

Increased exposure time of *Daphnia magna* to nicotine causes an increase in heart rate

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Abstract

Nicotine is a stimulant which is known to cause an increase in heart rate. Several studies describe multiple side effects that nicotine has on heart health, for example one side effect is that it increases blood pressure (Corotto et al., 2010). We proposed that having an increased exposure time to nicotine rather than a decreased or nonexistent exposure time may result in a higher heart rate which means that the cell is producing more action potentials. We conducted four trials comparing the effect different exposure times to nicotine have on the heart rate of *Daphnia magna*. We expect our results to be of interest to those who intake nicotine and want to understand the effect nicotine has on heart health. Our results conclude that an increased exposure time of *Daphnia magna* to nicotine causes an increase in heart rate. This is a significant finding because it can help explain why nicotine users often feel lightheaded or short of breath. These are symptoms caused by oxygen deprivation due to the heart not effectively pumping blood throughout the body because of an increased heart rhythm. This conclusion could open new avenues of research to understand the effect other stimulant drugs have on the heart.

Introduction

For the past several years, nicotine usage in the United States has grown with the increasing popularity of electronic cigarettes, which has led more adolescents to be exposed to the harmful side effects of nicotine. A study on the effects that nicotine has on human cardiovascular health showed that nicotine is positively correlated with cardiovascular disease and an increase in

atherosclerosis, caused by high blood pressure and high cholesterol (Benowitz, 2004). When used, nicotine affects the release of several neurotransmitters, such as epinephrine and acetylcholine, which high amounts of these chemicals can increase the risk of cardiovascular disease (Benowitz & Gourlay, 1997). However, it is still unclear if nicotine or other harmful substances that are being inhaled during smoking play a primary

role in the development of cardiovascular disease from smoking (Benowitz, 2004).

We used nicotine which is a drug to investigate why some cells produce more or fewer action potentials than others. Our experiment is designed to study the direct effect that nicotine has on the heart by testing nicotine on *Daphnia magna*, which are an invertebrate species that lack an autonomic nervous system. They are a good model to test our hypothesis on because without an autonomic nervous system they lack the ability to unconsciously control and regulate their heart rate so this will allow us to understand the effect nicotine has on heart rate without the interference of a confounding variable. We hypothesize that an increase in exposure time of nicotine to *Daphnia magna* causes an increase in heart rate which represents an increase in the production of action potentials because nicotine is a stimulant. We suspect this relationship because stimulants are a class of drugs that “stimulate” the body’s central and autonomic nervous systems. Nicotine stimulates the body’s adrenal glands to release epinephrine which increases heart rate, breathing rate, and blood pressure. We will know our hypothesis is supported if increased exposure time to nicotine increases the heart rate of *Daphnia magna*. We will know our hypothesis is not supported if increased exposure time to nicotine decreases or has no effect on the heart rate of *Daphnia magna*. An alternative hypothesis to explain an increase in heart rate due to an increase in exposure time to nicotine could be caused by the central nervous system’s interpretation of the external environment which would be the nicotine. Our experiment addresses the issue in a unique way because it allows us to visually observe the effect that

nicotine has on the heart without the influence of the autonomic nervous system.

Methods

In our experiment, we tested how various exposure times of *Daphnia magna* to nicotine affected their heart rate. We set up the experiment based on the guidelines given in the lab manual (French and Shaw, 2018). To begin, we first had to test the heart rate of *Daphnia magna* with no added stimulants to the water they were in. Next, we collected data with exposure times of 10, 15, and 20 minutes of *Daphnia magna* to three drops of nicotine in addition to the water they were in. We used a box and whiskers plot to display the data we collected for our experiment and we used a One-way ANOVA statistical test in order to analyze our results and evaluate the correlation between time of exposure to nicotine and heart rate.

