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Nasty Nicotine: Recognizing the Fluctuating Effect Nicotine has on the Heart Rate of *Daphnia magna* Over Time

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Abstract

Nicotine is a highly addictive drug that can influence the heart rate of those exposed to it by increasing the number of action potentials. *Daphnia magna* are comparable test models to that of humans to observe the drug's cardiac effects, because they have myogenic hearts that are similar to that of a human's heart. We believed that if the *Daphnia magna* was exposed to a nicotine solution over time, then the heart rate would peak at a certain time point before the parasympathetic nervous system would activate to decrease it. In this investigation, we observed the heart rate of *Daphnia magna* as it was submerged in a 10 nM nicotine solution for 20 minutes. For this experiment, we conducted five trials of measuring the *Daphnia magna* heart rate pre- and post- nicotine exposure every four minutes. Since nicotine is considered a stimulant in high doses, we thought it would peak at the 12-minute mark because the nicotine would be given enough time to influence action potential activation; however, our results showed that only three out of the five trials had the greatest heart rate at the 12-minute mark. After averaging our results at each time point, we found that the average line followed the general trend that heart rate increased until peaking at 12 minutes before gradually decreasing for the rest of the experiment.

Introduction

Nicotine is a highly addictive stimulant found in tobacco. Humans abuse nicotine in the main form of smoking cigarettes. This stimulant has an effect on the nervous system by causing an elevated sense of alertness and pleasurable sensation (Griesar et al., 2002). The relationship between nicotine and the nervous system affects the electrical activity within the cardiac system, as it is known to increase

heart rate (Gajewska et al., 2014). Due to ethical considerations, a comparable model, *Daphnia magna,* was chosen to test the effect nicotine has on the action potentials present within the model's cardiac system over a period of time.

Daphnia magna are small crustaceans with transparent bodies that allow investigators the chance to examine the effects of external substances on internal processes and structures, such as the heart (Ceballos et al, 2010). The *Daphnia magna* heart is unique in the sense they have myogenic hearts like vertebrates, instead of neurogenic hearts like arthropods (Stein et al, 1966). Myogenic hearts produce contractions spontaneously without neural stimulation, while neurogenic hearts are regulated by cardioregulatory nerves that use inhibitory and excitatory fibers on cardiac ganglion neurons (Yamagishi et al., 2000). Even though the heart contracts on its own, the presence of stimulants can influence additional cardiac activity. As sympathetic nervous system stimulation occurs, exposure to nicotine releases catecholamines and neurotransmitters. This stimulates cardiac contractions because it amplifies the effect of acetylcholine binding, which causes action potential activation (Haass et al., 1997). The hemodynamic actions of nicotine increase cardiac output by increasing heart rate and myocardial contractions because these chemicals have a direct release from vascular nerve endings (Benowitz et al., 1997). One unique trait paralleling human biology to the *Daphnia magna* is the presence of a nerve similar to that of a human's *vagus nerve*, the body's major parasympathetic nerve. When there is a sudden stimulation causing tachycardia, the *vagus nerve* produces a negative feedback loop causing the *Daphnia magna* to elicit a vasovagal reflex to lower the heart rate (van Lieshout et al., 1991).

Based off of this information, we are interested in how the nicotine stimulant affects the *Daphnia magna* heart rate over a time period. We hypothesized that the *Daphnia magna* will have the highest heart rate at the 12-minute mark, because the nicotine will have an optimal amount of time to influence the action potential for cardiac contractions. This is approximately half of the time of our study, which is similar to the timeframe as to where heart rate peaked in another experiment that focused on the action of acetylcholine activation on the *Daphnia magna* heart (Bekker et al., 1950). The hypothesis will be supported if the average heart rate for the *Daphnia magna* will be greatest at the 12-minute mark in comparison to the other time points that heart rate is being measured at. On the other hand, the hypothesis will not be supported if the nicotine causes the *Daphnia magna* heart rate to be greater at any of the other time points.

