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Caffeine concentration shows no significant effect on *Daphnia magna* heart rate

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Abstract

There are many different chemicals that can affect heart rate due to their molecular makeup. Also, the concentration of said chemicals can influence the severity of the effect. We investigated the effect of caffeine concentration on the heart rate of *Daphnia magna*. *D. magna* are an ideal species for examining heart rate because their myogenic heart causes it to perform similarly to humans. We conducted 3 trials, each trial comparing two different concentrations of caffeine on the change in daphnia heart rate. This experiment would be of interest to many because it offers an illustration of how human heart rate may be affected by caffeine (a significant chemical in human consumption), due to the similarities in daphnia and human heart function.

Introduction

Organisms react and respond using a network of nerves that runs, connected to the brain through the central nervous system. The brain sends and receives electrical impulses that travels through this network of nerve cells in the form of traveling action potentials. This is a complicated process in which a dendrite receives a signal that activates the voltage-gated ion channel and induces the depolarization and repolarization in axon. This propagates down the axon and as this reaches the terminal branch, neurotransmitters are released into the synapse and triggers another cycle for the next nerve cell (Jones, 2008).

In humans, the heart functions on a myogenic mechanism where the impulses originate within the heart muscles (in contrast to neurogenic processes). Although the heart rate is regulated within the heart, it can also be influenced by external signals such as nerve signals from the brain, endocrine signals and changes to blood pressure or CO_2 concentration.

There are many substances that impact the heart rate, whether an increase or decrease is

induced. One of these substances is a very commonly used psychoactive drug called caffeine. This is a stimulant that is unregulated and usually found in tea and coffee, that has become a big part of modern life to keep us awake and running. Caffeine has a similar chemical structure to adenosine and therefore competitively inhibits adenosine from binding to the adenosine receptors, inducing an increase in sympathetic nervous system activity (Gonzaga et al., 2017).

In this investigation, we will be exploring the effects of caffeine concentration on the change in heart rate of *D. magna*. *Daphnia* have a self-regulating heart, similar to most vertebrates and hence, we can test the effect of caffeine concentration on the heart without human subjects. We expect that the impact of caffeine on the *Daphnia* will be similar to the observed impact caffeine has on humans. Therefore, we hypothesize that an increase in concentration of caffeine will lead to a higher percent change in heart rate as more adenosine receptors may be blocked.

Our hypothesis will be supported if caffeine induces a significant difference to heart rate and a difference is observed between the two experimental groups of different concentration, given that the higher concentration of caffeine induces a larger percent change in heart rate. On the other hand, the hypothesis will be rejected if no significant difference is observed between the two experimental groups or if the group with lower concentration induces a higher percent change in heart rate.

Methods

The effects of different concentrations of caffeine were tested on the change in *Daphnia* heart rate. We began by retrieving several *Daphnia* from the aquarium and placed one on a slide with a small amount of aquarium water under a microscope using a modified pipette. A small amount of cotton was used to restrict the *Daphnia*'s movement to observe its heart easily. Using a phone adapter, we took a 10 second slow motion video of the *Daphnia* and counted the heart beats with a manual counter. To determine the daphnia's heart rate in beats per minute, the values collected by the counter were multiplied by 6. This initial heart rate was used as a control to determine the normal heart rate of the daphnia, before introducing the experimental caffeine solutions.

We then removed the water from the slide using a paper towel and added a few drops of caffeine solution, either 0.5% caffeine or 1.0% caffeine, to the dry slide with the daphnia. After 7 minutes of allowing the

$$Percent\ change = \frac{post\ caffeine\ heart\ rate - pre\ caffeine\ heart\ rate}{pre\ caffeine\ heart\ rate} \times 100\%$$

Formula 1.

