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0.2% Ethanol and 0.2% cannabidiol has no significant effect on the heart rate of *Daphnia magna*

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Abstract

Daphnia magna, a crustacean with a myogenic heart, has a heart that is easily visible and manipulated in order to test the effects that different solutions have on heart rate. In this study, we are studying the effect of CBD and ethanol on the heart rate of *Daphnia magna*. We relate a myogenic heart to action potentials in this study, which is not common when looking into the *Daphnia.* We proposed that the CBD solution would slow the heart rate the greatest amount. We conducted 5 trials for each solution and used a microscope and video recorder in order to accurately count heart rate in beats per minute. Although the *Daphnia* and human heart are different, our results may reflect useful information on how CBD and ethanol interferes with regular heart function.

Introduction

Both ethanol (beverage alcohol) and Cannabidiol (CBD) are substances that are commonly used when someone wants to relax. Consuming either of these substances causes slow response time of the body. The response time of the body is directly related

Studies have explored the effect that ethanol has on the heart rate of *Daphnia magna* but they have not studied the effect on action potentials (Leatherman et al., 2009). In addition, CBD is still being researched because it has recently grown in popularity

CBD is primarily used to relax the human body. Action potentials trigger the channels in the pre-synaptic neuron terminal (Ali et al., 2015). Without this process functioning properly, action potentials will cease or slow the transmission of signals to the brain. This results in the relaxation effect that is commonly associated with CBD.

Ethanol, is also used whenever someone wants to relax, however it is more closely associated with slurred speech, impaired vision, and even a lack of coordination. Like CBD, this is also caused by a direct relationship to action potentials. Alcohol increases the activity of gamma-Aminobutyric acid, which is the primary inhibitory neurotransmitter in the central nervous system (Siggins et al., 2005).

Action potentials and heart rate are intimately related in creatures with myogenic hearts. In an experiment conducted by Yamagishi (2003), the myogenic heart of the *Triops longicaudatus* had in increase in heart beat rate as well as action potential potential when exposed to dopamine, a known stimulant. We chose *Daphnia* not only because of the myogenic heart, but also because of the anatomy of the body. The body is transparent so it is possible to observe the heart beating when under a microscope. If the heart rate of the Daphnia is decreasing, than the number of action potentials will also be increasing.

We hypothesize that the heart rate of *Daphnia* exposed to CBD will be significantly lower than *Daphnia* exposed to alcohol because CBD will decrease the number of neurotransmitters by inhibiting significantly slower heart rate in the *Daphnia magna* than that of the CBD solution, or there is no significant difference.

Methods and Materials

In this experiment, we used an ethanol and cannabidiol solution in order to observe the changes that occurred in the heart rate of the *Daphnia magna.* One group of trials was observed without any additional substances in order to determine the normal heart rate of the *Daphnia*. One of the following groups was observed with 0.2% ethanol and the other group was observed with 0.2% CBD. Ethanol and CBD were our solutions of choice because they are both associated with slowed response times in the human body. Each group had five replicates in order to minimize the effect of outliers on the data.

We used a dropper with the tip cut off to effectively move the *Daphnia* from a beaker of aquarium water to a depression slide. A paper towel was used to remove excess water, and then strands of a cotton ball were placed over the *Daphnia* to hold it in place. For the control, we allowed the *Daphnia* 7 minutes on the depression slide before we started recording. For the trials with solutions, we also waited 7 minutes before we started recording to allow adequate time for the solution to affect the *Daphnia*. For CBD, 1 drop of the 0.2% solution was added and the heart rate was observed using a microscope and a phone come in contact with tap water as it can be harmful to them.

The *Daphnia magna* heart rate was calculated by multiplying the number of heart beats recorded over the 10 second span by 6 to get beats per minute for one trial. The beats per minute for the five trials of each of the 3 groups was used in order to create a box and whisker plot and compare the average beats per minute. The beats per minute were also used to run a one-way anova test because we have three groups with one variable that is normally distributed to determine if the differences in the averages were significant or not.

Results

The average for 0.2% ethanol was the highest at 555.2 beats per minute. CBD was the lowest at 522.4 beats per minute. The range for ethanol was also the highest at 168 beats per minute. The results of the control group were the most consistent with a range of 34 beats per minute. A One-Way ANOVA was conducted to compare the effect of different solutions on heart rate in *Daphnia Magna* exposed to 0.2% CBD, 0.2% ethanol, and standard conditions. There was not a significant effect of the solution on heart rate in beats per minute [F(2,12)=0.7174; p=0.5078]. A Shapiro Wilks test gave a p=0.7616 for control, 0.1215 for CBD, and 0.687 for ethanol.

Discussion

After conducting our research, we found that our hypothesis was not supported because there was no significant difference in the *Daphnia magna* heart rate between ethanol and cannabidiol. Our data showed that the effects of ethanol caused a higher maximum heart rate than the effects of cannabidiol; however, the data was found to be insignificant due to the close proximity of the overall averages between the affected heart rates caused by the two substances. These results could have occured due to the minimal amount of both substances that the *Daphnia* were exposed to. Another cause could be that the *Daphnia* reacted differently to the substances than humans typically would, due to them having different types of hearts and different sized hearts pertaining to their body size.

There are a few different possibilities for why the heart rate amongst the *Daphnia* could have varied for different trials of the same group. Like with any other creature, the age also affects the rate of heart rate of the *Daphnia*. *Daphnia* that are able to eat more food or higher quality food will also have a higher heart rate compared to a *Daphnia* eating less (Anderson and Jenkins, 1942).Another thing that could have affected the heart rate of *Daphnia* is how much they had been “excited” before we recorded the

Fig 1. The average heart rate in beats per minute for the control was 532.4, 522.4 for

heart rate. If a *Daphnia* had been scared and swam around rapidly, it's going to have a higher heart rate than if it was calm.

Although any animal could have been used, we chose the *Daphnia* for a specific reason. The heart rate of *Daphnia* is affected in a similar manner as the human heart is, so the effects on *Daphnia* heart rate is the same as the effect on human hearts (Gaikwad et al., 2012). The focus of future research could be directed towards why there is a difference in heart rates between *Daphnia magna* and humans. In this research, it could be discussed how the two hearts have similarities and differences and the implications of said differences. Implications of our study could provide background information for future studies that go further in depth about *Daphnia magna*'s heart rate.

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