

# Ups and downs of a tiny (organism's) life: relative effects of ethanol and caffeine on heart rate of *Daphnia magna*

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Caffeine and ethanol are some of the most widely consumed chemicals worldwide, yet little is known about their effects on heart rate. Many studies explore the effects of each solution separately, but not which one has a greater effect on heart rate than the other. *D. Magna* share a close resemblance to the human heart, making them a more ethical alternative for our research. We conducted 12 trials comparing the heart rate of *D. Magna* when exposed to ethanol or caffeine. Even though *D. Magna* have a large span of average heart rates, our findings suggest that both caffeine and ethanol increased heart rate.

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## Introduction

Unlike most crustaceans, *D. magna* has a myogenic heart that contracts on its own and is regulated by the central nervous system. This property in conjunction with their transparency allows for simple visualization of how the heart reacts to environmental conditions. The similarities of the heart of *D. Magna* and vertebrates make *D. Magna* an excellent experimental subject for the effects of heart rate (Stein *et. al* 1965, "Animal Taxonomy" 2016). While the effects of stimulants and depressants have been studied at length in the development of separate drugs, they have been seldom compared in magnitude. Additionally, the nature of stimulants and depressants each having a wide range of impacts underscores the importance of understanding their effects on physiology (Thomas 1994). Properly understanding the physiological impacts of different chemicals

increases the efficacy of medical treatments in overdose patients.

Ethanol and caffeine are both widely consumed in modern society. The respective depressant and stimulant effects of the chemicals are viewed favorably by many individuals, increasing the consumption of them. Despite their wide use, relatively little is known about how ethanol and caffeine affect the heart. Ethanol has been shown to increase heart rate in vertebrates and induce tachycardia in extremely high quantities (Sparrow *et. al* 1987, Kupari 1983). It has been noted that ethanol does not act directly on the heart in vertebrates due to the increase in heart rate; it would follow that ethanol affects the rate of cardiac contractions before the action potentials synapse to the heart (Kupari 1983). A proposed mechanism for this decrease in heart rate is through an inhibition of the vagus nerve. Ethanol binds to GABA receptors in the postsynaptic cells and allows chloride ions to

enter the cell, hyperpolarizing it and increasing the amount of sodium ions that must enter the cell to propagate the action potential (Davies 2003). When the function of the vagus nerve is inhibited, the myogenic heart is less regulated and increases its rate as a result. These depressant impacts are not limited to the vagus nerve, though, and hyperpolarize all postsynaptic cells resulting in impaired movement. This mechanism is not universally supported. Conversely, ethanol has been sometimes shown to decrease the heart rate in *D. Magna* (Leatherman *et. al* 2009). The authors did not propose a mechanism for this decrease, however this effect disputes Davies's mechanism.

Though caffeine is classified as a stimulant, it has been shown to have varying effects on heart rate. It has been shown to both increase and decrease heart rate unreliably (Green, 1996). In the body, caffeine allows calcium ions to diffuse through the presynaptic membrane, increasing neurotransmitter release, lowering the excitability threshold, and prolonging action potentials (Nehlig *et.al* 1992). If acting on the vagus nerve, this mechanism would support findings in which heart rate decreases since the increased firing of the nerve would restrict heart rate. However, caffeine has also been shown to strongly increase heart rate in *D. magna* (Leatherman *et. al* 2009).

Despite the varying effects of stimulants and depressants on heart rate, we hypothesized that depressants have a greater effect than stimulants on heart rate because the effect of depressing action potentials is more immediately felt than stimulating them. If our hypothesis is supported, the inhibiting effect of ethanol on neurotransmitter release due to its binding to GABA will affect the percent change of *D. magna* heart rate more than the stimulating effect of caffeine of lowering the threshold potential of presynaptic neurons. If our hypothesis is rejected, then caffeine will have a greater effect on heart rate.

## Methods

To measure the differing effects of ethanol and caffeine on heart rate of *D. Magna*, we

measured the percent change in heart rate of *D. magna* before and after introduction to caffeine and ethanol solutions.

*D. magna* were collected from a large tank with a sample cup. Using a modified dropper, a *D. magna* was placed onto a depression slide under a binocular light microscope. A paper towel was used to absorb excess water on the slide, restraining the *D. magna*'s movement to allow for easier measurement of heart rate. The lowest power lens was used on the microscope, and a phone lens was attached in place of one of the microscope's eye lenses. Due to the high heart rates of *D. magna*, using a phone camera to record the heart rate and play it back in slow motion increases the accuracy of measurement.

Each *D. magna* had an initial heart rate measured before the addition of any solution. A ten second video was taken of each *D. magna* and played back in slow motion. The number of heartbeats was measured in the ten second video using a hand counter to keep track of the number of heartbeats. After the number of heartbeats were measured, the result was multiplied by six to arrive at a beats per minute measurement of heart rate for *G. magna*.

After the initial heart rate was measured, two drops of stimulant or depressant solution were added to the depression slide. A solution of 5.0% ethanol served as our depressant and a 1.0% caffeine was used as our stimulant. The *D. Magna* sat in the solution for 10 minutes to ensure that the solution took effect. After 10 minutes, the final heart rate of *D. magna* was measured in the same manner as the initial heart rate. Once the measurements were complete, the *D. magna* were rinsed into a designated rinse container, allowing them to recover while different *D. magna* were used for other trials. Six trials of each solution were conducted.