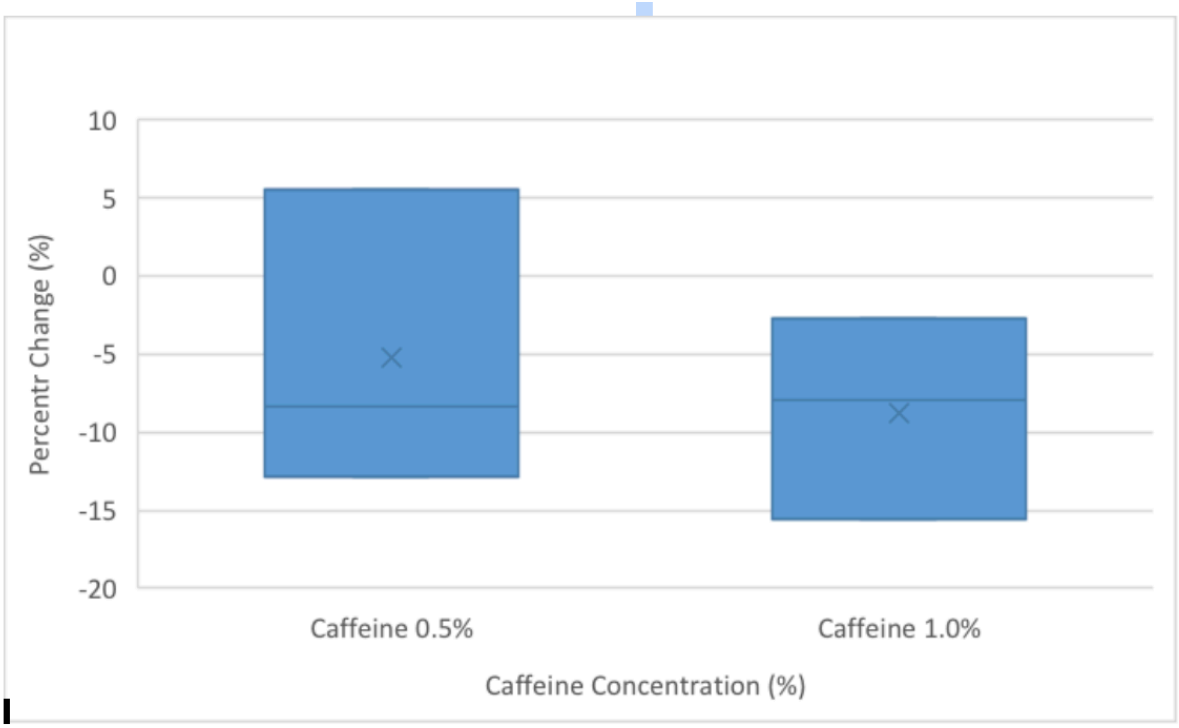

Figure 1: box and whisker plot showing the mean and distribution of values of percent change in daphnia heart rate for the 0.5% and 1.0% caffeine groups.

caffeine to affect the daphnia, and we took another 10 second slow motion video to determine the heart rate in beats per minute after exposure to the caffeine solution. We repeated the counting procedure mentioned above. We conducted 3 trials for each caffeine concentration (0.5% and 1.0%).

For each trial, we calculated the percent change in heart rate after adding the caffeine using Formula 1.

We compared the percent changes of the 0.5 % caffeine trials with the percent changes of the 1.0 % caffeine trials in a box-and-whisker plot. We also examined whether the two concentrations of caffeine had an effect on heart rate by performing a paired t-test on both groups.

Results

From Figure 1, we can see that in both groups, caffeine generally decreased the heart rates of the daphnia by observing that the mean (represented by the cross) and the median values (represented by the horizontal line in the box) were all below zero. We can also notice that there was a large variation in percent change values, with a range of 18,31 % for the 0.5% concentration group, and a range of 12.88 % for the 1% concentration group.

When comparing the two groups, we can observe that the 1.0% caffeine solution had a greater effect on heart rate than the 0.5 % concentration group, with mean percent changes of -8.76 % and -5.22%, respectively.

There appears to be an outlier in the 0.5 % group, with one percent change (5.49%) being significantly higher than the next highest percent change (-8.33%). Also, this percent change was the only increase observed of all of the trials of both concentrations.

A paired t-test was conducted to compare the effect of 0.5% caffeine solution on daphnia heart rate. There was no significant difference between the two condition: $t(2)=0.92155$, $p=0.45405$.

A paired t-test was conducted to compare the effect of 1.0% caffeine solution on daphnia heart rate. There was no significant difference between the two condition: $t(2)=2.2942$, $p=0.1487$.

Discussion

According to our data, no significant difference in daphnia heart rate is caused by any caffeine concentration and hence our hypothesis not supported. However, it is clear that a higher concentration of caffeine does impact heart rate to a greater degree, but in the opposite way of what we expected (larger decrease rather than a larger increase). The more significant impact of 1.0% caffeine could be due to the simple fact that there was a higher amount of caffeine that the daphnia was exposed to. The larger impact of higher dosage of caffeine could be attributed to the larger amount of the caffeine interfering with the natural homeostatic processes of the daphnia. In addition to this, the larger impact could make returning to homeostasis more difficult or time consuming and this could have been reflected in the data (Fig 1).

The data rejects our hypothesis as no significant difference could be observed between the before and after caffeine exposure daphnia. This could be due to several factors. One reason why this may be is due to the fact the daphnia was transferred to the slide where movements were restricted. The longer period of limited movement may have contributed to the lowering of heart rate. Additionally, since the initial measurements were taken straight after the transfer of daphnia, the heart rate may not have had time to slow down. A study by McClaran and Wetter also showed that at low stress levels caffeine could decrease heart rate (McClaran & Wetter, 2007) and so, it is a possibility that the slide was a low stress level setting for the daphnia.

Another is the limitations of the method or equipment used. When we were counting the daphnia heart rate after the 7 minutes of exposure to the caffeine solution, it was noticeably more difficult as each contraction was difficult to distinguish. In a study that found that caffeine had a clear obvious effect on daphnia heart, the experimenter calculated the caffeine dosages-based recommendations for humans and used a stroboscope to determine heart rate (Foster, 1997). However, it is difficult to determine if the caffeine concentration or the equipment used was the main cause of the unexpected results.

In a different study using a similar method to our study, no convincing effect of caffeine on daphnia heart rate could be observed (Corotto et al., 2010). This further suggests that there was a limitation in our method.

However, the observations made in this investigation reflected the results we obtained in our investigation, indicating that the error observed was not due to any human error or systematic error made by our apparatus during our experiment.

In addition, further research revealed that doses of approximately 200 mg of caffeine decreases heart rate slightly (Benowitz, 1990). However, it was also made clear that caffeine also induces a release of epinephrine and norepinephrine. It is known that these hormones increase heart rate and hence, theoretically, if measurements were over a longer period of time, we may have observed an increase. Despite this prediction, in a paper published in 1995, it was declared that most studies could not show any significant impact of caffeine on heart rate (Bracco et al., 1995).

Taking this into account, an investigation with more appropriate apparatus, setting waiting time before taking each measurement and taking more measurements over a longer time interval could reveal different results that may reflect a greater relevance to the effect of caffeine on the heart.

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