JUBLI
Journal of Undergraduate Biology Laboratory Investigations

# The circle of life: cyclic molecules increase the rate of heart's function as opposed to acyclic molecules among *Daphnia magna*

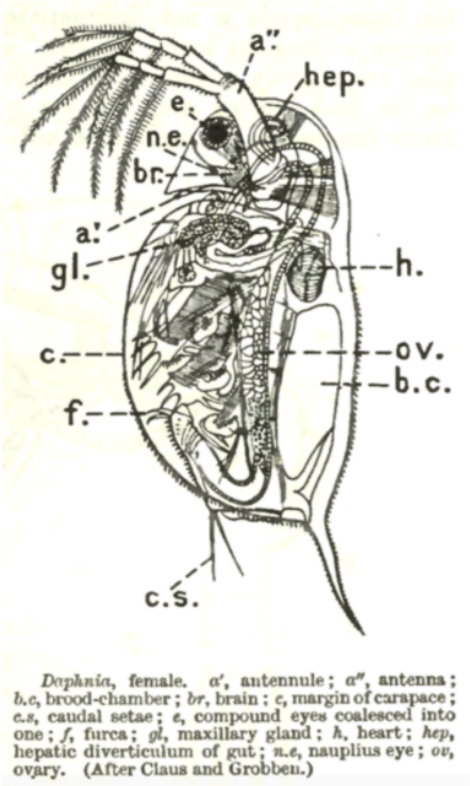
**Jazmyn Andre, Kaitlin Buie, Michael Ting, Traci Dubose***
**University of Oklahoma, Department of Biology, 730 Van Vleet Oval, Room 314**

## Abstract

*Daphnia magna*'s heart rate has been seen to differ with the use of certain solutions. During this experiment we showed how acyclic and cyclic molecules cause a different heart rate in *Daphnia magna*. We hypothesized that the reason that cells produce more action potentials is because they may have higher levels of acyclic molecules compared to cyclic molecules. The reason being, they are "simpler" and they are not as likely to bind to molecules during the action potential process. This was done by obtaining the bpm (beats per minute) of *Daphnia magna* before and after soaking them in MSG (monosodium glutamate-an acyclic molecule) and phenylephrine (a cyclic molecule) solutions. We found that there is the average percent change among the *Daphnia magna* that were soaked in the MSG solution resulted in a 9.54% change and average percent change among the *Daphnia magna* that were soaked in the phenylephrine solution resulted in a –17.0% change (Table 1). This confirmed our hypothesis however, other studies say otherwise. We also believe that there could be many other possibilities for the results we received.

## Introduction

*Daphnia magna* are commonly used to study certain drug's effects on the heart and its cardiac functions (Villegas-Navarro, 2003). This is due to the *Daphnia magna's* transparent body which, when light is shone through, most of its organs can be seen with clarity including its heart (see Figure 1). The usage of *Daphnia magna* is not only because of their transparency, but also because their ultrastructure is similar to that of a human's heart, being that the heart wall is proportionally thick for its size similar to that of humans, they contain epicardial and endocardial surfaces, and the location of the mitochondria of these cells is similar to that of humans, being on the surface (Stein et al., 1966). It is highly unethical and difficult to test within humans as we are checking the inner functions of the heart, making this model even more viable. However, much of why certain drugs effect the heart rate on an atomic or even molecular level are still unknown to this day.




*Daphnia*, female. *a'*, antennule; *a''*, antenna; *b.c*, brood-chamber; *br*, brain; *c*, margin of carapace; *c.s*, caudal setae; *e*, compound eyes coalesced into one; *f*, furca; *gl*, maxillary gland; *h*, heart; *hep*, hepatic diverticulum of gut; *n.e*, nauplius eye; *ov*, ovary. (After Claus and Grobben.)

**Figure 1** | Labeled Anatomy of *Daphnia magna* (New World Encyclopedia, 2017)

Acyclic and cyclic molecules are two classifications of organic molecules that are determined by whether or not the molecule has a carbon-based ring in it. The atoms of these molecules are connected via covalent bonds. Cyclic, molecule that contains a ring, are usually more complex and smaller in the third dimension relative to acyclic, molecules without a ring. Acyclic molecules are considered more complex due to the large aromatic rings resulting in interactions of π–π−stacking and edge-to-face contacts increasing contact area and energy (Meyer et al., 2003). A big factor in drug use among these molecules is that cyclic molecules used as drugs have the possibility of crossing over membranes, making it more readily faster to use (Newmann, 2018). Since acyclic molecules do not have the large aromatic rings, they may be even more so available to cross over membranes rather than cyclic molecules. The two chemicals we used to test the *Daphnia magna*'s heart rate were MSG (monosodium glutamate), an acyclic molecule and phenylephrine (a cyclic molecule containing 1 ring) (National Center for Biotechnology Information, 2005; National Center for Biotechnology Information, 2008). We hypothesized that some cells produce more action potentials because they may have higher levels of molecules that are acyclic rather than cyclic molecules because they are simple and less likely to bind to molecules in the action potential process. To test why some cells produce more action potentials than others, we will be comparing the change in heart rate using a pre-exposure heart rate and a post-exposure heart rate in our chemicals. We will know whether or not our hypothesis

is supported if the *Daphnia magna* in a MSG solution has the highest percent change in heart rate but will know if it is not supported if the *Daphnia magna* in a MSG solution does not have the highest percent change in heart rate or if the solutions have a very similar or the same change in heart rate.

