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The Effect of Caffeine on the Contractility of the Heart Muscle in Daphnia

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Many studies have shown that caffeine increases the contractility of the heart muscle in humans. Due to the fact that the human cardiac tissue functions similarly to that of Daphnia magna, we tested how caffeine affects the Daphnia heart rate and used the results to make assumptions about the human heart contractility in response to caffeine. We added various concentrations of caffeine to the environments of different Daphnia to determine how the caffeine levels affect the Daphnia's heart rate. We had two experimental groups and one control group and four trials were run for each of these groups. The findings were helpful in understanding the different factors affecting cardiac muscle contraction in humans; understanding these factors is useful in maintaining a more consistent heart rate in genetically engineered cardiac muscle cell prototypes.

Introduction

Scientists have encountered difficulty in maintaining a consistent rate of contraction in genetically engineered cardiac muscle cell prototypes. By understanding how caffeine affects heart rate, scientists will acquire a better understanding of how to make the prototype contractions more uniform. Our experiment was carried out on Daphnia magna. The Daphnia heart is similar to the human heart, and therefore the caffeine's effect on Daphnia cardiac contractions can also be applied to humans. Just like the human heart, the Daphnia heart is myogenic (Bekker and Krijgsman, 1951) , meaning that the contractions produced by the heart do not require stimulation from nerve cells (Encyclopedia 2004). Daphnia magna generally make a good study organism because their heart rate can be monitored and counted under different conditions, and their translucent bodies make it easy to do so. Therefore,the Daphnia hearts were used to answer the question of how caffeine affects heart rate. Caffeine is generally known to increase the level of adrenaline in an organism (Battram et al., 2005), which in turn increases the rate of cardiovascular function (Tuurala et al.,1982). Caffeine also increases the height of cardiac action potential in an organism (Bulbring and Whitteridge, 1941) by increasing the contraction strength and the duration of the action potential (Gubareff and Sleator, 1965). As this occurs, the heart rate also increases (di Bernardo et al., 2002). Cardiac action potential is a change in voltage across the cell membrane of the heart cells. This occurs within the ion channels when charged ions move in and out of the cell. The heart has a resting potential where the cell membrane of the cardiac muscle cell separates different concentrations of ions, such as sodium, potassium, and calcium. When an electrical impulse is generated by an electrical cell, the ions cross the

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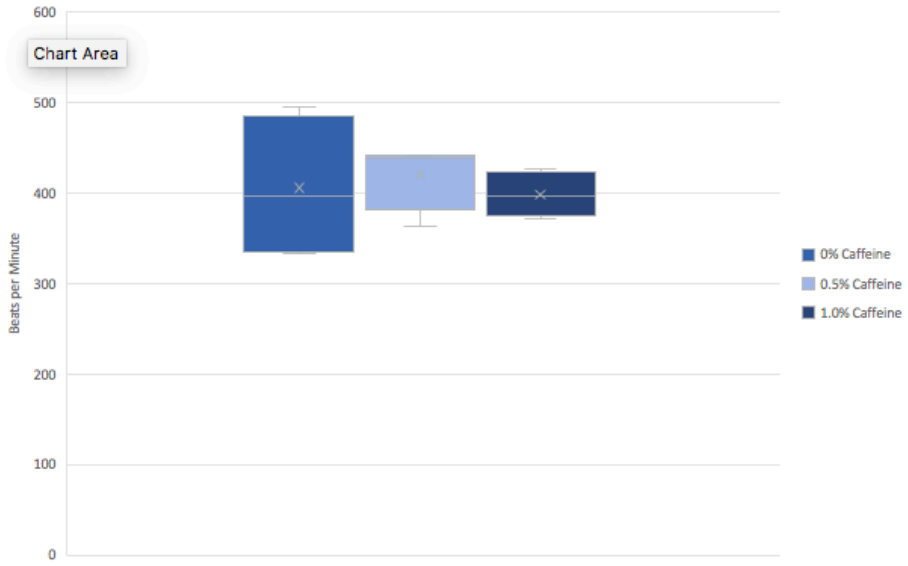

Figure 1.1. Shows the averages for the four Daphnia magna in each experimental group as well as the range of each group.

cell and cause the action potential. This movement of ions across the cell membrane through ion channels is the drive that causes contraction of the cardiac muscle (Ikonnikov and Yelle, 2009). When the ions return to their previous resting state, this is the relaxation of the heart, or resting potential. The ions also experience a plateau phase, where there is little electrical charge across the ion. This process of resting and action potentials is what causes contraction and relaxation of the cardiac muscle. However, caffeine increases the amplitude of action potential but reduces the duration of the plateau phase, meaning caffeine causes the action potentials to occur much quicker (Clark and Olson, 1973). With this idea in mind, we hypothesized that increasing caffeine concentrations in an artificial habitat of Daphnia will increase their heart rate because the caffeine will raise the level of adrenaline in their bodies, which increases contractility of the cardiac muscle. Our hypothesis is supported if increased caffeine concentrations cause a significant increase in the Daphnia's number of heart beats per minute. However, our hypothesis is rejected if the heart beats decrease with increased caffeine concentrations, or if the caffeine has no substantial effect on the contractility of the heart.