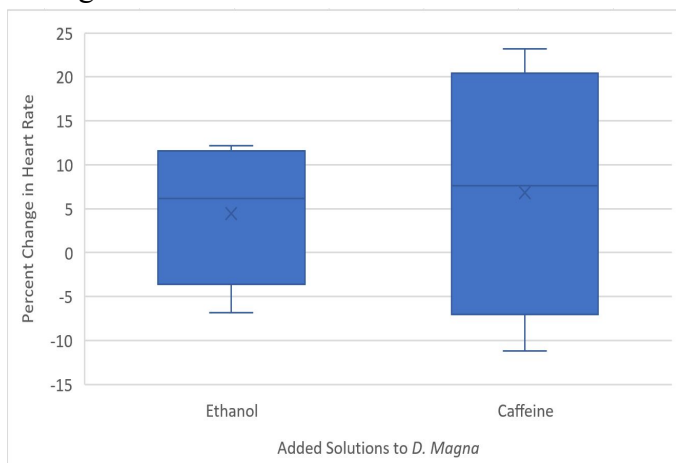
Once all data was collected, the percent change was calculated by subtracting the initial heart rate from the final, dividing by the initial heart rate, then multiplying the result by 100:

$$\text{Percent Change} = \frac{\text{Final Heart Rate} - \text{Initial Heart Rate}}{\text{Initial Heart Rate}} \times 100.$$

To analyze our data, a paired t-Test was used to test for a significance of difference between the percent changes of heart rate when affected by ethanol and caffeine.

## Results

A paired t-Test was conducted to compare the effects of ethanol and caffeine on heart rate of *D. magna*. Our data did not demonstrate a significant difference between the percent change in heart rate of ethanol- and caffeine-immersed *D. Magna*;  $t(1) = -0.450$ ,  $p = 0.671$  (See Figure 1, Appendix). Both ethanol and caffeine had varying effects on heart rate, but both tended to increase heart rate. No clear difference between them can be distinguished based on the data we collected.



**Figure 1.** Shows the range of percent changes of *D. Magna* in the ethanol and caffeine solutions. While the caffeine has a larger range, the statistical analysis found that there was no significant difference between the two solutions in terms of changed heart rate.

## Discussions

Inconsistent with our hypothesis, there was no significant difference in the percent change of heart rate of *D. magna* in ethanol and caffeine

solutions. Though both solutions had positive and negative percent change values, the overall trends of each solution were increases in heart rate of similar magnitudes for both ethanol and caffeine.

Our data is consistent with Leatherman's and Nehlig's findings that caffeine increases heart rate (Leatherman *et. al* 2009, Nehlig *et. al* 1992). Since *D. magna* have a nerve-regulated myogenic heart, it is assumed that they have a similar nerve to the vagus nerve in humans. Understanding caffeine's stimulant effects in the body, our data suggests that stimulation of the heart regulating nerve bundle is not occurring due to caffeine. If this nerve bundle was stimulated in our trials, the heart rate would have decreased uniformly. It follows, then, that stimulation occurs elsewhere in the heart-contraction mechanism. Perhaps the caffeine stimulates the cardiac muscles themselves by allowing  $\text{Ca}^{2+}$  to enter the cells, lowering the excitability threshold and facilitating contraction. This mechanism is possible in both humans and *D. magna* since the effects of caffeine are similar in both organisms.

The ethanol-affected *D. Magna* had an increase in heart rate instead of the predicted decrease. It is unclear why we observed ethanol having the opposite effect than Leatherman's which demonstrated a decrease in *D. magna* heart rate (Leatherman 2009). The depressant effects of ethanol we observed are, however, consistent with those of Sparrow and Kupari (Sparrow *et. al* 1987, Kupari 1983). Davies's mechanism of ethanol's effect on the vagus nerves is uniform with our observed results (Davies 2003). Though *D. magna* do not have a vagus nerve, the heart regulating nerve bundle is plausibly depressed by ethanol. The binding of ethanol to GABA receptors in post-synaptic cells--allowing chloride ions to hyperpolarize the post-synaptic cells and increase threshold potential--in the nerve bundle would decrease the activity of the regulatory nerve bundle and allow the cardiac muscles to contract with less regulation, thus increasing heart rate. This

mechanism would explain our observations of ethanol's positive chronotropic effect on heart rate.

Despite the similar trends of similar magnitude, some of our data had an opposite effect than the rest. While ethanol and caffeine have demonstrated a tendency to increase heart rate, some of our trials for each chemical demonstrated the opposite effect. This suggests that the effects of these chemicals are not consistent among all organisms--and supports Thomas's findings that the effects are not always consistent (Thomas 1944). The reasoning for this vacillation of results was not clear, but likely results from differences in individual organisms. Perhaps some of the *D. magna* became desensitized to the chemicals from repeated exposures over the course of 1,400 students examining their change in heart rate for three weeks.

The *D. Magna*'s similarity to the human heart makes it great for other studies relating to caffeine, ethanol, and heart rate. In a study conducted about dopamine's effect on heart rate in *D. magna* it was found that dopamine instantly increases heart rate when combined with caffeine, and when added to ethanol-affected *D. Magna* increased their heart rate (Kundu 2018). If applied to human hearts, we could determine how caffeine and dopamine work together to increase heart rate and have a greater understanding of the relationship between them. Examining how dopamine's stimulatory effects decrease the depressive effects of ethanol on heart rate could be studied further to possibly fashion a medicine to help reverse the effects of alcohol. Further, combining ethanol, caffeine, and dopamine in different proportions to study the effect on heart rate could help establish guidelines for responsible consumption of these drugs to reduce the amount of misinformed over-intoxication that often has detrimental consequences.

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