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It's coffee time: Caffeine across a range of concentrations is not associated with higher heart rates in *Daphnia magna*

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Abstract

There are many factors that can affect the contractibility of cardiac muscle, such as exposure to stimulants such as caffeine. Using Daphnia Magna as models, we exposed them to different concentrations of caffeine to study the change in heart rate contractibility by determining their heart beats per minute. We used two experimental groups, one control group, and three trials were run for each group to accurately compare the difference in heart rate. Our results showed that there was no significant increase in the heart rate in Daphnia as the concentration of caffeine was increased. Our experiment studies not only the effects of caffeine but also the effects of different concentrations on Daphnia's heart rate, separating it from previous research. Knowing this could greatly benefit the engineer of synthetic cardiac muscles and their ability to maintain a consistent heart rate when exposed to stimulants.

Introduction

Cardiovascular disease is a leading cause of death globally (Mohammed et. al, 2015). Cardiovascular disease can lead to disruption of blood flow to the heart, causing damage to cardiac muscle. Cardiac muscle cell contractions pump blood into the vessels of the circulatory system. These specialized contractions determine the heart beats per minute (heart rate). The duration of action potentials leads to a faster rate of myocardial tissue contraction, causing an increase in the heart rate (Belardinelli and Isenberg, 1983). Cardiac muscles have a highly specialized function, therefore they have lost the ability to proliferate and regeneration of myocardial cells is limited (Kajstura et. al, 1998). Thus the damage caused by heart disease cannot be repaired and can bring about worse ramifications, including heart rate irregularity and even death. There are many breakthroughs happening to help improve cardiac function such as cardiac cell transplantation, although the long term effects are unknown (2002, Muller-Ehmsen et. al). Regenerative medicine is working to engineer synthetic cardiac muscle cells for implantation into those that have suffered

from myocardial damage due to myocardial infarction or chronic myocardial failure (Mohammed et. al, 2015). These engineered cardiac muscles still have trouble maintaining a consistent rate of contraction, due to the many factors that can affect cardiac muscles. This inspired us to explore the effects of caffeine on cardiac muscle contraction. Specifically, if an increase in caffeine would cause an increase in the heart rate. To determine how caffeine would affect cardiac muscle contraction, we used *Daphnia Magna* as a model because they are transparent making their heart easily visible, are affected by solutions rapidly, and are commonly used for research (Foster, 2010; Corroto et. al, 2010). We exposed the Daphnia to two solutions with different concentrations of caffeine, then determine its heart beats per minute. The higher heart beats per minute, the more of an increase would be seen in the cardiac muscle contraction. We believe that caffeine will increase the rate of cardiac muscle contraction when the solution concentration increases because caffeine is a stimulant. If the results support our hypothesis the Daphnia's heart rate will increase with the increased caffeine concentration. If the results do not support our hypothesis the Daphnia's heart rate will show no effect or a decrease in the heart rate with the increased caffeine solution.

Methods

To test how the rate of cardiac muscle contraction in animals is affected by caffeine we used *Daphnia Magna* as a model. We manipulated the concentration of caffeine exposed to the Daphnia to observe how it affects heart rate. One of our experimental groups contained a 0.5% solution of caffeine and the second experimental group contained a 1% caffeine solution to show the difference in Daphnia heart rate as concentration increases. Our control group only contained aquarium water to obtain a base heart rate for daphnia to compare our experimental groups so we know what the effect of caffeine is on heart rate. In this experiment, we placed daphnia on a depression slide filling the depression with the aquarium water using a dropper and restricted its movement with cotton. For our experimental groups, we added one drop of caffeine solution either being 0.5% or 1%. We performed 3 trials soaking the daphnia in the solution for 7 minutes before collecting data. We used six total different Daphnia during our experiment, testing a new Daphnia each trial except for the control. We observed the Daphnia through the microscope and using a smartphone adapter connected to the microscope, we took a 10 second video of the daphnia in slow motion. From the video we used the hand counter to count the heartbeats and multiplied that by six to calculate heart rate per minute. We displayed our data using a box and whisker plot to compare the control, 0.5% solution, and 1% solution of caffeine. We also ran a One-way ANOVA using Past 3 because we had nominal and measurement data using three groups and only one variable and it was normally distributed (Hammer & Harper, 2018). Additionally, by using the One-way ANOVA and Past 3 we were able to find the differences between the mean of our one measurement and one nominal variable and able to test for significance of our data.

