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Need a reason to break the addiction?: Increasing caffeine concentrations increases heart rate and increasing nicotine concentrations decreases heart rate of *Daphnia magna*

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Abstract

Caffeine and nicotine are known to affect cardiac muscle cell receptors, and both chemicals have been studied recently due to the increase in caffeinated drinks and e-cigarettes being sold in the market. This experiment studies whether changing concentrations of caffeine and nicotine solutions affect the percent change in heart rate of the crustacean *Daphnia magnus*. We hypothesized that increasing the concentration of nicotine will cause a decrease in percent change in heart rate (giving a larger negative value), and increasing the concentration of caffeine will increase the percent change in heart rate. This study investigates how the concentration of the chemical solutions affect heart rate rather than simply comparing changes between the two. The heart beats of twelve *Daphnia magnus* were counted both before and after being introduced to a solution; the concentration and type of solution were compared by the calculated percent change in heart beats per minute of the *D. magnus.* Since the heart of the *Daphnia* are similar to those of vertebrates, further studies on the effects of caffeine and nicotine on the heart rate and health of humans could be created based on our results.

Introduction

Daphnia magna are small crustaceans with a single chambered heart. Although they have exoskeletons, their heart rate is self-regulating and initiated in the cardiac muscle cells like those of vertebrates (Kaas et al., 2009). This allows the effect of different chemical solutions on the *D.*

magna's cell receptors to be comparable to the effect that they have on human cardiac muscle cells, despite the fact that humans have a four-chambered heart. Chemical substances can either increase or decrease the frequency of action potentials between the brain's neurons and the heart's muscle cells depending on whether the chemical acts as an antagonist or agonist and which cell receptors the chemical acts upon. Antagonists inhibit cell receptor signals and agonists increase cell receptor signals (Hoefnagels, 2018). Thus, if a chemical acts as an agonist to an inhibitory neurotransmitter cell receptor, it will decrease an organism's heart rate, and if a chemical acts as an antagonist to the same receptor, it will increase heart rate. Opposite effects will occur if a chemical is applied to an excitatory neurotransmitter cell receptor.

In recent years, the usage of vapes and e-cigarettes have increased steadily among young adults. E-cigarette usage by high school students increased by 78 percent from last year according to a recent study (Nedelman, 2018). In addition, it was discovered that about 85 percent of U.S. citizens consumes caffeine at least once per day (Knights et al., 2014). Both caffeine and the nicotine contained in e-cigarettes are known to affect human cardiac muscle cell receptors. Studies have reported that nicotine decreases heart rate of the *D. magna* (Villegas-Navarro et al., 2003). Other investigations have concluded that caffeine is a known antagonist to adenosine cell receptors (which inhibits heart muscle cells and heart beats); caffeine therefore increases heart rate in humans (Daniels et al., 1998). It is important to note that *Daphnia* do not possess an automatic nervous system, a distinction that sets it apart from mammals like humans (Shaw and French, 2018). Since nicotine and caffeine both affect receptor cells of the sympathetic nervous system in humans, we should use caution before making conclusions about how these solutions will affect mammals based on the results of this study (Haass and Kubler, 1997).

In this experiment, we examined which chemical solutions acted as an agonist and which acted as an antagonist to cardiac muscle cell receptors of *Daphnia magna*. In addition, this study will explore the idea that increasing the concentrations of caffeine and nicotine also have an effect on the action potentials of muscle cell receptors. In order to do this, we investigated the effect that caffeine and nicotine solutions of varying concentrations have on the percent change in heart rate of the *D. magna*. We hypothesized that adding a greater concentration of nicotine to the *D. magna*'s environment will cause the percent of change in heart beats per minute (BPM) to decrease and adding caffeine will cause the *D. magna*'s percent change in BPM to increase, because nicotine is an antagonist and caffeine is an agonist to muscle cell receptors. If the hypothesis is supported, we predicted that the percent change in BPM of the *Daphnia* added to the caffeine solution will be positive and increase as the concentration of caffeine added increases. The hypothesis will also be supported if the percent change in heart rate of the *D. magna* introduced to the nicotine solution will be negative and decrease with increasing concentration in nicotine solution. The hypothesis will not be supported if changing the concentration in the two solutions has no effect on the percent change of heart BPM or if the caffeine solution yields a negative percent change and the nicotine solution yields a positive percent change.

