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Increasing heart rates of *Daphnia magna* in an excitatory monosodium glutamate solution versus decreasing heart rates in a depressive ethanol solution

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Abstract

Several experiments have been done using *Daphnia magna* to research the effects that different solutions such as ethanol and monosodium glutamate (MSG) may have on the heart. Much like the human heart, *D. magna* hearts are striated and neurogenic allowing for the comparison between the human heart and *D. magna* hearts. We exposed *D. magnas* to ethanol and MSG to observe the effects it may have on the heart rate, which was calculated by counting the heart beats per minute. Monosodiumglutimate significantly increased the heart rate with the highest increase going from 300 bpm to 384 bpm. Ethanol did the opposite and lowered the *D. Magnas* heart rate.

Introduction

Daphnia magna (*D. magna*) are small crustaceans that are found more commonly in standing freshwater, except for glacial and hot springs bodies of water as these specific habitats are not ideal for *D. magna* survival (Ebert, 2005).

The heart of a *D. magna* is myogenic meaning it is capable of creating its own cardiac contraction regardless of the absence of a nervous input much like the human heart. This is fascinating because *D. magna* are classified as arthropods whose heart normally depends on stimuli to create cardiac contraction, neurogenic heart, making them an exception. Previous research was done to study the structure of a *D. magna's* heart and it was observed that it has long striated myofibrils and that the cell wall is mostly one cell wall thick. (Stein et al. 1965). The use of *D. magna* to study the effects of different additives is further supported given that cardiac muscle in humans is also striated. The relative opaque biology of the organism allows us to measure heartbeat easily, while also being comparable to that of a human heart.

The heavily debated and prevalent component found in the American diet is monosodium glutamate (MSG). MSG, described by the U.S. Food & Drug Administration (FDA), is an additive food supplement for flavor that is generally recognized as safe (U.S. Food & Drug Administration, 2012). The controversy behind the addition of MSG in our diets may be due to anecdotal evidence submitted to the FDA being spread on social media and news outlets of the potential harmful effects of this additive. Due to this, the FDA mandates that foods be labeled that MSG has been added. The side-effects of MSG could be a result of its primary component- glutamate. Glutamate is a known excitatory neurotransmitter found in our central nervous system (Niciu et al. 2011). As glutamate is mostly found within the cell and rarely outside the cell, the high concentration that is required for fast synaptic transference is created (Miladinovic et al. 2015). By exciting the transmitters with increased action potential, heart contractions should increase, which may be the cause of the headaches, sweating, and heart palpitations seen in individuals who have consumed excess MSG (Horn, 2016).

Another substance found in diets across the globe is ethanol; the most desired ingredient in alcohol. The effects of ethanol can differ depending on the individual, creating various mannerisms in those intoxicated. Some individuals exhibit a somber, relaxed state of mind suggesting that alcohol will block neurotransmitters, thus inhibiting neurotransmitters and decreasing heart rate. Similar results were seen in an experiment testing the effects on the ventricles of rats. The introduction of ethanol both decreased the frequency of heartbeats (Bebarova et al. 2010). A possible theory on why alcohol decreases activity is due to ethanol increasing the activity of the inhibitory neurotransmitter GABA, thus decreasing action potentials (Leatherman et al. 2009).

With these effects in mind: how does the addition of the substances monosodium glutamate and ethanol affect the overall heart beats per minute of a *D. magna*? In order to give a fair comparison, the results will be compared to *D. magna* not yet exposed to the solution. The same *D. magna* will then be added into that specific trial's solution to keep information consistent. We hypothesize that introducing an excitatory additive, glutamate, will cause an increase in the heart beats per minute of the *D. magna*, whereas the ethanol solution will cause a decrease heart beats per minute due to the increased activeness GABA.

Methods

For the purpose of determining how different solutions affect the heart rate of D. magna, we exposed D. magna to both a 1% concentration MSG solution as well as a 1% concentration ethanol solution. We compared the heart rate of the D. magna after they had been exposed to these solutions for seven minutes. This was accomplished by observing the *D. magna* on a depression microscope slide under a microscope. The depression microscope slides allowed for us to keep the *D. magna* alive throughout the experimental procedure due to its ability to hold a sufficient amount of water in the depression. The two previously mentioned solutions were chosen because we wanted to analyze how the heart rate varied under different conditions and they were compared to *D. magna* that were only exposed to aquarium water.

For the experiment itself, we used a phone to readily record through the lens of a microscope adapter to record ten 10 second videos of a *D. magna* in the aquarium water. The video was then put into slow motion, allowing us to accurately observe and accurately calculate the heart rate of the *D. magna* during the ten second trials. Multiplying our ten-second results will give us the *D. magna's* heart rate in beats per minute. After collecting control data, the *D. magna* was exposed to one of the two solutions by adding four drops of the chemical dropwise then left alone for seven minutes. The same *D. magna* was only used twice and then replaced; as the control and testing one solution.

