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# Crushing on Nicotine: *Daphnia Magna* Heart Rate Rises at Low Concentrations

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## Abstract

This experiment analyzes the nature of nicotine. Our research focuses on nicotine's effect on the heart rate of *Daphnia magna.* There is extensive research on the effects of neurotransmitters and narcotics on the human brain but nicotine has always been an enigma because its effects vary depending on the user's mood and dosage. Our experiment keeps the exposure time constant so we can focus solely on the concentration to categorize nicotine as an agonist or antagonist. We introduced three different Daphnias to 1.0 mM of nicotine and the other three to 10.0 mM of nicotine, and their bpm was recorded at four, eight, twelve, sixtenteen, and twenty minutes. We hope this study will draw interest from those in the biology and psychology field, or anyone interested in the effects of nicotine on myogenic hearts, ultimately shedding more light on the categorization of nicotine and better understand its effects not only in Daphnia but also vertebrates.

## Introduction

Neurotransmitters are the messengers that relay the brain's signals to different parts of the body. The neurotransmitters send these signals from neuron to neuron and finally to the muscle for movement. On the molecular level, neurotransmitters get released from the presynaptic cell and are matched with receptor molecules on a postsynaptic cell's membrane. Receptor molecules are very particular and will only accept messages that are specific to the neurotransmitter. After the signal has been relayed and is no longer needed, the neurotransmitter must be removed from the synapse. This is possible by cleaving the neurotransmitter into an inactive form or the neurotransmitter is uptaken back into the presynaptic cell (Feher, 2012).

Neurotransmitters have been found to have excitatory and inhibitory qualities and in some cases can have both. Dopamine, a common neurotransmitter found in humans,

is considered both excitatory and inhibitory. Dopamine becomes excitatory at high levels which can cause a boost of energy, but it can also become an inhibitor at low levels to increase focus. The emotions a human feels from neurotransmitters can be replicated by drug usage. Drugs have the same effects as neurotransmitters because they mimic their characteristics. Excitatory drugs are referred to as agonists while inhibitory drugs are antagonists. Antagonists disguise themselves as a particular neurotransmitter to manipulate the neuron into firing. Antagonists also bind to receptor sites but block the neuron from firing. A commonly used drug is Nicotine which mimics acetylcholine in the human brain and is able to bind to acetylcholine receptors.

*Daphnia magna* have also been shown to have acetylcholine receptors in their neurons (Guilhermino et al., 2000). Additionally, both humans and *Daphnia magna* have a myogenic heart that beats independently from central nervous system involvement (Pirtle et al., 2018). These factors are important as they demonstrate that nicotine may affect both organisms in a similar way due to their similar heart physiology and the shared presence of acetylcholine receptors. In humans, nicotine stimulates the body at first but becomes a sedative once the "buzz" is gone (Ashton et al., 1973). This indicates that nicotine is considered an agonist and antagonist, so it should speed up heart rate then decrease it. Previous research observed that nicotine is toxic to *Daphnia magna* (Wallbank et al., 2016). This means that nicotine may bind to *Daphnia Magna*'s acetylcholine receptors in a similar way it does to humans . Although *Daphnia magna* is a much simpler species than *Homo sapiens*, and since it is unethical to test on humans, their shared acetylcholine receptors and myogenic heart means the effect of nicotine on *Daphnia magna* could be parallel to *Homo sapiens*. Therefore the purpose of our experiment is to use the *Daphnia magna*'s myogenic heart as a simplified model of the myogenic heart in humans.

When exposed to nicotine, we hypothesize that the heart rate of *Daphnia magna* will increase initially, but over time it will begin to decrease because the drug will begin as a exhibitor but start to act as an inhibitor of normal heart function. In addition, a higher concentration of nicotine will lead to a more drastic difference between the stimulated and depressed heart rates. Examining nicotine's effects on the heart rate by manipulating the concentration as well as time could shed more light on nicotine's effect on the cardiovascular system. The current amount of research on effects of nicotine on *Daphnia magna* is limited, so this experiment will contribute to the research on these organisms. Hopefully, it could also add to the existing research of nicotine's effects on humans and other vertebrates with a myogenic heart. The hypothesis will be supported if nicotine causes a rise and fall in the heart rate of the *Daphnia magna* as well as it being more drastic at higher concentrations. The hypothesis will be disproved if the trend of the rise then fall is not evident or if the heart rate falls first then rises.

# Methods

The effects of two different concentrations of nicotine, 1.0 mM and 10.0 mM, on the heart rate of *Daphnia magna* were tested in four minute intervals over twenty minutes. By using different solutions

and measuring the heart rate of *Daphnia magna* at different times, we were able to see the correlation between concentrations of nicotine, exposure time, and heart rate. We decided to use the four minute mark as a baseline for comparison since this mark would be considered pre-exposure. Because the solution is known to be fully absorbed after seven minutes, the eight, twelve, sixteen and twenty minute marks were where the heart rates were measured post-exposure. These time marks were used to see the effects of different nicotine concentration on the heart rate of the *Daphnia magna* over time.