Methods

In our experiment, we tested the percent change in heart BPM of *Daphnia* introduced to different concentrations of nicotine and caffeine. We performed this study in order to test the effects 0.5% of caffeine (CAFF) and 1% of caffeine versus 1 mM of nicotine (NIC) and 10 mM of nicotine had on BPM of Daphnia. Caffeine has an agonistic effect on the rate of action potentials which can cause an increase in heart rate while nicotine has an antagonistic effect on the rate of action potentials which can cause a decrease in heart rate. The percent change in heart beats per minute was calculated in order to account for the variability in size and heart rate between different *Daphnia*.

In our experiment we recorded two *Daphnia* per solution, 12 *Daphnia* in total, this allowed us to configure the average percent of change in heart rate between the two different *Daphnia*. We collected data by submerging the *Daphnia* in each concentration of nicotine (1mM and 10mM) and each concentration of caffeine (0.5% and 1%), using one drop of each concentration for every *Daphnia*, then video recorded the *Daphnia* in slow motion for 10 seconds after allowing each to be submerged in each solution for seven minutes. Finally we counted the number of heart beats of the *Daphnia* from the recording. We placed cotton balls into the water on the slides to stop the *Daphnia* from moving. Because we only recorded the *Daphnia* for 10 seconds, after counting the total number of heart beats, we multiplied the total by 6 so that we could calculate the total number of beats per minute of the *Daphnia*. Once each *Daphnia* was tested it was returned to the aquarium water.

The number of heart beats counted in those 10 seconds will be multiplied by six in order to find the heart BPM of the *Daphnia*. Change in heart beats per minute is calculated by subtracting BPM of the *Daphnia* after it is exposed to the experimental solution from its pre-solution BPM. Percent change is then found by dividing this change in BPM by the pre-solution BPM and multiplying the quotient by 100. We found the average percent change for each experimental solution. Using Excel, we graphed these averages using a split bar graph, one bar for each solution group. A two-way ANOVA test was taken using the PAST3 software to compare the significance between the two different experimental solutions and their varying concentrations.

Results

The *Daphnia* in the nicotine solution of the lowest concentration (1 mM NIC) had the lowest percent change in heart beats per minute, with an average of around 5.71% (Fig 1).The *Daphnia* in the solution of 1% CAFF had the second lowest average percent change in heart BPM, at around 8.90% (Fig 1). The *Daphnia* in the solution of 0.5 % CAFF had a slightly higher average percent change in heart BPM, at around 9.09% (Fig 1). Finally, the *Daphnia* in the nicotine solution of the highest concentration (10 mM

