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The opposite effects of caffeine and ethanol on *Daphnia magna*'s heart rate

James Wilson, Jessica Collette, Andrew Miller, Anh Hoang, Traci Dubose*
University of Oklahoma, Department of Biology, 730 Van Vleet Oval, Room 314 Norman, OK 73019

Abstract

Caffeine is used in everyday life for many to achieve the feeling of alertness; however, when ethanol (alcohol) is consumed the adverse effect is expected. Studies have varying results on how heart rates are affected when either caffeine or ethanol are introduced. We proposed that when caffeine was added to a solution, the heart rate would increase, and when ethanol was added to a solution, the heart rate would decrease. We first observed *Daphnia magna* heart rate under a slide in aquarium water, and then again after adding either solution to the water. We expect that our findings will be of interest to regular caffeine and/or alcohol consumers in understanding how these substances can increase or decrease heart rate in the short term.

Introduction

Caffeine and/or alcohol are consumed on a regular basis (Ferreira et al., 2006). Many studies and experiments have been done to determine the effects of both caffeine and ethanol consumption on the body. Caffeine is classified as a stimulant in humans, which is something that will temporarily increase efficiency of a specific function or organism (Bracco et al., 1995). Caffeine also blocks adenosine receptors, which stops adenosine from being processed and released. Adenosine is known to slow heart rate, so if caffeine blocks adenosine, then heart rate should increase (Belardinelli et al., 1989). Meanwhile, ethanol (contained in alcoholic beverages) acts as a depressant in humans, which will temporarily decrease or slow efficiency of a specific function or organism (Paradela et al., 2015). We want to test each substance's effect on heart rate because they are widely consumed by a majority of people. To do this, we will use *Daphnia magna*, a type of water flea, to determine the change in heart rate when caffeine and ethanol are added to the *daphnia* environment. *Daphnia* were chosen to compare to humans because they, unlike most invertebrates, have a self-regulating heart (Stein et al., 1966). *Daphnia* are favorable for this experiment because their heart is visible and recordable. Our

hypothesis is that the heart rate of *daphnia* increases when subjected to caffeine because caffeine is a stimulant and that the heart rate of *daphnia* slows when subjected to ethanol because it acts as a depressant. We know that our hypothesis will be supported if the heart rate of the *Daphnia* significantly increases because of the caffeine and significantly decreases because of the ethanol. It's also possible that half of our hypothesis will be true and the other half rejected. For example, the caffeine and the ethanol could both increase heart rate.

Methods

To analyze the effect of additives on the heart rate of *Daphnia* we retrieved eight *daphnia* from the aquarium, placed one on the depression slide, and placed the slide on a microscope for data collection using the method shown in French and Shaw (2018). We recorded the *Daphnia* in the control environment without any additives for fifteen seconds. After recording the control heart rate, a drop of one percent caffeine or one percent ethanol was added into the depression with the *Daphnia*. We waited ten minutes after adding the chemicals before recording the *Daphnia* again to allow the substances to be absorbed. The *Daphnia* was recorded again for fifteen seconds. The videos were slowed down to allow us to precisely count the number of heartbeats. This number was multiplied by four in order to obtain a heart rate in beats per minute (BPM). This process was repeated three more times using caffeine and four times using ethanol. We decided that using eight different *Daphnia* would allow us to account for heart rate differences in individuals.

The change in heart rate for each *Daphnia* was compared using the percent change formula (BPM after exposure minus BPM before exposure divided by BPM before exposure times 100). This quantitatively displayed the relationship between pre-exposure and post-exposure heart rates. The percents changed were then averaged together for each type of substance. Each group's percent changes were used to form a box and whisker plot. The two graphs were then combined to compare the percent change in heart rate and also the direction of the change (positive or negative).

Because our experiment measured pre-exposure and post-exposure heart rates for two treatments, we conducted two separate statistical tests. We used paired t-Tests for both sets of data because both were found to be normally distributed using a Shapiro-Wilk normality test (Hammer & Harper, 2013).