Firstly, we used a beaker to collect a sample of *Daphnia magna* from the aquarium. Then, we used a modified dropper to select a single *Daphnia magna* to place onto the depression slide. In order to restrict the movement of the *Daphnia magna*, we removed excess water around the depression and placed a whisk of cotton around the *Daphnia magna*. Once the trials for the control group data with no added stimulants was collected, we began our trials with the various exposure times to nicotine. For each experimental trial, about three drops of the nicotine solution were added into the daphnia’s water. Then, we placed each slide onto the stage of the microscope with all settings set to low power. Once the microscope was in focus, we connected the phone adapter to the microscope and recorded ten-second-long videos in slow motion of each trial for each of the control

and experimental groups. We counted the heart beats per minute of the daphnia's heart by counting the number of heartbeats in the ten seconds and then multiplying that number by six. In total, we performed four trials for the control group and for each experimental group. Once we finished experimenting with the *Daphnia magna*, we rinsed off the slide with aquarium water and gave them time to rest before placing them back into the aquarium.

Results

Our data shows that the *Daphnia magna* who were not exposed to any nicotine had the lowest heart rate, whereas the *Daphnia magna* who were exposed to nicotine for any duration of time had higher heart rates. The trend between the three experimental groups of the exposure times for 10, 15, and 20 minutes was that heart rate increased with increasing exposure times to

nicotine (Figure 1). Our data did not include any outliers. The percent change for an exposure time of 10 minutes was 31.9%. The percent change for an exposure time of 15 minutes was 41.8%. The percent change for an exposure time of 20 minutes was 59%. The percent change was almost double for an exposure time of 20 minutes compared to an exposure time of 10 minutes. A One-Way ANOVA was conducted to compare the effect of nicotine on heart-rate in 10, 15, and 20-minute exposure time conditions. There was a significant effect of the nicotine solution on heart-rate between the four exposure conditions, [p-value = 9.655E-12].

Discussion

The data from our experiment showed that nicotine increases the heart rate of *Daphnia magna* with increasing exposure times which means that our data supported our hypothesis. The more exposure the