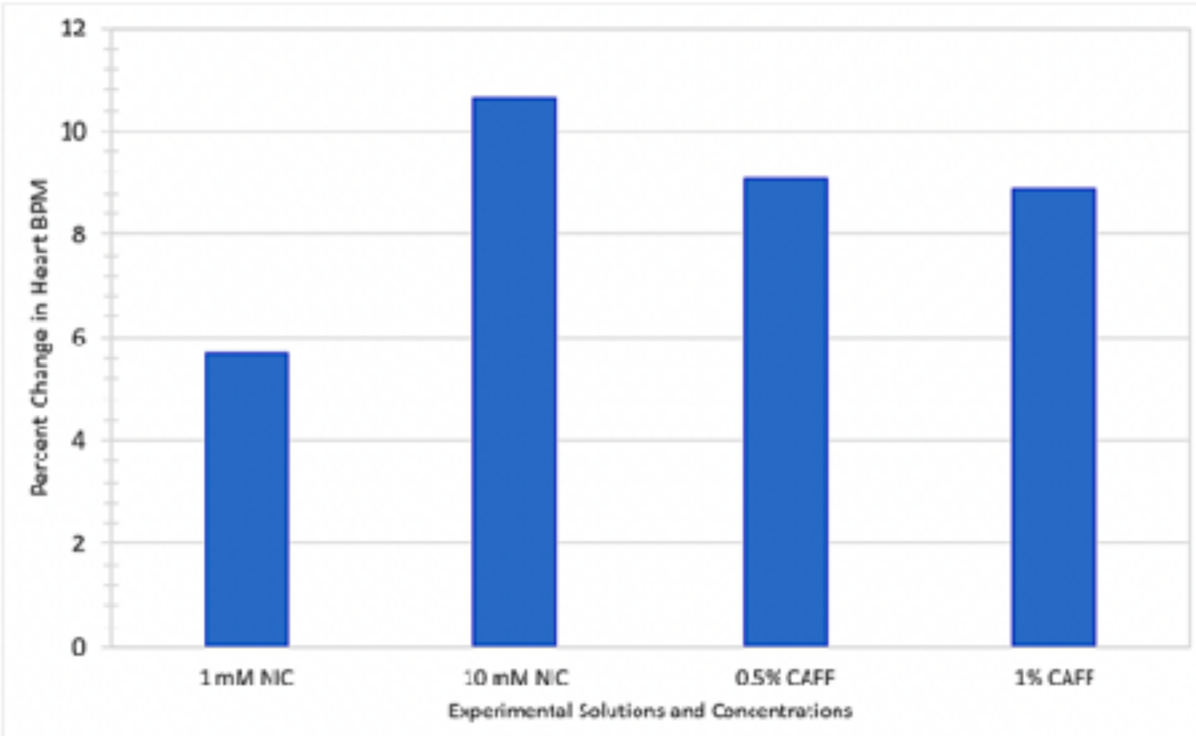

Figure 1 — A comparative graph of average percent change in heart BPM of Daphnia magnus introduced to different chemical solutions of varying solutions (0.5% CAFF, 1% CAFF, 1 mM NIC, and 10 mM of NIC). The solution of 1mM Nicotine had the lowest percent change in heart beats per minute (at around 5.71%) while the 10mM solution of Nicotine had the highest (about 10.7%). The 1% concentration of CAFF had a lower average of percent change (around 8.90%) than the lower concentration of CAFF (0.5 %) which was a 9.09% change.

NIC) had the highest average percent change in heart BPM, at around 10.7% (Fig 1). It is important to note that while two of the *Daphnia* of the 10 mM NIC solution had percent changes of 6.90% and 5.08%, the third *Daphnia* had a percent change of 20.0%, which makes it an outlier. The average percent change in heart BPM of the *Daphnia* for each solution did not correspond with our expected values. The percent change in heart BPM was positive for all tested solutions (Fig 1). Increasing the concentration of nicotine had a positive correlation with percent change in heart rate (Fig 1). Increasing the concentration of caffeine had almost no effect on percent change in heart beats per minute. A Two-way ANOVA was conducted on the influence of type and concentration of the chemical solutions on the percent change of heart rate of the *Daphnia magnus* using solutions of 1 mM NIC, 10 mM NIC, 0.5% CAFF, and 1% CAFF. There was not a significant effect of solution type (caffeine or nicotine) on percent change of heart rate; [F (3, 11) = 0.70; p = 0.59]. There was not a significant effect of solution concentration on percent change of heart rate; [F (2, 11) = 4.93; p = 0.054].

Discussion

Our hypothesis that increasing nicotine concentration added to the *D. magna*'s environment will cause the percent of change in heart BPM to decrease, and increasing caffeine concentration will cause the *D. magna*'s percent change in BPM to increase was rejected. The percent change in heart BPM for both nicotine and caffeine were positive, showing that both solutions increased heart rate of the *Daphnia*. In addition, we discovered that increasing the concentration of nicotine increased the percent change in *Daphnia* heart BPM. We found that increasing the concentration of caffeine decreased the percent change of heart BPM in *Daphnia* by 0.1%, concluding that changing concentration of caffeine doesn't have a strong effect on *Daphnia* heart rate.

