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The Effect of Caffeine and Ethanol on the BPM of Daphnia magna

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Daphnia magna are known to have hearts that are comparable to human hearts. The Daphnia are used to test the effect of different substances on their heart rate to see a similar effect that would be in the human heart. We hypothesized that the addition of caffeine to the Daphnia would cause an increase in their beats per minute, while the addition of ethanol would cause a decrease in the beats per minute of the Daphnia. The experiment consisted of five trials for each solution. Our data suggests that caffeine caused a significant increase in their heart rate ($p=0.005024$), and that ethanol caused a significant decrease in the heart rate of the Daphnia ($p=0.028729$). The results can be used to determine the effect ethanol and caffeine would have on humans.

Introduction

The body is a complex combination of systems which seek to maintain homeostasis. A vital part of this is communication via neurons. Motor and sensory neurons convey information to and from the brain by the use of action potentials. When a stimulus causes a graded potential to reach threshold, an action potential is fired down the axon. (Sanata et al., 2010) One of the key components of a successful action potential is the release of neurotransmitters. Neurotransmitters release into the synaptic cleft from the synapse and travel to the next neuron along the chain. Neurotransmitters bind to ligand gated channels allowing sodium to pass from one neuron to another. (Süudhof, 2008)

Caffeine and ethanol (among other drugs) influence these neurotransmitters, which in turn will affect the action potentials and thus signals received and sent by the nervous system. This will directly affect many key functions in the body including the heart rate. Ethanol acts as a repressor to heart rate. By manipulating the baroreflex sensitivity, which controls the heart rate, ethanol can depress the heart rate. It becomes more impaired as more ethanol is consumed (Abdel-Rahman, et al., 1987). It is shown to hinder calcium dependent neurotransmitter release, so it will also hinder excitatory and inhibitory postsynaptic potentials. This inhibition causes the suppression of functions like production of adrenaline (Amanda, et al., 2009). Adrenaline is part of the sympathetic nervous system. An adrenergic stimulation increases permeability of ion channels and levels of calcium, which regulate and increasing heart rates. (Ju, et al., 1999) Contrary to ethanol, Caffeine increases the sympathetic activity. Increasing the levels of caffeine can cause tachycardia (Starr et al., 1937), a substantial rise in plasma epinephrine (Izzo et al., 1983) and an increase in plasma renin activity (Robertson et al., 1978). By influencing the adrenergic stimulation, permeability of ion channels, giving rise to

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epinephrine, caffeine manipulates the autonomic nervous system (Hibino et al., 1997).

A daphnia heart is myogenic, meaning its heart rhythm is generated by a specialized muscle in the heart. As a daphnia heart is similar to a human heart, we predicted their hearts will react in a similar way to humans. When ethanol is introduced, the heart rate will decrease and Dwhen caffeine is introduced heart rate will increase. We tested a daphnia because it has a transparent body that allows us to see the heart with a microscope and observe its heart beat. We hypothesized that introducing ethanol will decrease the heart rate because this is a depressant which will lower their adrenaline concentration. Alternatively, introducing caffeine will increase the heart rate because it is a stimulant which changes the levels of the neurotransmitter Epinephrine (adrenaline).

Methods

In this experiment, we tested how ethanol and caffeine would affect heart rate. By recording a baseline heart rate (pre-exposure) of each daphnia and then introducing concentrations of ethanol and caffeine we saw how they each affect the heart rate (post-exposure). We used 5% ethanol and 1% caffeine concentrations consistently in each trial. We did this in order to eliminate some of random variability.

We tested five different daphnia for both ethanol and caffeine trials. Each daphnia was placed in water and their heart rate was recorded to establish a pre-exposure baseline relative to that specific daphnia because of the variability between individual's heart rates. After recording the baseline either ethanol or caffeine was added (approximately 5 drops) to the slide containing the daphnia. It was then exposed to the substance for 7 minutes to allow the solution to have an effect on the daphnia's heart rate. Its heart rate was recorded after the exposure. The data for each set of five daphnia were recorded so the results could be compared in the results section.

The goal of the experiment was to observe how both ethanol and caffeine affect heart rate. By recording baseline heart rates of daphnia and exposing them to each substance, we could monitor the actual effects of the substances on their heart. We exposed the daphnia to ethanol and caffeine on microscope slides. A microscope was used to magnify the daphnia so the heart could be seen. A phone was attached and set to video to record the daphnia's heart beat. (French et al., 2018) This allowed us to record the daphnia for ten seconds. Then this value was multiplied by six to determine the heart rate in beats/min.

A paired T-test was used to compare the pre-exposure and post-exposure heart rates for each solution for a total of two tests, and to test the significance of the data. (Hammer & Harper, 2013) The paired T-test was used because the same daphnia were used before and after exposure to each substance. The percentage change was calculated from the data obtained for each trial. The percent change formula used was (post-exposure BPM – pre-exposure (control) BPM) / pre-exposure BPM) x 100.

Results

The addition of ethanol decreased the heart beats per minute of the Daphnia in each trial. Ethanol also made the Daphnia move more slowly in general. The addition of caffeine increased the heart beats per minute of the Daphnia in each trial. When the caffeine was added, their speed and movement remained the same.

