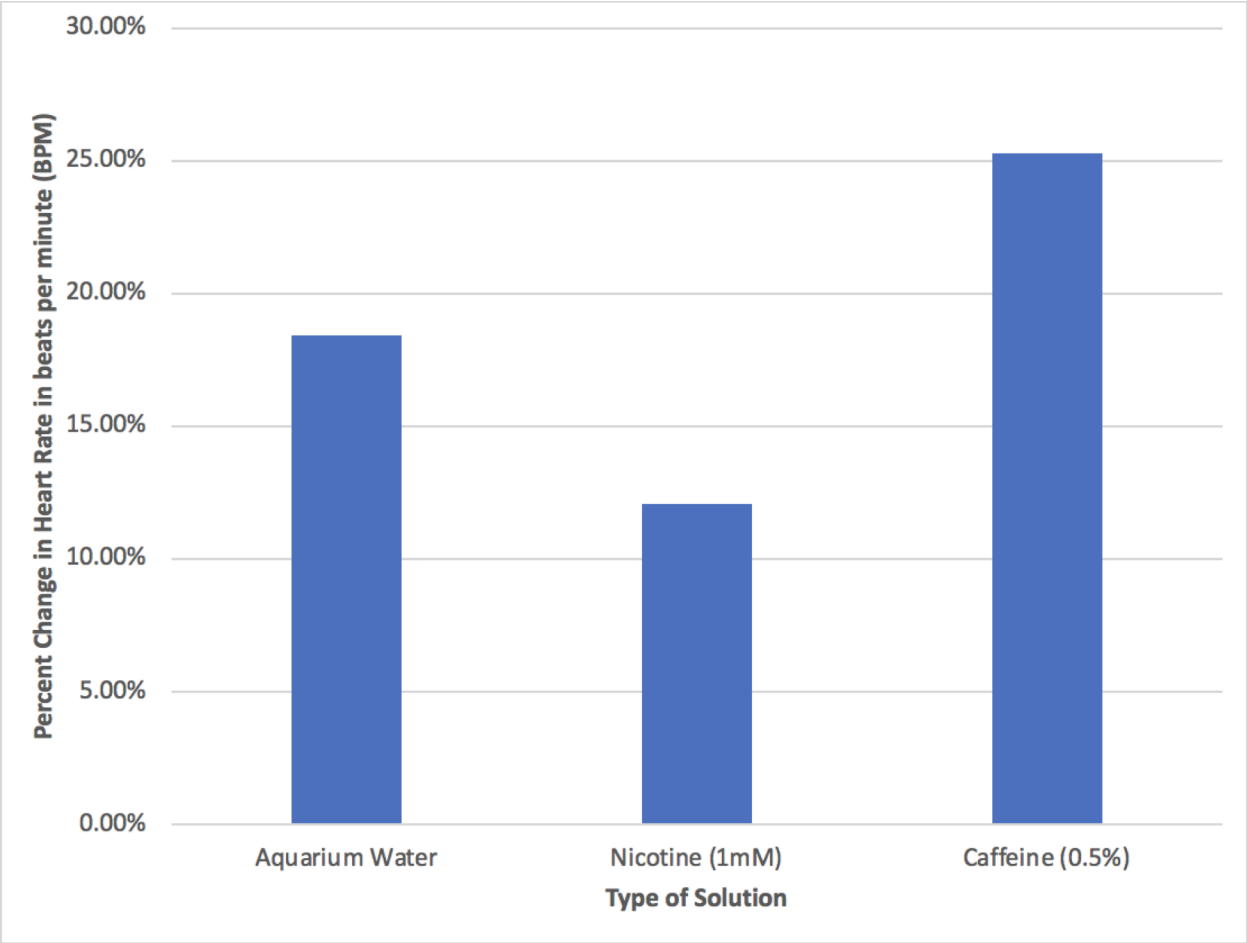

Figure 1.1: A baseline percent heart rate change was established with the water. Nicotine caused the heart rate to decrease while caffeine caused the heart rate to increase.

A test for normality was conducted and it showed the p-values for the three solutions. Aquarium water had a p-value of 0.8542, nicotine had a p-value of 0.4847, and caffeine had a p-value of 0.9189. Therefore, all of the data was normally distributed. A One-Way ANOVA was conducted to compare the effect of type of solution on the percent change in Daphnia heart rate in aquarium water, nicotine, and caffeine. There was not a significant difference of type of solution on the percent change in Daphnia heart rate between the three conditions; [F (2,9) =1.741; p = 0.2295].

Discussion

Our data partially supported our hypothesis that nicotine and caffeine increased heart rate in Daphnia just as they do in humans. Caffeine increased while the nicotine decreased the Daphnia heart rate. Due to the fact that Daphnia have similar reactions as humans do to caffeine, and nicotine, Daphnia are a viable specimen to use to test how these drugs can affect humans. In humans, after the vagus nerve is stimulated in the heart, the nerve will work to counteract a high heart rate by regulating innate immune responses (Rosas-Ballina, et.al, 2011). In the case of caffeine, the vagus nerve is inhibited and is not able to regulate heart rate. Although Daphnia do not have a vagus nerve, they have an equivalent nerve that is affected in the same way as the vagus nerve is by caffeine.

Other interpretations of the increased heart rate could be due to the stress placed on the

Daphnia during transport and a new confined environment. In addition, the Daphnia that we utilized during our experiment may have been previously exposed to nicotine, or other solutions because the Daphnia in the aquarium were constantly being reused. If the Daphnia used was not given the allotted time to allow for the effects of the previous solution used on it to wear off, this could have affected how the Daphnia responded to the nicotine in our experiment. Since nicotine is dose dependent, the concentration will determine the effect that it has on humans, which could also be applied to Daphnia, due to the similarities between the two. In small doses, nicotine serves as a depressant, while in larger doses it functions as a stimulant (Ashton).

In the future, the Daphnia should be observed in controlled environments for a set amount of time in order to ensure that previous solutions have had the appropriate amount of time to wear off. Moreover, instead of only testing stimulants on the Daphnia heart rate, we could also investigate how depressants affect heart rate in Daphnia. This would be beneficial because it would help experimenters to have a better understanding of the differences between the effects of stimulants and depressants on heart rate in humans.

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