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THE EFFECT OF ETHANOL, NICOTINE, AND CAFFEINE SOLUTIONS ON CHANGE IN HEART RATE IN ORGANISM *DAPHNIA MAGNA*

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The rate of contraction in cardiac muscle cells relies heavily on the conditions of the environment that the muscle cell is being exposed to. Several studies analyze how stimulants and depressants alter the heart rate of animals, but these studies lack experimentation on hearts that have a similar physical structure to humans (Hille, 2001). Our study focuses on the crustacean, *Daphnia Magna*, as a model to determine the effects that ethanol, nicotine, and caffeine solutions have on an organism's heart rate, an indication of the firing of action potentials within cardiac muscle cells. We proposed that caffeine and nicotine will increase the heartbeat of the organism, while ethanol will decrease it because the caffeine and nicotine are stimulants that agitate the neuroreceptors causing the stimulation of action potentials, whereas the ethanol is an inhibitor that blocks the firing of neuroreceptors. We determined the heart rate of five different *Daphnia Magna* pre-exposure and post-exposure to a solution of either caffeine, nicotine, or ethanol, five times per solution, while the heart rate was recorded in intervals of six and the beats per minute were calculated to make comparisons between the effects. We expect that this study will be useful to scientist in the field of tissue engineering and regenerative medicine looking to replace damaged tissues of heart attack victims.

Introduction

Heart disease is the leading cause of death in the U.S (Rosamond, 2008), and scientists desire to create synthetic cardiac muscle tissues to replace damaged ones and prolong the life of those with this medical condition (Shaw & French, 2018). Studying the effect of certain conditions on the rate of contraction can provide explanations to heart abnormalities like arrhythmia; the improper beating of the heart. Cardiac action potentials are specific to the heart and how it performs contractions to beat; these action potentials are created through electrical impulses, stimulated for neurons to communicate between one another (Shih, 1994). This can be exacerbated by the addition or absence of certain solutions. By understanding which solutions affect action potential in these cardiac cells, along with the direction the action potential is sent, we can discover what substances are best to avoid if prone to heart disease or arrhythmia.

The design of the experiment was inspired by a study previously recorded which studied how varying drugs affected the heart rate of frogs (Hille,

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2001); analyzing this study motivated us to determine how substances could also have an effect on the hearts of humans. The heart of a frog contains chambers that lead to the same ventricle, whereas the heart of a human contains chambers that lead to separate ventricles, making their metabolisms more efficient (Kimball, 2010). These anatomical differences affect the respiration of the organism, which is positively correlated to the rate that the heart beats. *Daphnia Magna* was chosen for this experiment as an organism with a heart more similar to that of a human; their self-regulating properties (Shaw & French, 2018), as well as their myogenic heart tissues (Stein, 1966), corresponding to that of a human. These factors, along with a transparent exoskeleton that make their hearts visible to the human eye using a microscope make this specific organism a considerable specimen and complementary to studies. Our experiment will study how caffeine, nicotine, and ethanol affect the cardiovascular muscles of *Daphnia Magna,* while Hille's study analyzes the correlation between tetrodotoxin, saxitoxin, tetraethylammonium and their effects on the cardiovascular muscles of frogs (Hille 2001). Although Hille's study provided a positive relationship between the listed chemical compounds and heart rate, conducting further research on the topic would be beneficial in order to expand the understanding of how different additives (ie: caffeine, nicotine, and ethanol) affect the heart rate of an organism with a heart more similar to that of humans.

This design tested how different substances affected action potential between different neurotransmitters, which did, in turn, affect the heart rate of organisms. The design's goal was to understand how these substances can affect the rate of the heart following a stimulating or depressing effect. This provided knowledge as to what these substances are doing to the human body and could possibly explain how to prevent problems such as heart disease or arrhythmias. The initial information deriving from the lab manual, along with additional citations gathered through a series of scientific journals created the platform for our objective; we wanted to discover whether caffeine, nicotine, and ethanol release/ inhibit/ provide effects to the ion channel and action potential of a neuron, further affecting the heart rate. Nicotine and caffeine are stimulants that are known to activate the neurotransmission of dopamine and acetylcholine; these neurotransmitters excite the central nervous system, as well as bind to the receptors and inhibit the neurotransmission of adenosine which has sedative effects on the central nervous system (Vinader-Caerols, 2012). Ethanol has similar sedative effects as adenosine through the inhibition of dopamine and norepinephrine (Vinader-Caerols, 2012). Due to these effects, we believe the presence of nicotine and caffeine in water containing *Daphnia Magna* will result in an increased heart rate of the *Daphnia Magna*, while ethanol will do the opposite and decrease the heart rate of the *Daphnia Magna* because caffeine and nicotine will activate neurotransmitters that excite the central nervous system, while ethanol will inhibit these neurotransmitters.