For each of the six trials, an unexposed *Daphnia magna* was exposed to nicotine. Three were exposed to 1.0 mM and three were exposed to 10.0 mM. The *Daphnia magna* each received three drops of the nicotine. By having three trials for each concentration, we could average the beats per minute rather than base our data on one *Daphnia magn*a. The heart rates were measured at the specified intervals by taking a ten second slow motion video of the *Daphnia magna* through a microscope. The videos were shot in slow motion to be able to count every heartbeat with a hand clicker. Two experimenters individually counted the heartbeats in the videos and multiplied the number of heartbeats by six to convert it to beats per minute (bpm). Two experimenters viewed each video to ensure the heartbeats counted were accurate. After the twenty minute trial, each *Daphnia magna* was rinsed and placed back into the aquarium where they were collected.

The data was displayed on a scatter graph with a linear trend line to have a visualization of trends within the data. The different concentrations of nicotine were represented on individual graphs because the range for the concentrations were not close in bpm. Having two graphs allowed the trends in the data to be clear and comparable to each other. A correlation test was done for both concentrations and their respective set of data points. This was necessary to determine if there is a relationship between the two variables. The correlation test also eliminates the possibility of a null hypothesis.

# Results

In the trials with exposure to 1.0 mM nicotine, the general trend was an increase in heart rate over time. While there were drops in heart rate around the five minute mark, the heart rate increased for a majority of the trial (See Figure 1). In the trials with exposure to 10.0mM nicotine, the general trend was a decrease in heart rate over time (See Figure 2). There were no significant increases in heart rate over any of the 10.0 mM trials. Both sets of data showed the most amount of change in heart rate between the eight and twelve minute mark. However, the average heart rate of the Daphnia exposed to 1.0 mM nicotine was 142 bpm higher than the Daphnia exposed to 10.0 mM nicotine. Trial 3 produced the highest average heart rate, 100.8bpm faster, compared to all the other Daphnia in the 1.0 mM group. After performing a correlation test on the 1.0 mM of nicotine experimental group, we found a strong direct correlation value of 0.966148584. On the other hand, 10.0 mM of nicotine experimental group had a strong indirect correlation value of -0.958994093.

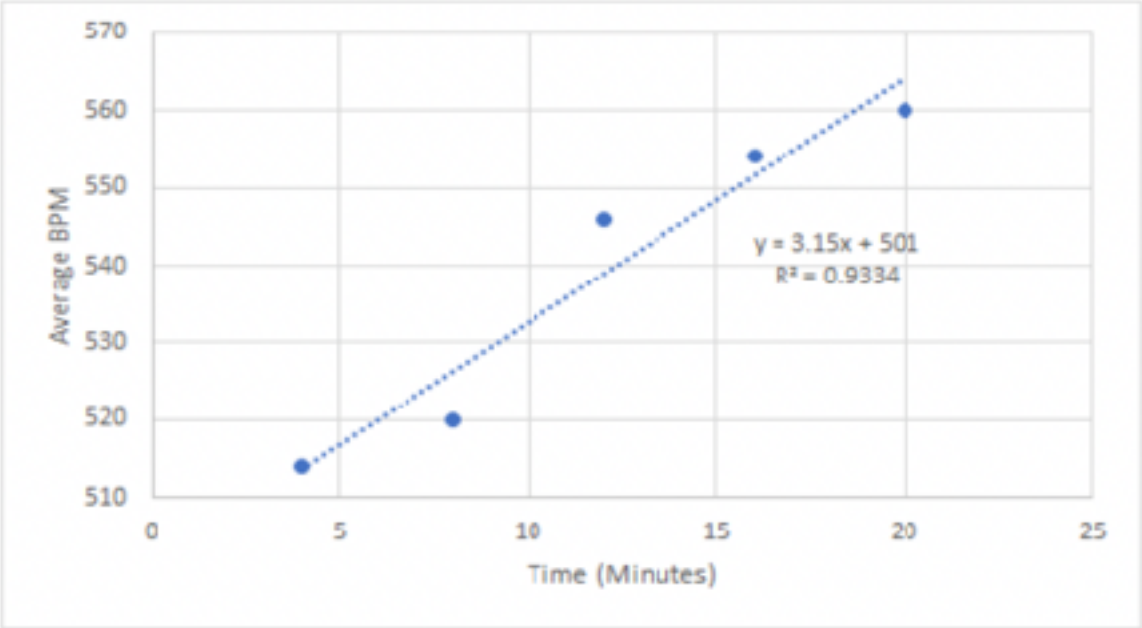


**Figure 1** | Heart rate in BPM of *Daphnia magna* placed into a 1.0 mM nicotine solution with measurements recorded at four minute intervals for twenty minutes. The longer the *Daphnia magna* remained in the solution, the higher their heart rate was measured as. The rate of change in average heart rate was highest between the eight and twelve minute marks.

