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Blame it on the Alcohol: An Investigation on Increasing Ethanol Concentrations Lowering *Daphnia magna* Heart Rate

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Action potential has a direct relationship with heart rate for organisms. Exposure to stimulants and depressants will impact rates of action potentials, resulting in varying effects on heart rate. In this lab experiment, the effects of ethanol will be observed for *Daphnia magna,* which will be exposed to various concentrations of ethanol. The data collected in the experiment suggested that exposure to heightened concentrations of ethanol resulted in lower heart rate. Other research has suggested that ethanol is a depressant for organisms with myogenic cardiovascular systems. These findings can be applied in the healthcare world relating to the development of pharmaceuticals.

Introduction

Pharmacology is essential in the healthcare industry. The effects that various drugs have on heart rate is extremely significant as it helps determine the side effects of drugs on organisms (Arturo et al., 2003). By understanding the effects that different drugs such as stimulants and depressants have on organisms, we are able to make strides in the world of pharmacology. All of the factors that will be investigated in this report play a key role in the development of effective drugs.

Organisms respond to stimulants and depressants very differently, various factors contribute to this. Research suggests that ethanol behaves as a depressant for organisms with myogenic cardiovascular systems (Kohn et al., 2009). A study conducted by Ursinus College showcases a relationship between ethanol exposure and decreased heart rate for *Daphnia magna*; as well as an increased heart rate with exposure to stimulants found in energy drinks (Kohn et al., 2009). This suggests that alcohol is a depressant for myogenic organisms. It also suggests that depressants decrease heart rate for myogenic organisms while stimulants increase heart rate.

Heart rate directly correlates to Action potential. Action potential is the change of electric potential in neurons that allows for various functions organisms need to survive (Hodgkin and Huxley, 1939). For neurogenic organisms, one nerve fire results in one heart beat while for myogenic organisms, nerve fires result in a decreased heart beat (Brook and Lange, 1977). We will be observing *Daphnia magna*: myogenic

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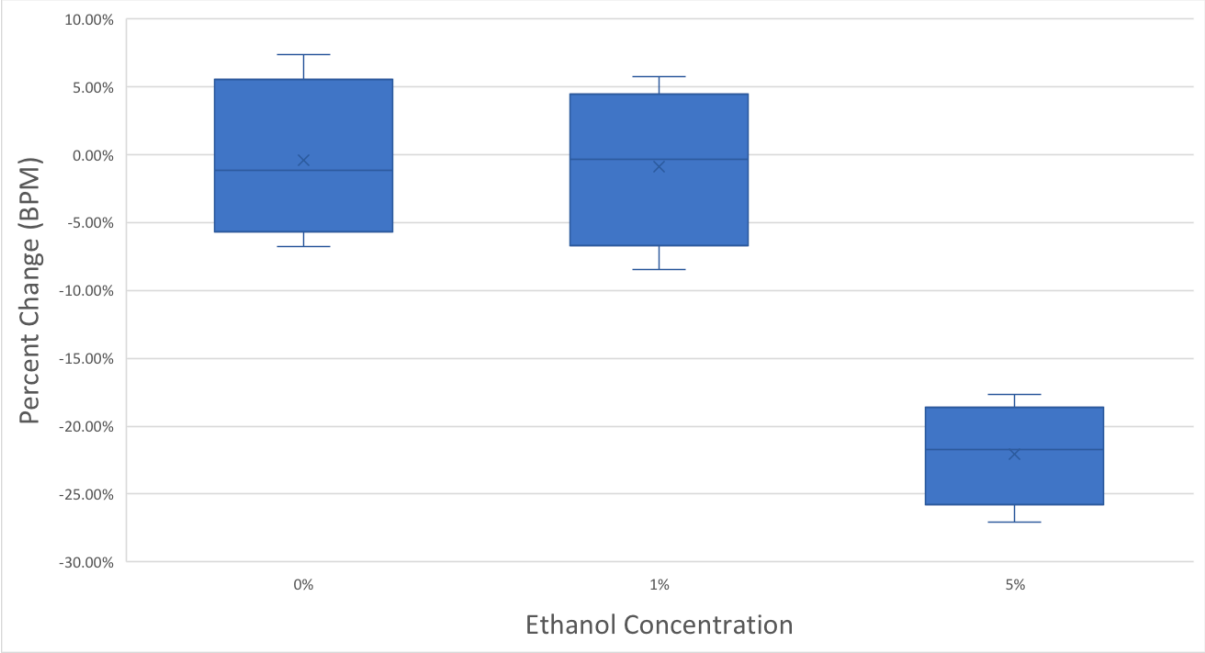

Figure 1.1 The figure shows the average rate of change (BPM) when *Daphnia magna* are exposed to solutions of 0% ethanol (average change of -0.43%), 1% ethanol (average change of -0.99%), and 5% ethanol (average change of -22.06%).

organisms. *Daphnia magna* and humans are both myogenic organisms this allows us to make predictions for *Daphnia magna* through how humans react and vise versa (Jofesson et al., 1966) The inverse relationship between neural firing and heart rate allows us to observe one factor and obtain information about the other. While we will count heart rate of the *Daphnia magna* we will also be able to infer changes in action potential with changing heart rate.

