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ETHANOL DECREASES HEART RATE OF DAPHNIA COMPARED TO WATER

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Various substances can have multiple effects on the number of action potentials taking place in a neuron, which can directly impact the heart rate of a myogenic organism. Does ethanol alcohol decrease or increase action potentials in a neuron? We tested this question using daphnia as our organism, making it unique because daphnia are a myogenic organism, just like humans. Throughout five trials, we tested these organisms and 5% ethanol combined with aquarium water, we found that ethanol alcohol caused the heart rate of the daphnia to decrease, in this case, due to a decrease in action potentials in the organism.

Introduction

The leading cause of death in the United States is heart disease. As scientists have begun developing synthetic cardiac muscle cells, they have run into a problem with getting the genetically engineered cells to beat at the same rate as the original heart cells. By experimenting with different substances, scientists hope to develop a drug which can suppress or increase heart rate as needed (Shaw & French, 2018). The heart beats because its' pacemaker cells increase the membrane voltage enough to generate rhythmic action potentials. This then generates contraction of the heart muscle, commonly known as a heartbeat (DiFrancesco, 2012). In a rat, ethanol was shown to decrease the number of heart beats per minute due to depressing action potentials in the membrane (Gimeno, 1962). This decrease in action potentials is due to the postsynaptic receptors being much less sensitive (Wallner, 2003). Curious as to whether ethanol has the same effect on all myogenic organisms, we tested the effect of ethanol on Daphnia heart rate. Although most arthropods have neurogenic hearts, or hearts controlled by their nervous system, daphnia are an exception to this because they are myogenic (Stein, 1966). This means that their hearts beat due to spontaneous heart muscle activity rather than in response to the pacemaker activity of the cardiac ganglion (Hill, 1992). Humans are also myogenic, which makes daphnia an exceptional candidate for testing the effects of drugs on the heart (Stein, 1966.) They are also small and relatively transparent, making the heart easily accessible for viewing without using dissection or other methods which would in turn harm the organism. Using information from these past journals, we have hypothesized that heart rate will decrease in Daphnia submerged in 5% ethanol compared to Daphnia submerged in their original aquarium water due to the fact that ethanol depresses action potentials in the membrane. We

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will know that this hypothesis is supported if there is a lower heart rate after daphnia submersion in ethanol compared to water. However, a higher heart rate after ethanol submersion would disprove our hypothesis.

Methods

To test the effect of ethanol on the heart rate of daphnia, we used a solution of ethanol and aquarium water and a solution of 100% aquarium water. We used the protocol for handling daphnia found on Canvas (Shaw & French, 2018). We recorded the daphnia under a microscope for 15 seconds then watched this video in slow motion while using a clicker to count the heart rate of the daphnia. By multiplying this number by four, we calculated the heart rate in beats per minute, or bpm. After this, we used a dropper to add one drop of 5% ethanol alcohol to the aquarium water solution that the daphnia was submerged in on the slide. The daphnia was submerged in the water/alcohol solution for seven minutes in order to give the alcohol time to affect the daphnia. After the seven minutes passed, the daphnia was again observed under the microscope and heart rate was calculated in the same way that it was before the alcohol was added. Heart rate was recorded before and after the ethanol was added in order to accurately measure the rate of change. We repeated this using five daphnia in order to increase the accuracy and size of our experimental group. As our control, we repeated this entire process but with one difference: rather than adding a drop of alcohol, a drop of aquarium water was added. This sham control was used to improve the accuracy of our data by showing whether the alcohol affected the heart rate or the heart rate was simply the time spent in a slide, which obviously causes some stress on the organism.

We now found the percentage rate of change between the first and second observed heart rates in each of the ten daphnia. These percentages were then averaged and placed onto a bar graph for ease of viewing. This was done using the software Microsoft Excel. Due to the fact that our data was both nominal and measurement, and that we had two unpaired groups, analysis is conducted by running an unpaired T-test on the percent change values.

Results

An unpaired t-Test was conducted to compare the effect of ethanol on daphnia heart rate. There was a significant difference between the two groups; with the degree of freedom is 4, p= .0167. Daphnia submerged in a solution of 5% ethanol showed a significant decrease in the average percent change in daphnia heart rate compared to the daphnia in the control group, which were submerged only in aquarium water. However, even though ethanol had a significant effect on heart rate, there was not a consistent percent change between trials. There were outliers above and below the average percent change in the groups submerged in 5% ethanol, trial 3 (-8.76%) and trail 4 (-47.25%). This means that although the percent change between ethanol and aquarium water showed an obvious difference, there is still significant difference between different daphnia in each trial because of independent heart rates of each daphnia. When the daphnia were in 5% ethanol, the rate of their heart change percent decreased, on average, by 21.70%, compared to when they were in water, as seen in Figure 1. When Daphnia was submerged in 5% ethanol, their heart rate decreased every time, while the percent heart rate of Daphnia in water was overall consistent (Figure 1). Although in some trials there was a slight decrease of heart rate and in other trials there was a slight increase, the change it heart rate had little to no change.