Methods

This experiment was designed to measure the heart rate of Daphnia magna in beats per minute. The manipulated variable was the concentration of caffeine in the Daphnia's environment. By determining the contractility of the cardiac muscle in these different environments, we were able to establish a relationship between caffeine and heart rate. To ensure that differences in contractility could be attributed solely to the caffeine, we also measured the heart rate of Daphnia in an unaltered environment where no caffeine was added at all. We handled the Daphnia using the protocol in Shaw French (2018). For the first experimental group, one Daphnia was placed on a microscope slide and three drops of 0.5% caffeine were added to the environment. The daphnia set in the caffeine-water solution for ten minutes to ensure the caffeine had time to have an effect on the daphnia. The Daphnia's heart was then observed under a microscope. The heart beats per minute were recorded with an iPhone in slow motion for twenty seconds. The video was used to accurately measure the amount beats per minute the Daphnia's heart contracted. The beats were counted with a hand-held counter. To calculate the bpm, the number of heartbeats in the twenty second period were

multiplied by three. Upon finishing, the Daphnia was removed from the slide and placed back into the beaker. Three more trials were run with the same process taking place, but a new Daphnia magna was being used for each trial. For the second experimental group, the same process was repeated as for the first group; however, three drops of 1.0% caffeine were added this time to test a different caffeine concentration. Four trials were run for the second experimental group. The control group underwent the exact same procedures as the two experimental groups; however, caffeine was not added to the slides for the control group, because Daphnia heart rate was being tested in a natural environment. Four trials were run for the control group. Our data consisted of Daphnia heartbeats per minute. We then measured the average heart rate using this data. The results were summarized by displaying the data in a bar graph with error bars to see the variation of beats per minute amongst the Daphnia Magna in different caffeine solutions; a One-Way ANOVA test was conducted to find differences between means of the one nominal variable and one measurement variable, and the statistical significance of the data was tested by using PAST3.

Results

The data in Figure 1.1 shows the average heart rate of the Daphnia in each group. The 0% caffeine group had an average of 405.75 bpm. The 0.5% caffeine concentration had a 420 bpm average and the 1.0% caffeine concentration had an average of 398.25 bpm. The figure also provides the ranges of the four Daphnia magna heart rates in each group. Trials were consistent throughout with no outliers affecting the averages of the results. A One-Way ANOVA was conducted to compare the effect of caffeine solution of heart rate of daphnia magna in 0 %, 0.5%, and 1.0% caffeine conditions. There was not a significant effect of caffeine on daphnia heart rate between the given caffeine solutions; [$F(2,9)] = 0.165$; $p=0.850$].

Discussion

The experiment began with the hypothesis that higher concentrations of caffeine would increase Daphnia magna's heart rate. The experiment was run to get an idea about the effect that caffeine would have on the human heart rate. However, the collected data fails to support our hypothesis. The ANOVA shows that after the Daphnia were exposed to the various concentrations of caffeine, there was little difference between the bpm of the control and experimental groups. This indicates that the caffeine concentrations had little to no effect on the Daphnia heart rate. Therefore, concerning our original question of whether caffeine affects heart rate, results conclude that the particular concentrations of caffeine we used did not have an effect on heart rate. It is possible that we ended up with such results because the caffeine concentrations we used were too small to cause any significant effect. Using higher caffeine concentrations might have given us more accurate results about the effect of caffeine on bpm. Gubareff and Sleator's experiment addresses the use of a much larger range of caffeine concentrations. In their experiment, they used 1.0% and 0.5% as their lower caffeine concentrations, and their higher caffeine concentrations were essentially much greater than the ones we worked with. Therefore, Gubareff and Sleator's experimental results stated that increased caffeine concentrations significantly affected mammal heart rates (Gubareff and Sleator, 1965). Future research could involve using higher concentrations of caffeine to study its effects on Daphnia magna heart rate similar to those used but Gubareff and Sleator. More experiments could also be run using human test subject to test effects of caffeine more accurately instead of using Daphnia and concluding the same results about humans. Future hypotheses could include: Higher concentrations of caffeine will have more substantial effects of increasing heart rate in humans. Essentially, this question should continue being studied because understanding the effect that caffeine has on human heart rate allows scientists to effectively develop genetically engineered cardiac muscle cell prototypes that will maintain a consistent heart rate.

Literature Cited

Battram, D., Graham, T., Dela, F. (2005). The Effect of Caffeine on Glucose Kinetics in Humans-

Influence of Adrenaline. *The Journal of Physiology.* 569(1): 347-355).

Bekker, J., Krigsman, B. (1951). Physiological Investigations Into The Heart Function of Daphnia. *The Journal of Physiology.* 115: 249-257.
Bulbring, E., Whitteridge, D. (1941). The Effect of Adrenaline on Nerve Action Potentials. *The Journal of Physiology.* 99: 201-207.

Clark, A., & Olson, C. B. (1973). Effects of caffeine and isoprenaline on mammalian ventricular muscle. *British journal of pharmacology*, 47(1), 1-11.

Di Bernardo, D., Langley, P., Murray, a. (2002). Effect of Changes in Heart Rate and in Action Potential Duration on the Electrocardiogram T Wave Shape. *Physiol Meas.* 23(2): 355-364.
Encyclopedia. (2004). Myogenic. https://www.encyclopedia.com/science/dictionaries-thesauruses-pictures-and-press-releases/myogenic (accessed 10/30/18).

Gubareff, T., Sleator, W. (1965). Effect of Caffeine on Mammalian Atrial Muscle, and its interaction with adenosine and calcium. *The Journal of Pharmacology and Experimental Therapeutics. Vol. 148(2).* 202-214.

Tuurala, H., Soivio, A., Nikinmaa, M. (1982). The Effects of Adrenaline on Heart Rate and Blood Pressure in Salmo Gairdneri at Two Temperatures. Ann. Zool. Fennici. 19: 47-51.

Hammer and Harper. (2013). PAST3 (3.2) [Computer software]. Oslo, Norway: https://folk.uio.no/ohammer/past/

Ikonnikov, G., & Yelle, D. (2009, January 22). Physiology of cardiac conduction and contractility. Retrieved October 30, 2018, from http://www.pathophys.org/physiology-of-cardiac-conduction-and-contractility/

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