A paired T-test was conducted to compare the effect of 5%ethanol on the heart rate of Daphnia. There was a significant decrease between the pre-exposure and post-exposure heart rates; $t(4)= 3.344$, $p=0.02$. A paired T-test was also conducted to compare the effect of 1% caffeine on the heart rate of the Daphnia. There was a significant increase between the pre-exposure and post-exposure heart rates; $t(4)=-5.5902$, $p=0.005024$.

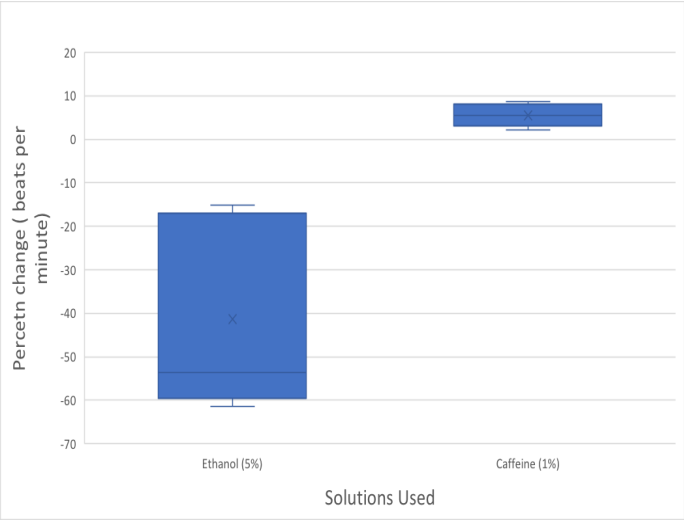

Figure 1: Demonstrates the percent change (BPM) of ethanol and caffeine. Overall ethanol decreased heart rate (with significant spread in data) while caffeine increased heart rate.

Discussion

Our hypothesis was confirmed by the data and trends we found in the results section. We were attempting to determine how ethanol and caffeine affect heart rate. Specifically, we hypothesized that ethanol would slow the heart rate and caffeine would increase the heart rate of the daphnia. Figure 1 displayed the collected values. It can be seen that ethanol had an inversely proportional relationship with the heart rate. The statistical T tests found a .02 p value which was significant. Conversely caffeine had a proportional relationship with the heart rate. When introduced to caffeine, the heart rates increased and the percent change was related to each daphnia. The statistical T test found a .005024 p value which is significant.

While each daphnia we tested had an expected change in their heart rate, there were several that experienced a larger or smaller percent change. This could be due to biological differences between the daphnia, different tolerances, and pregnancy. Additionally, the periods of exposure for each daphnia were not uniform. The goal was to monitor the daphnia after 7 minutes of exposure, but several daphnia were exposed much longer as it took us several attempts to determine their heart rates (often approximately 1 minute extra). The first daphnia in the ethanol trials had to be exposed to ethanol as it had to be removed from the initial solution.

It was possible that the change in heart rate could be due to the excitement or harm due to rough handling. When observing the daphnia in ethanol, their movement decreased. It could be that the daphnia stopped moving due to less water and restricted space which in turn lowered the heart rate. With such a small change in heart rate due to the introduction of caffeine, it could be assumed these values are just simple natural changes. Another cause for the increased heart rate could have been stress due to our handling of the daphnia.

These results correspond to the results and implications conducted by previous experiments and reports. As found in the study of Effect of Acute Ethanol Administration on the Baroreceptor Reflex Control of Heart Rate in Normotensive Human Volunteer, ethanol depresses the heart rate. It is assumed based on the similar results we found, that ethanol caused a decreased heart rate because it suppressed the sensitivity of the baroreceptor reflex (Abdel-Rahman et al., 1987). This accounts for the bradycardia found in both the daphnia and the humans. We found ethanol to be a central nervous system depressant (Uzbay, 2007).

Caffeine was found to increase heart rate in our experiment. It caused the sympathetic nervous system to produce more activity. Other studies have found that caffeine ingestion increases systolic blood pressure and arterial stiffness. (Sondermeijer, et al., 2002) It increases the heart rate by causing an increase in the production and release of the neurotransmitter Dopamine (Acquas, 2002). It also binds to adenosine ligand channel receptors and inhibits methylxanthines (Boulenger et al,. 1987). Adrenaline specifically activates beta-1 adrenoceptor in the sinoatrial node in the heart. This activation increases cAMP in that node which causes an increase in heart rate (British Pharmacological Society, 2012). Caffeine is a central nervous system stimulant as it activates noradrenaline neurons and affects the local release of dopamine (Nehlig et al,. 1992).

As a Daphnia has an anatomically comparable heart to a human, it can be tested and assumed that its response will be mirrored if a human was exposed to the same conditions. This has in fact proved to be the case. In the future, it could be possible to use daphnia to study and test effects of drugs and stimulus on the heart. Based on

their similarity with the human anatomy (with regards to the heart) it is likely scientists can infer how a human would react to the same drug or stimulus. However, despite some similarities, daphnia are not the most comparable anatomical species to humans. For instance, rats share more anatomical similarities to humans (vertebrae). Testing animals with more anatomical similarity than daphnia will likely bear stronger resemblance to the human response.

Caffeine and ethanol are fairly abundant substances. In the chance that they spill into and contaminate a water supply, it would be beneficial to understand how daphnia would be affected. Daphnia reside at the base of the food chain and are an integral part of their ecosystem. If something effected daphnia, it could have significant impacts on the ecosystem.

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