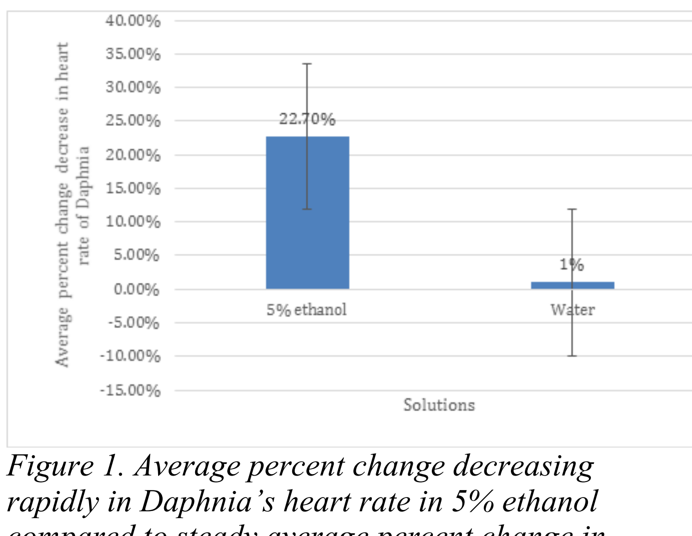

Figure 1. Average percent change decreasing rapidly in Daphnia's heart rate in 5% ethanol compared to steady average percent change in Daphnia's heart rate in aquarium water.

Discussion

As we hypothesized, the average heart rate of daphnia is significantly lower after 5% ethanol alcohol submergence, compared to submergence in aquarium water (as seen on figure 1). This correlation was consistent throughout all five daphnia in our experiment. Our control group, the daphnia not submerged in ethanol, had no significant heart rate increase before and after the seven minutes of sitting on the slide. The Fisher study supports our conclusion that alcohol consumption decreases action potentials in myogenic organisms (Fisher, 1975). Because humans and daphnia are both myogenic, it is a reasonable conclusion that alcohol decreases action potentials, and therefore heart rate, in humans.

As an alternative interpretation, one could argue that the decreased heart rate is more dependent on the lab setting and. stress that the organism was placed under rather than the alcohol. However, our control group goes to disprove this interpretation by showing no decrease in heart rate when no ethanol was used. Another alternative interpretation of our results could be that the vagus nerve regulated the lowering of the heart rate, rather than the alcohol consumption.

The reason for decreased action potentials in daphnia due to ethanol consumption is unknown, as it could be decreased acetylcholine release, lowered sensitivity of acetylcholine receptors, or a combination of both (Kaas, 2009). As a future experiment, the amount of acetylcholine released in the synapse could be measured in order to help determine the exact reason for the decrease in action potentials caused by ethanol alcohol. This could help to show how exactly what kind of heart problems could be combated with ethanol.

Literature Cited

DiFrancesco, D. & Camm, J. A. (2012). Heart Rate Lowering by Specific and Selective I f Current Inhibition with Ivabradine. A New Therapeutic Perspective in Cardiovascular Disease. 64(16): 1757-1765.

Fisher, V. J., & Kavaler, F. (1975). The action of ethanol upon the action potential and contraction of ventricular muscle. *Recent advances in studies on cardiac structure and metabolism*, *5*, 415-422.

Gimeno, A. L., Gimeno, M. F., & Webb, J. L. (1962). Effects of ethanol on cellular membrane potentials and contractility of isolated rat atrium. American Journal of Physiology. 203(1): 194-196.

Hill, R. B., et al. "Nervous regulation of the myogenic heart in early juveniles of the isopod c rustacean, Ligia exotica." *Phylogenetic Models in Functional Coupling of the CNS and the Cardiovascular System: 3rd International Congress of Comparative Physiology and Biochemistry, Satellite Symposium, Shimoda, August 31-September 2, 1991*. Vol. 11. Karger Medical and Scientific Publishers, 1992.

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*.

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

Stein, R. J., Richter, W. R., Zussman, R. A., & Brynjolfsson, G. (1966). Ultrastructural characterization of Daphnia heart muscle. *The Journal of cell biology*, *29*(1), 168.

Wallner, M., H. J. Hanchar, and R. W. Olsen. "Ethanol enhances α4β3δ and α6β3δ γ-aminobutyric acid type A receptors at low concentrations known to affect humans." *Proceedings of the National Academy of Sciences* 100.25 (2003): 15218-15223.