Methods

To conduct this experiment, small crustaceans, *Daphnia Magna*, were used as model organisms. Five of these organisms were used in each treatment to ensure that their reactions were common since heart rates of organisms vary between individuals. We documented the depending factor of the experiment, the *Daphnia Magna's* heart rate, using the special instructions outlined in the procedural video (Shaw & French, 2018). The *Daphnia Magna* used in the study were contained in an aquarium of water from its original habitat. The first solution was used to determine the baseline heart rate of each organism, containing just the organism with aquarium water to stay alive; this served as a comparison group for the experimental groups. Since the additives caused an effect of change in the heart rate of the *Daphnia Magna*, the other three solutions were experimental and independent of other variables; containing the aquarium water and two drops of either 1% caffeine, 1% ethanol, or 1 mM nicotine.

To perform this experiment, a beaker was used to retrieve five *Daphnia Magna* from the aquarium, as well as a dropper with the tip cut off, used to obtain each single organism from the beaker. The tip was removed from the dropper to allow the organism to be easily acquired. The *Daphnia Magna* and water were then compressed out of the dropper onto the concavity of the

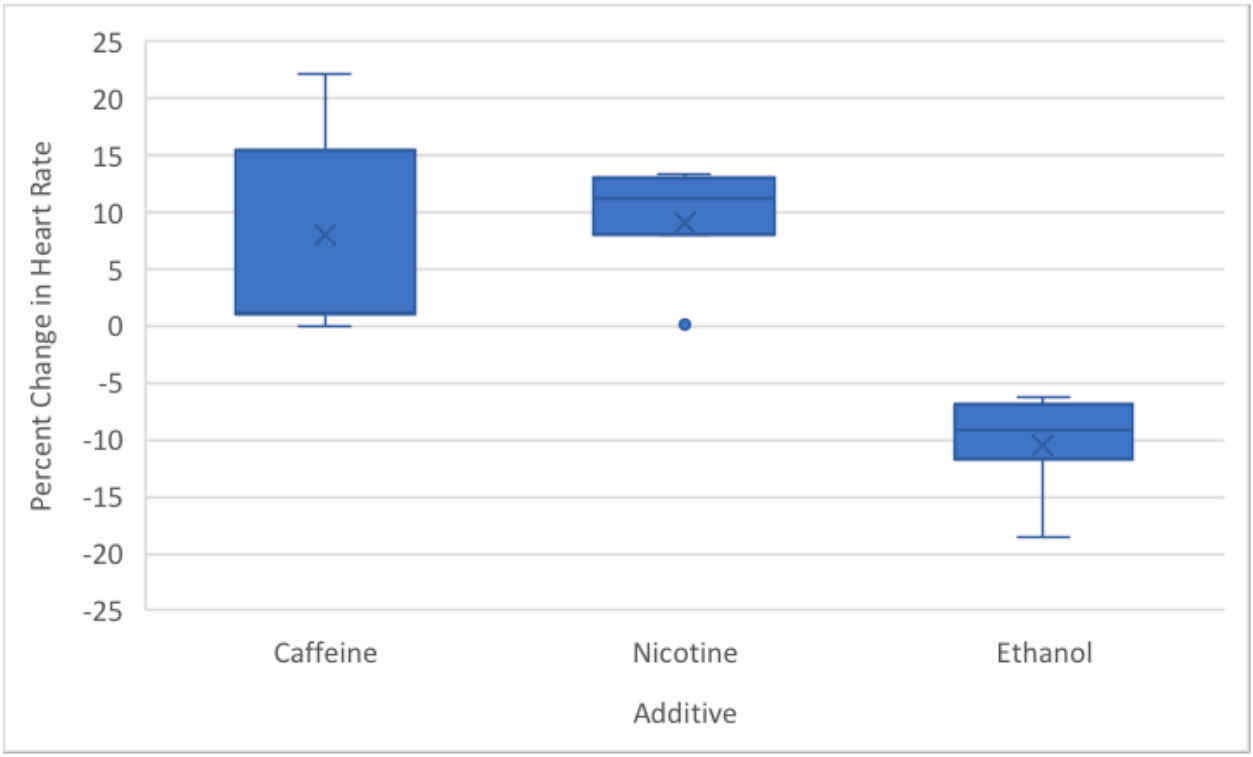

Figure 1. The box and whisker plot shows the range and median of percent change in heart rate due to the corresponding additives. As shown above, the caffeine and nicotine trials had similar positive results in the change of heart rate, while the ethanol was significantly different from the other two additives, as it caused a decrease in the organism's heart rate.

depression slide. A paper towel removed excess water from the depression slide to limit the movement of the organism while keeping it alive. Although we were able to see the *Daphnia Magna*, a microscope was used to identify the heart of the organism and accurately determine its heart rate. To calculate the heart rate of the *Daphnia Magna* effectively, a phone adapter was used in place of the eyepieces on the microscope. The knob on the side of the adapter was used to fasten the phone in place while the knob on the arm of the adapter was used to match the phone and adapter lens up and keep it in place.