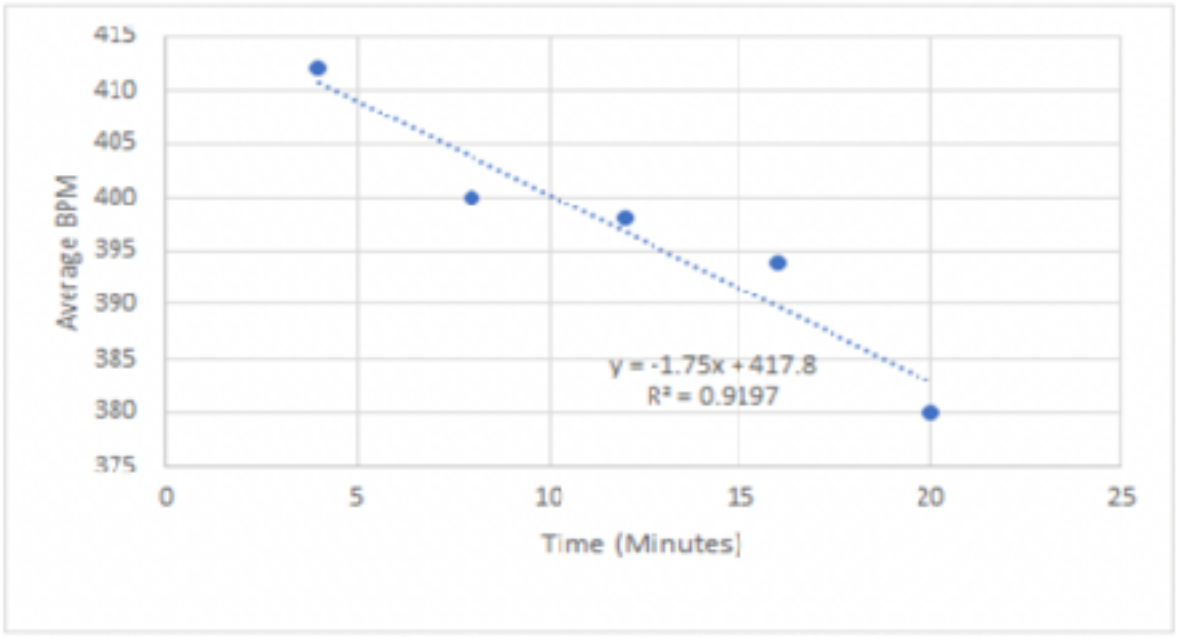


**Figure 2** | Heart rate in BP M of *Daphnia magna* placed into a 10.0 mM nicotine solution with measurements recorded at four minute intervals for twenty minutes. The longer the *Daphnia magna* remained in the solution, the lower their heart rate was measured as. The rate of change in heart rate was lowest between the eight and twelve minute marks.

## Discussion

The result of our experiment disproves our first hypothesis, as the heart rate of the *Daphnia magnas* did not show a

trend of increasing then decreasing for both concentrations. Our second hypothesis, higher concentrations of nicotine will lead to more drastic changes in heart rate, was also disproved. If our data supported our hypothesis, we would have seen similar trend of the heart rate increasing then decreasing between the two concentrations as well as more drastic differences in the 10.0 mM trials. Instead, our data displayed opposite trends between the 1.0 mM and 10.0 mM nicotine solutions and the 10.0 mM trial had an overall less drastic change in heart rate compared to the 1.0 mM trial.The anomaly in trial three of the 1.0 mM solution of nicotine was caused by a pregnant Daphnia because pregnancy results in a cardiac output increase of 30%-50% (Brown, 2016). This could explain why the heart rates of *Daphnia magna* from the 1.0 mM trials were on average, 142 bpm higher than the heart rates of *Daphnia magna* from the 10.0 mM trials. Because there is an overall bpm increase in the 1.0mM group and an overall bpm decrease in 10.0mM group, we conclude that within a twenty minute timeframe nicotine depresses Daphnia magna heart rate at low concentration and accelerate heart rate at high concentrations. With the correlation value being extremely close to 1.0, there is a significant direct correlation between the 1.0 mM nicotine and the increase in heart rate over time. With the correlation value being extremely close to -1.0, there is a significant indirect correlation between the 10.0 mM nicotine and the decrease in heart rate over time.

This data conflicts with previous research on human heart rate changes when exposed to nicotine. This could be because, although humans and *Daphnia magna* both have myogenic hearts, *Daphnia magna* do not have the complex central nervous systems that humans do. The main link between the human and *Daphnia magna* nervous system is only the vagus nerve. Additionally, Nicotine is also processed in the brains of humans, and not just the heart (Ashton et al.,1973). Whereas in *Daphnia magna*, the nicotine is processed solely by the myogenic heart because of the lack of nervous system complexity which can affect its definition as a stimulate or depressant. The data from this experiment accurately represents the effect of nicotine on heart function without any central nervous system involvement, which could shed light on how human brains process and are affected by nicotine and how the processing effects other physiological functions.

Future researchers that want to replicate this experiment should consider using wider ranges of concentrations. For example, higher concentrations of nicotine could be used to further test the indirect correlation between higher concentrations of nicotine and decrease in heart rate. Additionally, lower concentrations of nicotine could be used to further test the direct correlation between lower concentrations of nicotine and increase in heart rate. Having concentrations in between 1.0 mM and 10.0 mM can also test at exactly what concentration nicotine will switch from being an agonist to an antagonist.

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