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Caffeine and acetylcholine decrease *Daphnia magna* heart rate

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Abstract

We are researching why some cells produce more or fewer action potentials than others. Substances that people ingest may affect the activity of specific cells and therefore may affect their health. We are studying the effects of caffeine and acetylcholine, which are known to influence cardiac function in humans. We conducted four trials using a 1% caffeine solution and a .01M acetylcholine solution on *Daphnia magna,* measuring their change in heart rate from before and after 7 minutes of exposure. Our results may influence future research on caffeine, as well as future research on *Daphnia magna*.

Introduction

Caffeine and acetylcholine are two chemicals that affect human beings and their everyday health. More specifically, they affect cardiac function. It was found in humans that when caffeine is released into the system, there are more fatty acids in the blood, which raises the heart rate and energy reserves are readily accessible (Hofenagels 2018). Acetylcholine is known to inhibit muscle receptors in humans (Liu et al., 2012). One study on canines found that acetylcholine is responsible for the relaxation of contractions in arteries, although this can be altered by other factors (DeMey et al., 1982). *Daphnia magna* have an autonomic nervous system (ANS) similar to the ANS of mammals. Although they have a similar ANS, *Daphnia* do have many anatomical differences from mammals such as a ventral nerve cord, a 3-section brain, and neuronal cells located in their antenna (Tollrian et al., 2012). Since we are measuring *Daphnia* heart rates, we felt it was important to research how their hearts work. *Daphnia* hearts are very thin, with some areas being only one cell thick and others reaching only four to five cells thick (Stein et al., 1966). The myocardial contraction in *Daphnia* is initiated in the cardiac ganglion and is regulated by the

central nervous system (McMahon 2001). One study examining the effects of nicotine and ethanol on *Daphnia* found the solutions had the same effect on the *Daphnia* as they do on humans (Corrotto et al., 2010). *Daphnia magna* have proved useful for experiments like this because they are transparent, making measuring effects of solutions a more efficient process (Villegas-Navarro et al., 2003). For this investigation, we are observing why some cells produce different amounts of action potentials than others. To narrow down this question, we decided to observe the effects of two specific solutions on heart rate. Since we can't conduct this experiment on humans, we are using *Daphnia magna*. Examining heart rate will allow us to draw conclusions about what is happening with the action potentials, whether that means increasing or decreasing. The solutions we chose to experiment with were 1% caffeine and .01M Acetylcholine. Our hypothesis is inspired by the observations of the effects of caffeine and acetylcholine in mammals. Our hypothesis is that caffeine causes an increase in heart rate because it increases adrenaline and acetylcholine causes a decrease in heart rate because it binds to M2 muscarinic receptors. If our hypothesis is supported, then caffeine will increase the heart rate of the *Daphnia* and acetylcholine will decrease the heart rate. If our hypothesis is rejected, then caffeine will have no effect on the heart rate of the Daphnia or it will decrease the heart rate and acetylcholine will increase the heart rate or have no effect.

Methods

We tested the effects of .01M acetylcholine and 1% caffeine on Daphnia because they have systems somewhat similar to those of humans. In order to determine how the Daphnia were affected, we decided to examine changes in their heart rates after being in their normal aquarium water, then after being exposed to the respective solution for seven minutes. To set up the experiment we collected a Daphnia using a dropper with the tip cut off and then deposited one into a microscope slide depression, making sure not to overfill so that the Daphnia couldn't swim out. We used a very small piece of a cotton ball to lay on the depression and limit the Daphnia's movement, while still allowing it to have enough water to survive. In preparation to view the *Daphnia* under the microscope, we put the lowest magnification lens into the front position and adjusted the light for appropriate viewing without harming the *Daphnia*. We adjusted the microscope into focus then utilized a phone adapter to record a 10-second video of the Daphnia. We played the video back in slow motion and counted the heartbeats using a hand-held counter. We multiplied the number by 6 to get the BPM. We then dried up the aquarium water in the slide using the corner of a paper towel and inserted one of our solutions into the slide depression using a dropper. We allowed the solution to set for 7 minutes, with the microscope light off so the Daphnia wasn't harmed and to avoid evaporation, and then followed the same procedure to determine the beats per minute as we did for the aquarium water. After our data was collected, we rinsed the used *Daphnia* out of the slide depression and into a container of aquarium water, and then got a new *Daphnia* for each trial. We completed 4 trials for each solution, acetylcholine and caffeine. To understand the influence of the solutions specifically, we measured the Daphnia's BPM while in aquarium water,

establishing a baseline heart rate, and then measured again after 7 minutes of exposure to the solution. After determining the BPM both in aquarium water and in the solution, we calculated the Percent Change in heart rate by subtracting the pre-exposure BPM from the post-exposure BPM, dividing that value by the pre-exposure BPM, and multiplying by 100. Essentially, the formula is: (Change in BPM / pre-exposure BPM) x 100. To analyze our data after the trials we used a box-and-whisker plot, allowing us to easily compare the solutions through descriptive statistics. Additionally we conducted a Mann-Whitney U-Test as our data was not normally distributed.