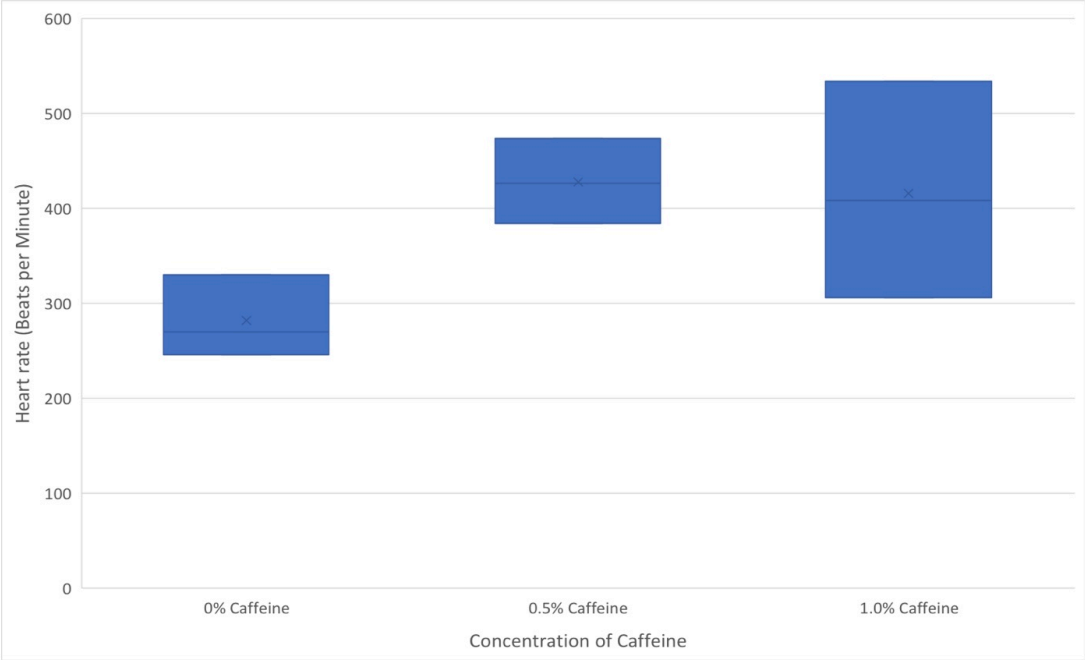

Figure 1. Shows the average and the range of the Daphnia's beats per minute as they were exposed to either 0%, 0.5%, or 1% caffeine solutions.

Results

The data in **figure 1** shows the average heart rate of Daphnia in each group. The average heart rate increased from our control group to our 0.5% group but then decreased slightly to our 1.0% group. The control group had an average of 282 bpm. The .5% caffeine group had an average of 428 bpm. The 1.0% group had an average of 416 bpm. A One-Way ANOVA was conducted to compare the effect of caffeine concentration on the heart rate of Daphnia in the control, 0.5%, and 1% caffeine solutions. There was not a significant effect of caffeine concentration on Daphnia heart rate between three conditions; [(2,6) =3.489; p=0.0988].

Discussion

This experiment began with the idea that an increase in caffeine exposure would lead to a higher heart rate of Daphnia. This, in turn, would provide answers to the effect of caffeine on human cardiac muscle contraction. Our results did not support our original hypothesis. The One-Way ANOVA test showed there was little to no significant change in the heart beats per minute of the Daphnia when exposed to our control and our experimental groups. Therefore, this proves that the Daphnia heart did not increase as they were exposed to the specific higher concentrations of caffeine we used, as we had previously hypothesized. This could have been due to an initial homeostatic response to prevent an increase in blood pressure as the Daphnia were exposed to the caffeine (Bichler et. al, 2006). Using higher concentrations of caffeine could have yielded a more significant change in the Daphnia heart rate. Gubareff and Sleator's (1965) experiment addresses the use of higher concentrations of caffeine and their effect on the Daphnia heart rate. Their experiment stated that at higher concentrations of caffeine there was an increase in the duration

of action potentials, reaching twice the normal duration at a concentration of 2.5 nM. A longer duration of action potentials leads to a faster rate of myocardial tissue contraction therefore, there is an increase in heart rate at higher caffeine concentrations (Gubareff & Sleator, 1965). Exposing the Daphnia to the caffeine solutions for a longer period of time could have also provided more accurate results of the long term effects on the Daphnia heart rate. Green and Suls's (1996) experiment addresses the long term monitoring of their test subjects as they are exposed to caffeine, monitoring their beats per minute for 24 hours. They found that the heart rate of those exposed to caffeine increased overnight, compared to shortly after exposure (Green & Suls, 1996). Further experiments could be performed using humans as test subjects to provide the most accurate results of caffeine's effect. This question should continue to be researched to benefit the engineering of synthetic cardiac muscles and their ability to maintain a consistent heart rate when exposed to stimulants.

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