There are other factors that account for the effects of depressants on organisms. GABA (Gamma-aminobutyric acid) is the main inhibitory transmitter for humans (Evert et al., 2015). This neurotransmitter is responsible for the effect that depressants such as alcohol have on myogenic organisms. Since humans and *Daphnia magna* both have myogenic cardiovascular systems we can assume that GABA will play a role in the impact ethanol has on daphnia (Jofesson et al., 1966) .

In order to further examine the relationship between depressants and heart rate laboratory experiments can be conducted. With our background information we hypothesize that higher concentrations of ethanol will lead to lower heart beats per minute since ethanol binds to GABA receptors inhibiting neurological response. If our hypothesis is supported exposure to higher concentrations of ethanol will result in lower heart rates for *Daphnia magna*. If our hypothesis is unsupported, exposure to ethanol will not impact the heart rate of *Daphnia magna*.

Methods

In this investigation, we utilized ethanol solutions at various concentrations and the base, aquarium water, in order to measure the effect that the solutions had on the number of heartbeats produced by *Daphnia magna* per minute. The number of heartbeats produced per minute can be used to determine the effect of ethanol on the nervous system.

To test the hypothesis, the *Daphnia magna* were taken out of the aquarium water and their initial heart rate was recorded for fifteen seconds using a microscope and a smartphone camera adapter. Following the initial recording, the *Daphnia magna* were submerged in various

concentrations of ethanol for 10 minutes. After letting the *Daphnia magna* sit in the solutions, the heart rate was measured again. The group of *Daphnia magna* that was submerged just using aquarium water (0% ethanol) was used to ensure that heart rate is actually affected by the ethanol and not just the time in between recordings. Along with the *Daphnia magna* submerged in 0% ethanol, we placed the *Daphnia magna* in solutions of 1% and 5% concentrations of ethanol. The heart rate of the *Daphnia magna* after exposure to the various concentrations of ethanol was then recorded for fifteen seconds. We used a hand counter to observe the number of heart beats in fifteen seconds, which then were multiplied by four to find their heart rate per minute. This was performed for four trials for solutions of a 0%, 1%, and 5% ethanol concentration, resulting in three total experiments. The data collected from these trials was used to calculate the average change in heart rate of the *Daphnia magna.* To figure the statistical analysis, we conducted a One-Way ANOVA and determined that the effect of ethanol on the heart rate was significant. We then used Tukey's pairwise test, which revealed the depth of difference in heart rate correlated to the concentration of ethanol.

Results

The data we collected showed the effects of ethanol on *Daphnia magna* heart rate. These results were displayed in the box and whisker plot (Figure 1.1). The solution 0% ethanol showed an average decrease of -0.43%, 1% ethanol solutions resulted in an average decrease of -0.99%, and 5% ethanol solutions displayed an average decrease of -22.06%. A One-Way ANOVA was conducted to compare the effect of concentration of ethanol solution on heart rate of the daphnia in 0%, 1%, and 5% concentrations. There was a significant effect of concentration of ethanol solution on heart rate between the 0%, 1% and 5% ethanol concentrations; [$F(2,9) = 21.74$; $p = .0003584$]. A Tukey's pairwise test revealed heart rate was statistically lower in the 5% concentration of ethanol than in 0% [.0007098] and 1% [.0008141] ethanol concentration.

Discussion

As hypothesised, higher concentrations of ethanol resulted in a lowered heart rate (Figure 1.1) . Our data suggests, as predicted, ethanol behaves as a depressant for *Daphnia magna*. Our results clearly show that as the concentration of ethanol increased, the heart rate for the *Daphnia magna* decreased.

There were two instances where the heart rate of the *Daphnia magna* increased in the presence of 1% ethanol. This didn't follow the trend we were expecting. However, these results can be justified. For certain drugs, concentrations can have huge impacts on whether it acts as a stimulant or depressant. Ethanol is one of these drugs. The majority of humans feel stimulating effects with low doses of alcohol but depressant effects with high doses (New Bridge, 2018). Since both humans and *Daphnia magna* have myogenic cardiovascular systems, the *Daphnia magna* can be used as a suitable substitute for a human cardiovascular system (Kohn et al., 2009). This helps us make sense of the two *Daphnia magna* that experienced increased heart rate in the presence of 1% ethanol.

In further experiments, more trails should be conducted. With more trails we would be able to support our findings even further. To improve upon the accuracy of the data, a computer program with a faster ability to count the heart rate of a *Daphnia magna* would be used. Human counting can be too slow to accurately record all heart beats. Double counting and hand counter malfunctions could have occurred which would have caused errors in our data.

Another possible experiment that would provide valuable information is testing other drugs such as nicotine and caffeine. Conducting trails with other drugs with allow us to categorize them as stimulants and/or depressants. Continuing to research in this field will allow us to predict the side effects of various drugs efficiently before testing them on human subjects.

Other studies have been conducted that support our findings. Ursinus College conducted an experiment in which *Daphnia magna* where exposed to energy drinks and ethanol, it was found that energy drinks were stimulants and alcohol was a depressant (Kohn et al., 2009). Similar to our findings, researchers at Ursinus College found that increasing concentrations of alcohol lead to

decreased beats per minute for the *Daphnia magna*. The support from other reliable sources further validates our findings.

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