The two sets of results account for one trial and was repeated until we had eight trials of data: four of diluted MSG and four of diluted ethanol. The heart rate averages collected from the trials will be displayed with a box and whisker plot, allowing us to display the average heart rate and potential outliers in our data for analysis. The results of each trial will also be demonstrated in a bar graph comparing the differences specific to each individual *D. magna* and different trial. Further analysis was conducted by performing a One-way ANOVA, due to our data consisting of both nominal and measurement data with different variables. The One-way ANOVA allows differences between averages to be seen between the differing variables.

Results

According to Figure 1, the control aquarium water demonstrates that the *D. magna* had heart rates that were noticeably higher than other studies have shown. To compare, the diluted 1% ethanol solution yielded the smallest amount of heartbeats per minute, whereas the diluted monosodium glutamate solution averaged the highest total heartbeats per minute. The control solution had the greatest outlier, but also yielded the most similar results (Figure 1).

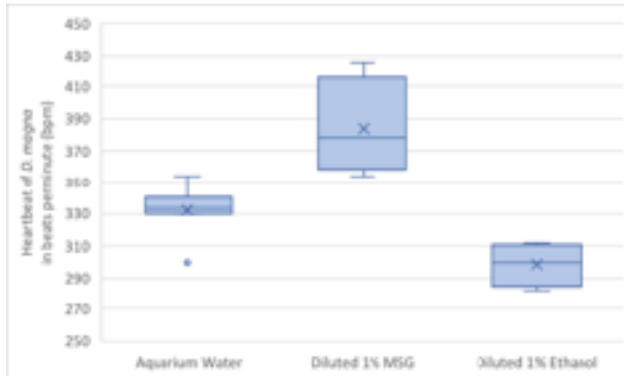

Fig 1. The box and whisker plot compares the rates of heartbeats of *D. magna* in different solutions: aquarium water, diluted 1% monosodium glutamate, and diluted 1% ethanol. The control aquarium water resulted *D. magna* had heart rates higher than other studies with an average of 332 bpm. The control also had the greatest outlier, with a minimum value of 300 bpm, however they also had the most similar with the majority falling into the 330-336 bpm range. The diluted monosodium glutamate solution averaged the highest total heartbeats per minute with a rate of 384 bpm and a high of 426 bpm. The diluted 1% ethanol solution yielded the smallest amount of heartbeats per minute, with an average of bpm of 298 and a low of 282.

According to Figure 2, each trial correlated with our hypothesis that when MSG was introduced, heart rate increased. Conversely, the addition of ethanol decreased heart rate (Figure 2).

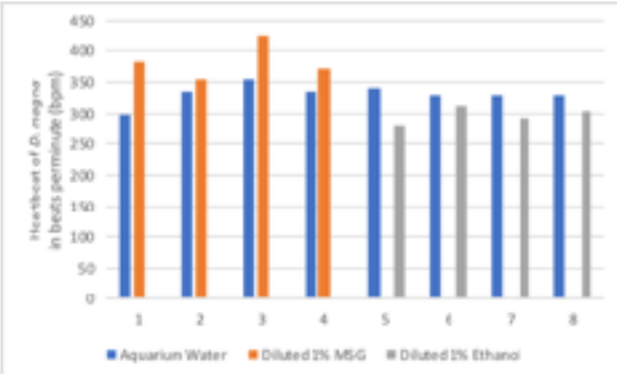

Fig 2. The bar graph compares the rates of heartbeats of *D. magna* in different solutions: aquarium water, diluted 1% monosodium glutamate, and diluted 1% ethanol. The *D. magna* from trial 3 in the diluted monosodium glutamate solution had the highest heartbeat rate with a high of 426 bpm. The *D. magna* from trial 5 in the diluted ethanol solution had the lowest heartbeat rate with a low of 282 bpm. The data correlates that the introduction of MSG increases heartbeats per minute, but ethanol decreases heartbeats per minute

Discussion

As shown in clearly Figure 2, the results agree with our hypothesis. The results show that introducing an excitatory additive, glutamate, caused an increase in the beats per minute of the *D. magna*. Conversely, the ethanol solution caused a decrease of beats per minute by introducing the active GABA (Figure 2).

According to other research performing similar tests on *D. magna*, the resting heart rate of *D. magna* under normal conditions should be approximately 200 bpm (Ebert, 2005). However, the results shown in Figure 1 show that our tested *D. magna* have a much higher heart rate with an average of 332 bpm (Figure 1). A possible confounding variable that could highly influence the outcome of our experiment is stress. The *D. magna* had to be manipulated physically and kept still under cotton to more easily measure their heartbeat under the light of the microscope. To attempt to counteract any further possible conflicting variables, the same *D. magna* was used for each trial, but a different one was selected when testing the different solution allowing for differences due to age or size to be minimized.

The results of our experiment can be applied and addressed in the food industry as American appetites especially are becoming more under scrutiny. With the similarities between the *D. magna* and human heart mentioned previously, the correlation both between the additives and their relative effects on heart rate can be applied to other physical effects the solutions may have on people. Further analyzing the effects of increased action potential firing from glutamate, and the decreased activity from ethanol, may help introduce why certain negative side effects are occurring after the consumption of both MSG and ethanol, and whether or not there are positive side effects as well. Should both additives be excluded from our diets? Could a complete removal of these substances be worse for our bodies? These are both questions that could be tested to better understand how these chemicals are actually affecting our bodies.

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