Methods

For this experiment, we are interested in determining how *Daphnia magna's* heart rate in beats-per-minute (BPM) is influenced by nicotine over a 20-minute time period. The pre-exposure heart rate was used as the base comparison model since the *Daphnia manga* did not have a stimulant present to have any cardiac influence. Once the organism was exposed to nicotine, the time of the study allowed for the post-exposure heart-rate to be measured to determine its longitudinal effects.

For the setup of the experiment, we gathered 20 mL of aquarium water containing *Daphnia magna.* For each trial, one *Daphnia magna* of approximately the same size was used to measure its heart rate in a depression slide underneath a microscope. To limit the amount of movement, a small piece of cotton was used to soak up excess amounts of aquarium water. Using an appropriate amount of light emitted from the microscope, the heart rate was initially recorded for ten seconds on a phone's slow-motion camera setting. The number of beats were measured using a hand counter. The number of beats was multiplied by six to get the heart rate in beats-per-minute. The aquarium water was soaked up and replaced with a 10 mM nicotine solution. Over a 20 minute-time interval, the heart rate was recorded and measured every four minutes using the same protocol to determine heart rate as when the *Daphnia magna* was in aquarium water. Afterwards, the *Daphnia magna* was washed off the depression slide into its original environment using aquarium water. This was repeated for a total of 5 trials.

For our conditions, we will create a line graph showcasing the average heart rate of the *Daphnia magna* in pre- and post-exposure with the x-axis representing time in minutes and the y-axis being heart rate in beats-per-minute. Due to the fact that there is not a statistical test that compares each trial and each time point simultaneously, descriptive statistics were utilized to analyze the general trends present within our dataset, specifically with a trendline based on the average of the five trials to illustrate central tendency.

Results

Figure 1 was used to visually display the heart rate data over a period of time. The aquarium water (0 minutes) was used as a base comparison for heart rate before nicotine inoculation with the rest of the

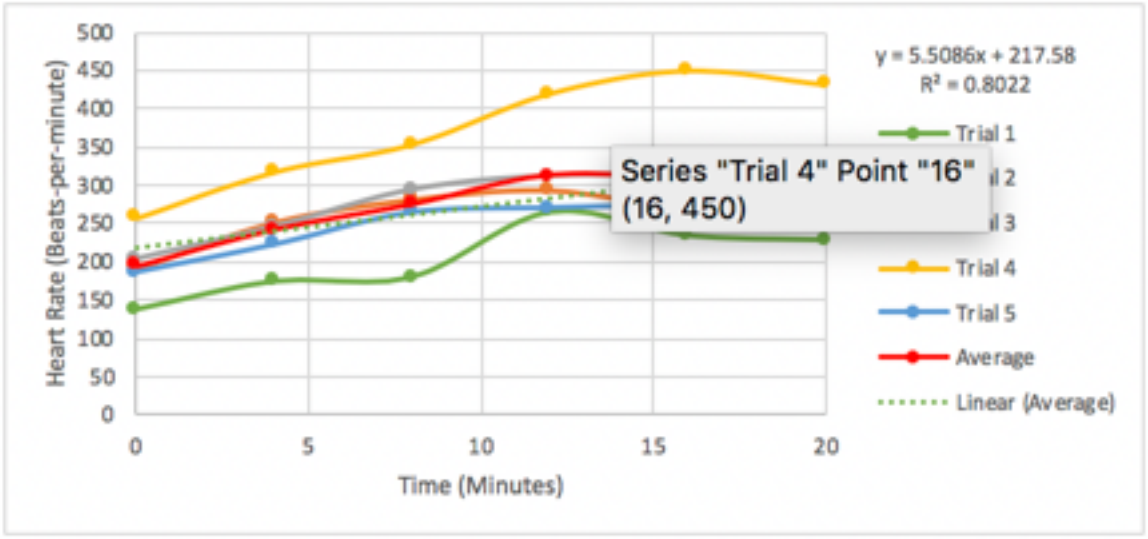

Figure 1: Line graph depicting the *Daphnia magna* average heart rate (beats-per-minute) over a 20-minute time period (n = 5).