Results

Three of the four *Daphnia* exposed to caffeine exhibited an increase in heart rate. The average percent change of heart rate for the caffeine treatment was 4.7955%. The range of percent change for the caffeine treatment was 16.043%. Three of the four *Daphnia* exposed to ethanol exhibited a decrease in heart rate. The average percent change of heart rate for the ethanol treatment was -5.0355%. The range of percent change for the ethanol treatment was 19.03%.

A paired t-Test was conducted to compare the effect of caffeine on *Daphnia* heart rate before and after caffeine exposure. There was not a significant difference between the two conditions; $t(3) = -0.6934$, $p = 0.5379$. A second paired t-Test was conducted to compare the effect of ethanol on *Daphnia* heart rate before and after ethanol exposure. There was not a significant

difference between the two conditions; t(3) = 1.3937, p = 0.2577.

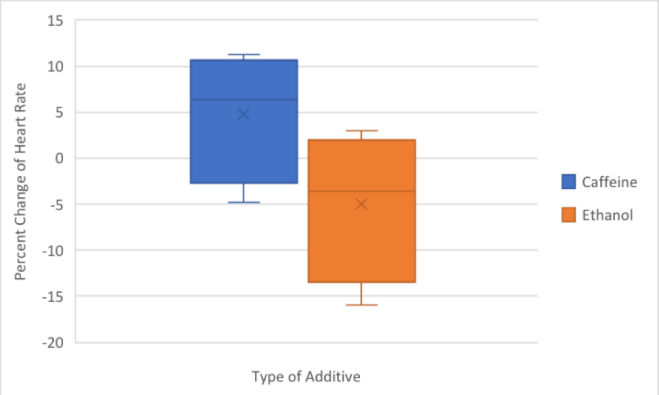

Fig 1. Comparison of percent change of *Daphnia* heart rate before and after exposure to an additive. Four trials were performed with caffeine, and four trials were performed with ethanol. The average percent change for caffeine was 4.7955%, and the average percent change for caffeine was -5.0355%.

Discussion

The results from our statistical analysis test show there was no significant trend between the four trials which indicates there is no proof that there is a correlation between ethanol/caffeine and *Daphnia* heart rate. Although our raw data did show a small trend that validates our hypothesis, it was not significant enough that we can accept our hypothesis. There is a possibility that if we had added more trials to our experiment then the data could have been significant. The concentration of the chemical solution could have also not been enough to have an effect. In many substances, there is a concentration threshold of when the substance will start to have an effect. The *Daphnia* was in the experimental solution for eight minutes which could have either been too little time for the chemical to affect the heart rate or too much time for the *Daphnia* to acclimate to the solution and return to its baseline heart rate.

One potential error that could have affected the results were the concentration of the chemicals in the aquarium water because the amount of aquarium water on the slide varied slightly but the amount of chemical added stayed at one drop. This factor could have affected our data. Another unaccounted for variable is the size variation between the *Daphnia* in each trial. The *Daphnia* in Caffeine Trial 3 was significantly bigger than others used in the trial and had a slower heart rate compared to the smaller *Daphnia*. Additionally, the *Daphnia* in Ethanol Trial 4 had some kind of heart arrhythmia. Its heart stopped beating briefly during both the control trial and the experimental trial. However, both the bigger *Daphnia* and the *daphnia* with arrhythmia heart beat supports our hypothesis even though they had an abnormal baseline heart rate compared to the other *Daphnia.*

While our research shows no significant trends in heart rate of *Daphnia* exposed to caffeine or ethanol, different results have been shown in other species and/or humans (Green & Suls, 1996). In addition, if our results are proven to be accurate in both *Daphnia* and mammals with repeated experiments and trials, then alcohol and caffeine can be taken out as a significant variable in affecting cardiovascular medicine.

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