

## **Caffeine and Nicotine of Different Concentrations Result in Varying Heart Rates of *Daphnia magna***

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### **Abstract**

In cardiac muscle cells of most vertebrates, the sympathetic nervous system is stimulated when agonists bind to adrenergic receptors (Shaw & French, 2018). The *Daphnia magna* heart and the autonomic nervous system of many vertebrates have similar properties; therefore, using *Daphnia magna* as a model organism is justifiable way to determine the effects of stimulants on heart rate. In this investigation, we studied the effects of two central nervous system stimulants, caffeine and nicotine, on *Daphnia magna*. For four trials each, we manipulated concentrations of 0.5% caffeine, 1% caffeine, 1 mM nicotine, and 10 mM nicotine in order to measure the effects on *Daphnia magna* heart rate. In the end, we found varying trends in our data. The 0.5% caffeine and 10 mM nicotine showed a positive slope in percent change; on the other hand, the 1% caffeine and 1 mM nicotine showed a negative slope. This is significant because it shows that *Daphnia magna* have inconsistent responses to common stimulants. We expect this experiment to be of interest to researchers and future experimenters who study the excitatory effects of these common stimulants on other organisms.

### **Introduction**

The consumption of caffeine and nicotine is rising among adolescents. A stimulant is a chemical that produces more action potentials which, in turn, raises the physiological or nervous activity in the body. Nicotine, an addictive central nervous system (CNS) stimulant, releases several neurotransmitters—including acetylcholine, dopamine, serotonin, and norepinephrine. These chemicals play a substantial role in the

reward center of the brain. Dopamine, for example, is a catecholamine drug that results in more intense heart contractions and an increased blood pressure. Studies have shown that nicotine stimulates the dopamine-secreting cells in the limbic structures of the brain, such as the prefrontal cortex and nucleus accumbens (Gilbert et al., 1989). In addition, researchers studied the effects of nicotine on rats; they found that small injections of nicotine increased plasma

corticosterone, a glucocorticoid involved in the regulation of energy and stress (Balfour & Ridley, 2000). Moreover, caffeine is a methylxanthine which is prevalent in various beverages and is also a central nervous system stimulant. Caffeine is also known to block the binding of adenosine to adenosine receptors. When adenosine acts as an agonist, neural activity slows down and blood vessels dilate. Caffeine, however, acts as an adenosine receptor antagonist; through inhibition of adenosine receptors, caffeine alters several brain functions (e.g. sleep, cognition, and memory) and increases heart rate (Bracco et al., 1995). These findings suggest that the intake of nicotine and caffeine increases excitatory effects in the brain and alters certain physiological responses, such as in the heart.

This led to our experiment where we want to find out if nicotine and caffeine increases the heart rate in *Daphnia magna*. *Daphnia Magna* have unique anatomical features; in one experiment, the heart of *Daphnia magna* was studied using light and electron microscopy. Researchers found that the heart wall is incredibly thin and contains long myofibrils, an abundance of sarcoplasm, and multiple mitochondria (Stein et al., 1966). Moreover, many biologists have utilized *Daphnia magna* to identify changes in the functioning of the heart because of their size and transparency. *Daphnia magna* possess a neurogenic mechanism that has similar properties to the autonomic nervous system in vertebrate animals; these similarities indicate that *Daphnia magna* and most vertebrates have comparable responses in the presence of drugs. Therefore, using *Daphnia magna* as a model organism is a feasible way to observe the effect of toxins on heart rate (Leatherman

et al., 2009). We hypothesized that nicotine will result in a higher percent change in *Daphnia magna* heart rate than caffeine because it binds to the adrenergic receptors, thus stimulating the sympathetic nervous system. If our hypothesis is supported, then the *Daphnia magna* will have a faster heart rate when placed in the nicotine solution; however, if our hypothesis is inaccurate, then caffeine will result in a faster heart rate when submerged into the caffeine solution. Also, no significant difference in heart rate would indicate that nicotine and caffeine have similar effects on heart rate.