Our hypothesis that nicotine would decrease heart rate in the *Daphnia magnus* was based on a study by Villegas-Navarro, et al. (2003). During the conduction of this experiment, further research revealed that nicotine is known to increase heart rate in humans. One potential alternative interpretation for the difference in data between their study and ours is that *Daphnia* do not possess an automatic nervous system. Since nicotine is known to affect the synapses of sympathetic neurons, nicotine may have varying and unreliable effects on the *Daphnia*.

Studies have also revealed mixed results regarding the effect of caffeine on heart rate. Foster (1997) confirmed that caffeine increases heart rate of a *Daphnia* and that heart rate decreases proportionately to a decrease in caffeine concentration. However, Corotto et al. (2010) stated that caffeine is not supposed to increase the heart rate of a *D. magnus*, and any increase in heart rate would reveal a bias in the lab study. One major outlier regarded one *Daphnia* of 10mM NIC, the percent change in BPM was 20.0%. This greatly skewed the average of the 10 mM NIC solution because the normal percent change was between 5-7% and this percent change was 20% causing an increase in the overall average. Other discrepancies could be there were two different generations of *Daphnia*, the first generation seemed to have beginning quicker heart rates and the second generation had beginning slower heart rates. However, the increase and decrease of the heart rates were consistent. The size of the *Daphnia* could affect the *Daphnia* heart rates as well, the smaller the *Daphnia* the quicker the heart rate compared to a larger size *Daphnia.*

From this study, future investigations can be conducted on the effect of nicotine and caffeine on the heart rate of humans. Since *Daphnia* have similar heart muscle cell receptors as that of vertebrates, it is possible that nicotine and caffeine could increase the heart rate of humans as well. To test this, willing human participants could have their heart BPM recorded both before and after consuming nicotine and caffeine. This information could be very useful to the FDA in their study on e-cigarettes. Conclusions made from these experiments would be of most interest to young adults, who are more likely to smoke vapes and consume high doses of caffeine.

Literature Cited

Corotto, F., Ceballos, D., Lee, A. & Vinson, L. (2010) Making the Most of the *Daphnia* Heart Rate Lab: Optimizing the Use of Ethanol, Nicotine & Caffeine. The American Biology Teacher. 72(3):176-179

Daniels, J. W., Mole, P. A., Shaffrath, J. D. & Stebbens, C. L. (1998). Effects of caffeine on blood pressure, heart rate, and forearm blood flow during dynamic leg exercise. Journal of applied physiology. 85(1): 154-9

Haass, M. & Kubler, W. (1997). Nicotine and Sympathetic Neurotransmission. Cardiovascular Drugs and Therapy. 10(6): 657–665

Hoefnagels, M. (2018). Biology Concepts and Investigations, Fourth Edition. McGraw-Hill Education, New York.

Kaas, B., Krishnarao, K., Marion, E., Stuckey, L. & Kohn, R. (2009). Effects of melatonin and ethanol on the heart rate of *Daphnia magna*. Impulse: The Premier Journal for Undergraduate Publications in the Neurosciences. 1-8

Knights, A., Hockenberry, J., Teplansky, R. & Hartman, T. J. (2014). Beverage caffeine intakes in the U.S. Food and Chemical Toxicology. 63: 136-142

Nedelman, M. (2018). Teen Vaping up 80%; FDA Proposes New Rules. www.cnn.com/2018/11/15/health/fda-vaping-ecigarette-regulation/index.html. (accessed 3/13/19).

Rachel Foster (1997) A stroboscopic method to investigate the effect of caffeine on Daphnia heart rate. Journal of Biological Education. 31(4): 253-255

Shaw, T. & French, D. (2018). Authentic Research in Introductory Biology, 2018 Ed. Fountainhead, Fort Worth.

Villegas-Navarro, A., Rosas-L, E. & Reyes, J. L. (2003). The heart of Daphnia magna: effects of four cardioactive drugs. Comparative Biochemistry and Physiology Part C. 136: 127–134