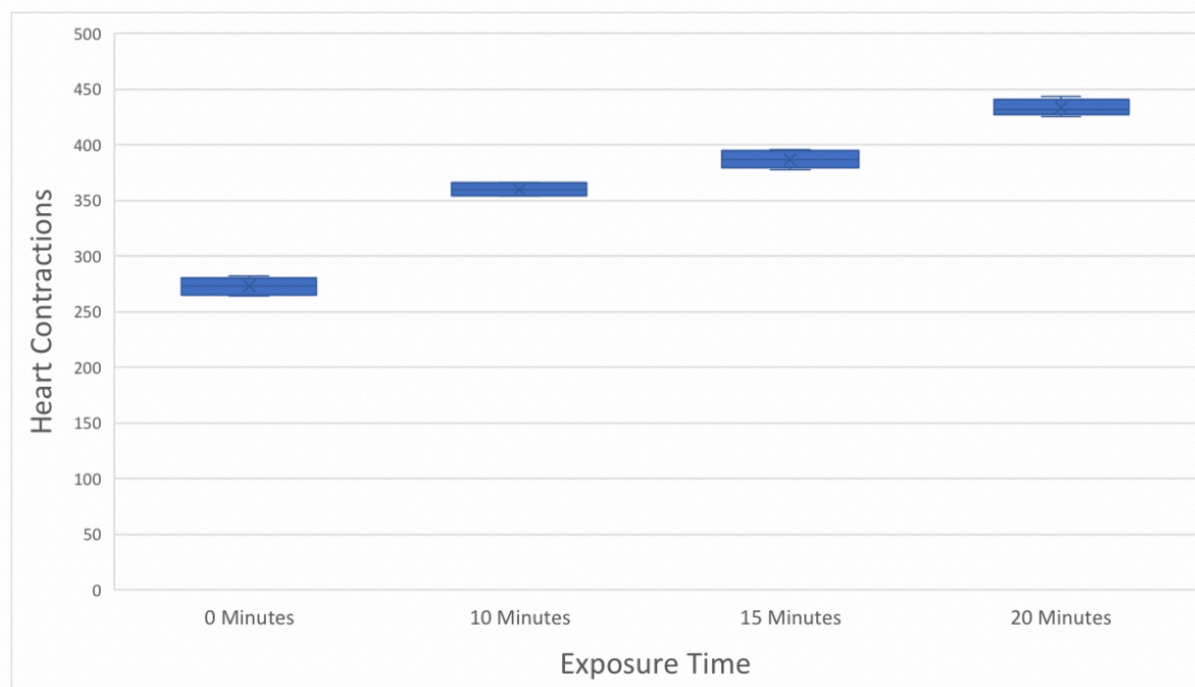


Figure 1. Comparison between heart rate and the amount of time *Daphnia* were exposed to the nicotine solution.

Daphnia magna had to nicotine, the higher their heart rate. The results show that nicotine affects the heart rate without the interference of the autonomic nervous system to regulate the heart rate. The daphnia heart rate had a larger percent rate of change when exposed to nicotine for 20 minutes > 15 minutes > 10 minutes.

The adenosine receptors for the daphnia's body size may explain the results we collected (El-Mas et al., 2011). For example, in humans' different doses of medicine affect everyone differently depending on their respective body size (Pai, 2012). The general pattern observed in mammals is that the smaller the body size, the greater the effect that stimulants have on their heart rate. It is possible that this same phenomenon occurred with the daphnia. Another possible explanation could be that the dosage of nicotine the daphnia were exposed to was not sufficient enough to make a connection to the effect nicotine has on the heart rate of humans. For example, humans require a large amount of nicotine to impact their heart rate rather than a small amount (Sofuoglu et al., 2012). Biologically, daphnia could be more receptive to nicotine meaning that their bodies intake the nicotine easier. This would help support our hypothesis because an increase in exposure time would explain a larger percent change compared to the control group.

The results we collected in this experiment are impactful on the current society today because nicotine use is extremely prevalent. Our results showed that increased exposure time to nicotine has a significant effect on heart rate. This information provides us with a better understanding of how nicotine can affect the

human heart. In future research, showing that nicotine does have the same effect on humans could possibly lower the percentage of people using nicotine. Another idea for future research could be to show the long-term effects that nicotine has on the heart health. Instead of experimenting with different exposure times to nicotine, we could experiment to see how nicotine affects heart health over a longitudinal period of time. By lowering the percentage of people using nicotine, overall heart risk will decrease, and heart health may improve across the world.

Literature Cited

- Benowitz, N.L., & Gourlay, S.G. (1997). Cardiovascular Toxicity of Nicotine: Implications for Nicotine Replacement Therapy. *Journal of the American College of Cardiology*. 29(7).
- Benowitz, N.L. (2004) Nicotine and coronary heart disease. *Trends in Cardiovascular Medicine*. 1(8): 315-321
- Corotto, F., Ceballos, D., Lee, A., Vinson, L. (2010). Making the Most of the Daphnia Heart Rate Lab: Optimizing the Use of Ethanol, Nicotine & Caffeine. *The American Biology Teacher*. 72(3): 176-179
- El-Mas, M.M., El- Gowilly, S.M., Founda, M.A., Saad, E.I. (2011). Role of adenosine A2A receptor signaling in the nicotine-evoked attenuation of reflex cardiac sympathetic control. *Toxicology and Applied Pharmacology*. 254(3): 229-237

Pai, M.P. (2012). Drug Dosing Based on Weight and Body Surface Area: Mathematical Assumptions and Limitations in Obese Adults. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*. 32(9): 856-868

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

Sofuoglu, M., Herman A.I., Nadim, H., Jatlow, P. (2012). Rapid nicotine clearance is associated with greater reward and heart rate increases from intravenous nicotine. *Neuropsychopharmacology*. 37(6): 1509-1516