Results

Both caffeine and acetylcholine caused a decrease in heart rate in the *Daphnia magna*. The average percent change in heart rate after exposure to acetylcholine was -12.5424, with a minimum of -18.445, a maximum of -4.6975. The average percent change in heart rate after exposure to caffeine was -19.0875, with a minimum of -25.7125, a maximum of -8.085 (See Figure 1). The results for the effects of acetylcholine were normally distributed but the results for the effects of caffeine were not. A Mann-Whitney U-Test was conducted to compare the effects of our solutions on Daphnia magna. There was not a significant difference between the two conditions; Mann-Whitney U = 3, P=0.19393.

Discussion

A portion of our hypothesis was supported and a portion was not. We got the results we expected for acetylcholine, however this was not the case for caffeine. Acetylcholine caused a decrease in heart rate in the Daphnia, telling us there were fewer action potentials. Some cells may have different amounts of action potentials depending on the conditions they are subjected to. There wasn't as much variance in the percent change in heart rate of the *Daphnia* in acetylcholine as there was in caffeine. This shows more consistency in the effects of acetylcholine than in the effects of caffeine. Caffeine lowered the heart rate in the *Daphnia*. Caffeine also had a larger variance in results as seen in Figure 1, but overall the effect was consistent. The similarities in the effects of the caffeine and acetylcholine can

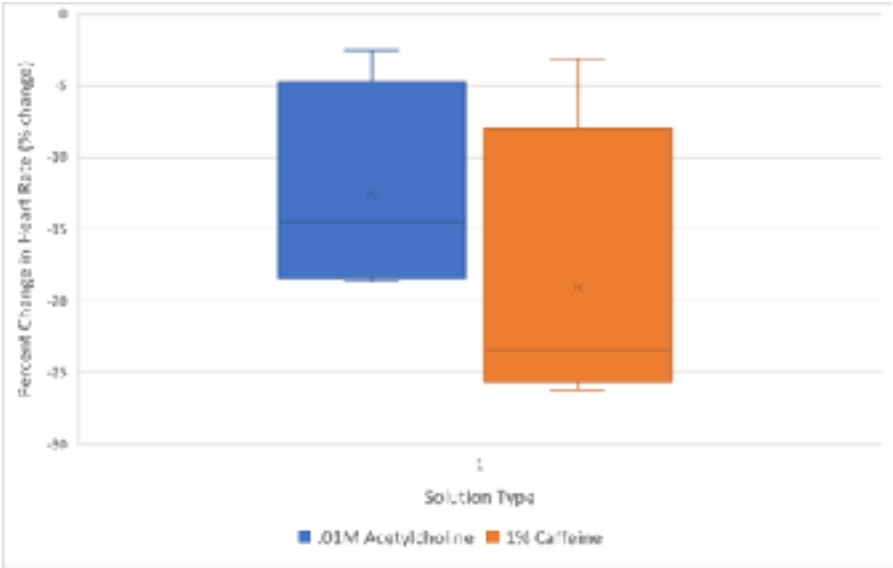

Fig 1. Comparison of percent change in heart rate of Daphnia Magna after exposure to .01M Acetylcholine and 1% Caffeine.

be seen by the results of the Mann-Whitney U-Test, which showed that there was no significant difference between the effects of the two solutions. We speculated some possible causes for our data that we did not specifically test for. For example, the *Daphnia* could still have effects from previous experiments that used different solutions, therefore, it could impact the *Daphnia's* heart rate when exposed to the two solutions we tested. Another example could be that each *Daphnia* could respond differently to each solution based on their size, age, and previous exposures. When recording our ten second slow motion video, there could have been inconsistency in quality and technological difficulties. In addition, there could have been human error in measuring the *Daphnia's* heart beat with the hand counter. Alternative interpretations of our results may include the effects of the amount of light the *Daphnia* were exposed to by the microscope, and the restriction of the Daphnia by the cotton on the depression slide which resulted in limited movement. For future experiments we would recommend focusing on the effects of caffeine on *Daphnia*, including different concentrations. We would then encourage further examination of the anatomy and physiology of *Daphnia* to understand how they respond to different solutions. There are many experiments testing the effects of different solutions on *Daphnia.* Some of these solutions have the same effects on *Daphnia* as they do on humans, but some do not. It may be beneficial to review all of these studies and then compare the solutions that have the same effects to those that don't. This information would be useful to look at the differences in the solutions themselves, and their results. Overall, this could help further research on the differences between cells and the frequency of their action potentials. Knowledge of how these many solutions affect cells, action potentials, and systems in the body could be helpful for those in the healthcare industry.

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