## Methods

In our experiment, we manipulated nicotine and caffeine in order to measure the effect on the heart rate of *Daphnia magna*. By measuring the heart rate of *Daphnia magna*, we were able to determine which stimulant resulted in the quickest heart rate. Nicotine and caffeine are central nervous system stimulants that alter neural pathways resulting in several physiological changes; both of these served as our experimental groups. The distinct chemical properties are the distinguishable factors affecting the heart rate of *Daphnia magna*. In addition, for our control group, we measured the heart of each daphnia prior to the addition of a chemical substance in order to obtain a base level heart rate (i.e. for each trial, the heart rate of *Daphnia* was measured when placed in water exclusively).

To begin the procedure, we followed the protocol provided to us in the laboratory (Shaw & French, 2018). We first obtained one *Daphnia magna* from the aquarium using a small beaker. We then placed the daphnia onto a depression slide using a modified pipette; we used a paper towel to

remove excess water from the slide and placed small fibers of cotton to limit movement of the daphnia. In each trial, the heart rate of each daphnia was taken before a chemical was added. After measuring the initial heart rate, we added 4 drops of a given chemical using a dropper. Before determining the heart rate *Daphnia magna*, we waited for 7 minutes so the chemicals could assert a significant effect. For four trials each, we utilized concentrations of 1% caffeine, 0.5% caffeine, 1 mM nicotine, and 10 mM nicotine. We collected data in bpm using a hand counter and video of daphnia to observe the heart beats. Using a cellular device, we recorded the *Daphnia magna* for 15 seconds; we then played the video back in slow motion in order to obtain an accurate count of heart beats. Upon data collection, we calculated the percent change in bpm of each group  $((\text{Change} / \text{pre-exposure BPM}) \times 100)$  and illustrated the data using a box and whisker plot to show the range of data as well as the averages. We also ran a Two-way

ANOVA statistical analysis to find significant differences of means between the groups.

## Results

Our results suggest several trends. For the 0.5% caffeine, the change in heart rate increased and showed a positive slope; however, the 1% caffeine solution decreased the heart rate and resulted in a negative slope. In addition, the trials with the 1 mM nicotine decreased the heart rate by 7.17% while the trials with 10 mM nicotine increased the heart rate by 17.31%, as shown in Figure 1. For the 10 mM nicotine solution, however, there was a significant outlier that may have skewed the data. A Two-way ANOVA was conducted on the influence of caffeine and nicotine as well as the varying concentrations on *Daphnia magna* heart rate. There was not a significant effect of the differences of solutions on heart rate ( $F=1.28$ ;  $p=0.338$ ). There was not a