The baseline heart rate of the organism was calculated using a handheld tracker which counted the heart rate in 20 second intervals; since the heart rate of *Daphnia Magna* is extremely fast, the heart rate was documented by clicking every six beats and then multiplying the number presented on the clicker by six after each trial. After conducting the baseline heart rate, two drops of either caffeine, nicotine, or ethanol were added to the water with the *Daphnia Magna;* we waited 7 minutes before calculations to allow exposure of the solution to the organism. Again, the heart rate of each organism is calculated in intervals of 6 for 20 seconds. This process was executed five times to justify similar results. Once every trial for each solution is conducted, these numbers are converted to beats per minute by multiplying the heart rates and intervals by 3 because they are each 20 seconds long. At the end of each trial, the *Daphnia Magna* were rinsed off of the slide into a second container, using aquarium water, as tap water would kill the organism. After the *Daphnia Magna* readjusted to their preexposed conditions, they were placed back into the original aquarium.

A Paired T-Test was used to express the data developed in the experiment and show significant differences between variables. To enhance the information provided in the paired T-test, a box and whisker plot was used to show the range and median of the data collected.

Results

Our raw data showed that the addition of caffeine and nicotine in the solution that the *Daphnia Magna* resides in, caused an increase in heart rate while the addition of ethanol caused a decrease in heart rate. There was one case where there was no percent change in rate with the addition of caffeine, resulting in an outlier in our data. The percent change with the addition of caffeine ranged from 0% to 22.2%, the percent change with the addition of nicotine ranged from 0.12% to 13.3%, and the percent change with the addition of ethanol ranged from -18.6% to -6.3% (Figure 1). However, our hypothesis was partially negated based on the statistics the caffeine produced. A paired t-test was conducted to compare the effect of the additive on the percent change in heart rate for each additive; caffeine, nicotine, and ethanol. There was not a significant difference between the caffeine and the percent change in heart rate; t=-1.7338, p=0.15798. There was a significant difference between nicotine and the percent change in heart rate; t=-3.6397, p=0.02197. There was also a significant different between ethanol and the percent change in heart rate; t=4.5364, p=0.010526.

Discussion

Our raw results show that the addition of caffeine, nicotine, and ethanol have an effect on the heart rate of *Daphnia Magna.* On average, caffeine and nicotine caused an increase in heart rate while ethanol caused a decrease in heart rate, therefore our hypothesis is supported by our raw data. Though the percent change varied in number and created a large range, it still followed our predicted trend on how it would affect heart rate; the stimulating solutions increased the heart rate of the organisms while the ethanol decreased them. Statistically the only substances that had significant differences were the nicotine and the ethanol, thus, our hypothesis was only partially supported.

The reason that the caffeine and nicotine may have caused the heart rate to increase could be the classification of the compound. Caffeine and nicotine are both stimulants which are categorized by their ability to excite the neural transmission of dopamine and acetylcholine; these neurotransmitters increase action potentials of motor control in our body (Calabresi, 1989). Caffeine and nicotine also inhibit the action potentials of the neurotransmitter adenosine, which affects the body similar to alcohol (Vinader-Caerols, 2012). When alcohol or ethanol is present, dopamine and norepinephrine are inhibited, these neurotransmitters play an essential role in thinking and attention and without the firing of these, motor function decreases (Hunt, 2006). These ideas are also apparent through the behavior of individuals who have used these substances. After use of nicotine and caffeine, the increased release of dopamine and acetylcholine are apparent through increased alertness and mood; in opposition, after use of alcohol, the inhibited release of dopamine and norepinephrine are expressed through the impairment of cognitive functions. Understanding what substances stimulate or depress the neurotransmision of action potentials and thus affect heart rate, can help scientists develop advancements in their approach to create synthetic cardiac tissues to aid individuals who have suffered from heart conditions.

Since *Daphnia Magna* are ectotherms, ambient temperature can have a major effect on the organism's heart rate because of its association with metabolism (Khan, 2008). Metabolism plays a huge role in the rate of chemical reactions happening in the body, therefore endotherms, like humans, are much better at regulating this. This can cause results that these substances have on the organism to vary from that of humans because warmer temperatures increase oxygen production and carbon dioxide consumption, therefore the rate of respiration is sped up and corresponds to an increase in heart rate (Bruning, 2013). The relationship suggests that when the ambient temperature is higher than the internal temperature of the organism, their metabolism speeds up and increases respiration, thus, increasing the heart rate; this relationship is reversed for cold ambient temperatures. For this reason, the temperature of the solution that the *Daphnia Magna* is contained in can be a confounding variable in this specific experiment and can cause variations in the results as *Daphnia Magna* lack the regulatory systems that humans possess. For future experiments, temperature should be measured and kept as a control variable when ectotherms are tested in order to rid of the metabolic

factor that affects heart rate as well. This would isolate the data to only the additives rather than other outside variables.

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