experimental time. Every one of the trials experienced an increase in heart rate after the nicotine was inoculated into the system. Gradually over the 20-minute interval, heart rate increased. For trials 1 – 3, the heart rate peaked at the 12-minute mark before gradually decreasing; however, trial 4 continued to increase past the 12-minute mark up to the 20 minutes, while trial 5 increased at the 16-minute mark and decreased very slightly at the 20-minute mark. The maximum heart rate produced out of the four trials was 450 BPM (trial 4, 16 minutes), while the minimum heart rate was 138 BPM (trial 1, 0 minutes).

Using a descriptive statistics approach, we averaged the five trials' heart rates at each time point to see a general trend. By looking at this average line, we can see that it peaks at the 12-minute mark before decreasing slightly. A trendline was created to determine the coefficient of determination and correlation coefficient to correlate our variables. 80.22% of the variability in pre- and post- nicotine-exposed heart rate (BPM) can be explained by the linear association between pre- and post-nicotine-exposed heart rate (BPM) and time (minutes). Our correlation coefficient was a value of .89 indicating a strong, positive, and linear correlation between these variables in our experiment.

Discussion

We hypothesized that the *Daphnia magna* will have the highest heart rate at the 12-minute mark, because the nicotine will have an optimal amount of time to influence the action potential for cardiac contractions. *Daphnia magna* have similar biological reactions to that of humans because of their myogenic hearts, therefore, they serve as a comparable model to determine the effects of nicotine products (Yamagishi et al., 2000). By having a longer time exposed to the nicotine, sodium channels will open at a fast rate, thus increasing action potentials (Purves et al., 2001). Our data partially supports our hypothesis that the 12-minute mark would have the highest heart rate post-nicotine exposure. Three of the five trials experienced the peak heart rate at this time point, while the other two had maximum heart rates that were slightly higher than that at their respective 12-minute mark. We expected the *vagus nerve*-like nerve in the *Daphnia magna* to decrease the heart rate to unelevated levels; however, this did not appear to happen. We are considering *Trial 4* to be an outlier in this experiment, because of the drastic difference in heart rate in comparison to the other trials performed. We attempted to get *Daphnia magna* of equal size to eliminate this confounding variable.

Aside from the nicotine, there are other possibilities that could have influenced the heart rate. One alternative factor comes from the *Daphnia magna's* size. Smaller animals have the potential to have a higher heart rate because there is a shorter duration of diastole to parallel diastolic pressure decay and have good coronary perfusion present (Westernhof et al., 1993). Also, the *Daphnia magna* could be experiencing external stress from being placed in a new environment. Since *Daphnia magna* conditions can be related to that of humans, one study done on human subjects showed that acute amounts of stress correlated to an increase in heart rate (Torpy, 2007). One other possible factor that could have played a

role in the heart rate of our *Daphnia magna* was the way it responded to the nicotine itself. The *Daphnia magna* could have adjusted to the solution it was in that could have caused the heart rate to slow down in the trials where heart rate decreased.

For future experiments, the experiment should be carried out over a longer period of time, so the *Daphnia magna* can have a longer exposure time to observe any longitudinal effects. This controlled environment will allow observers the chance to see if nicotine does have any effects on heart rate or if it is external factors. Another variable that could be altered would be the concentration of the nicotine itself. Nicotine is a unique drug that is dose dependent. It can act as a stimulant in high dosages or as a depressant in small dosages (Ashton et al., 1973). By finding the concentration as to where the drug inflects from one condition to another will allow researchers the chance to determine the point to where nicotine can positively or negatively impact heart rate over a period of time.

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