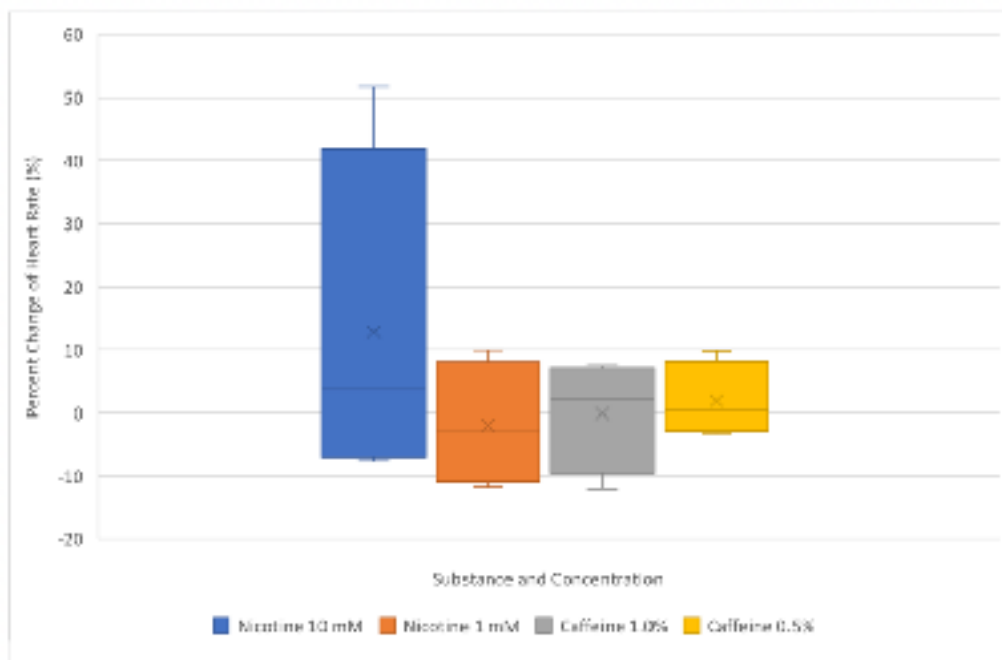


Figure 1: The percentage change in heart rate of *Daphnia magna* when placed in different concentrations of nicotine and caffeine.

significant effect between the concentrations ( $F=0.798$ ;  $p=0.525$ ).

## Discussion

Our findings show varying results in the heart rate of *Daphnia magna*. We originally predicted that nicotine would result in a higher percent change in heart rate than caffeine; however, our data is inconsistent with this prediction. Since 0.5% caffeine increased the heart rate while the 1 mM nicotine decreased the heart rate, our hypothesis was not supported. In another experiment, researchers hypothesized that a higher concentration of nicotine or caffeine would increase the number of action potentials thus increasing the heart rate of *Daphnia magna*. They found that the 0.5% of caffeine increased the heart by 52.3%, while the 1% caffeine resulted in either a constant or declining heart rate. They also studied the effects of nicotine on *Daphnia magna* heart rate and found that 1 mM nicotine resulted in a negative percent change, unlike the effects of 0.5% caffeine (Oujesky et. al, 2018). These findings are comparable to the trends in our investigation. Moreover, other researchers have studied the heart function of *Daphnia magna*. They found that acetylcholine, tetraethyl pyrophosphate, and pilocarpine result in a decreased heart rate; however, in other arthropods, acetylcholine stimulated the heart. The varying effects on the heart rate, as the researchers suggest, is due to the fact that *Daphnia magna* possesses a myogenic pacemaker in the heart as opposed to the neurogenic pacemaker—a heart requiring nervous input to contract—of other arthropods. This provides a feasible

explanation as to why the effects of drugs on *Daphnia magna* heart rate is inconsistent with common agonists of other organisms (Berker & Krijgsman, 1951).

Furthermore, we noticed that 10 mM nicotine increased the heart rate whereas the 1 mM nicotine decreased it. In another experiment, adrenaline was also found to slow the heart rate of *Daphnia magna* at low concentrations while increasing the heart rate at high concentrations. (Berker & Krijgsman, 1951). Therefore, since the heart rate is raised with increased nicotine concentration, nicotine might assert its effect directly on the heart muscle when used at high concentrations. Also, the data may be a misinterpretation of a stressful environment on the *Daphnia magna* which could result in varying heart rates. Moreover, there could have been several limitations to the experiment. *Daphnia magna* vary based on size and age; some *Daphnia magna*, additionally, could have been pregnant. These variables could either rapidly increase or decrease the heart rate and, in turn, alter the percent change in heart rate.

For future studies, we recommend using higher concentration levels of nicotine and caffeine in order to see if the results are any different. In addition, a shorter exposure time would be beneficial since *Daphnia magna* experience the effects of toxins much quicker compared to humans (Oujesky et. al, 2018). Despite these changes, future researchers should be cautious when comparing the effects of drugs on *Daphnia magna* heart rate to humans because *Daphnia magna* have differences in heart function, as found in the results of this experiment. We also recommend testing nicotine and caffeine on other mammals in

order to further investigate the physiological effects of stimulants.

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