# Methods

In order to test the effect of cyclic and acyclic molecules on change in heart rate, we obtained the pre-exposure and post-exposure bpm of the *Daphnia magna* using a light microscope to determine the change and percent change in the effects of that solution. This exposure consisted of soaking the Daphnia in solutions of MSG and phenylephrine for 7 minutes. We counted the bpm by taking a video of the heart beating in slow-motion for twenty seconds and multiplied the number of beats by three. Our control for the experiment was each *Daphnia magna*'s pre-exposure heart rate.

First, we were provided with the solutions of MSG and phenylephrine. At least three drops of this solution was needed for each trial. For MSG, a 1% concentration solution was used. For the phenylephrine solution, a 0.05% phenylephrine and 1.63% acetaminophen solution were dissolved in water and used.

We gathered 9 *Daphnia magna* from an aquarium in a 250mL beaker. Using a pair of scissors, cut the tip of a pipette about 1 inch from the tip at a 45-degree angle. Use this specially modified pipette to pipette one *Daphnia magna* onto a depression slide (we made sure the depression slide was concave down). A tiny wisp of cotton was used to restrict the movement of the *Daphnia magna* and to absorb some liquid. The slide was slipped into the microscope which was then turned on to the lowest magnification and a low light setting. We lowered the slide platform until the slide was in focus and used fine-tuning knobs for further clarity. A smartphone was attached to the microscope phone adaptor and was adjusted so that the camera was aligned with the lens. This phone mechanism was attached to the left lens of the microscope and we began to record in slow-motion for 20 seconds. This video was played back in slow-motion and the number of heartbeats were counted with a hand counter. We took out the slide and absorbed the liquid with a clean pipette. Then, 3 drops of the methacholine solution was placed into the solution and we let the *Daphnia magna* to rest in the solution for 7 minutes. After the allotted time had passed, this slide was placed back in the microscope, adjusted for clarity, recorded the *Daphnia magna* for twenty seconds, and noted the number of heartbeats using the steps listed above. This was done two more times using the 1% MSG solution and three more times each for the . 05% phenylephrine and 1% caffeine solutions. A different organism of *Daphnia magna* was used for each trial.

We first began analyzing our data by checking for normality using a Shapiro-Wilk W normality test on the averages of the trials. After, we concluded that all of our data followed a normal distribution and utilized a Paired t-test for further statistical analysis (see results). This was because we obtained our data using pre-exposure and post-exposure view. It was also because our data was considered normally distributed. Our

data was considered normally distributed because null hypotheses were all accepted at p>0.05. This was done using the PAST3 software.
The heart rate was calculated for each trial by multiplying the number of heartbeats in 20 seconds by three. The percent change was calculated using the equations: change= (post-exposure BPM – pre-exposure BPM) and % Change = (Change/pre-exposure BPM) x100. This percent change was averaged for each experimental group.

# Results

The percent changes in the bpm of *Daphnia magna* differed when different chemicals were used as expected. The average percent change among the *Daphnia magna* that were soaked in the MSG solution resulted in a 9.54% change (See Table 1). The average percent change among the *Daphnia magna* that were soaked in the phenylephrine solution resulted in a –17.0% change (See Table 1). This means that the heart rate of the *Daphnia magna* increased on an average of 9.54% when afflicted with the MSG solution but decreased on an average of 17% when afflicted with the phenylephrine solution.

The pre-exposure and post-exposure beats per minute (bpm) of *Daphnia magna* under the chemicals, monosodium glutamate and a phenylephrine, were ran using a Shapiro-Wilk W normality test. For the pre-exposure and post-exposure data when MSG was used, probability-values (p-values) of p=0.7735 and of p=0.1832were obtained. For the pre-exposure and post-exposure data when phenylephrine was used, p-values of p=0.7391 and of 0.6369 were obtained. Since p>0.05 among all of the data, the values were considered non-significant. Therefore, null hypotheses were accepted. This confirmed that all of our data was normally distributed.
A Paired t-Test was conducted to compare the effect of drugs with aromatic rings on the bpm of *Daphnia magna* in MSG and phenylephrine conditions. There was a significant difference between the two conditions; t (5)=0.33513, p=0.75113.

| | Trial 1 | Trial 2 | Trial 3 | Average |
|---|---|---|---|---|
| The % Change of bpm for *Daphnia magna* in MSG solution | 19.4% | 8.43% | 0.781% | 9.54% |
| The % Change of bpm for *Daphnia magna* in Phenylephrine solution | -15.6% | 5.71% | -29.8% | -17% |

Table 1: The data table above shows the percent change of bpm for *Daphnia magna* for three trials and its average in MSG and phenylephrine solutions. On average, the MSG solution caused the heart rate to rise whereas the phenylephrine solution caused the heart rate to lower.

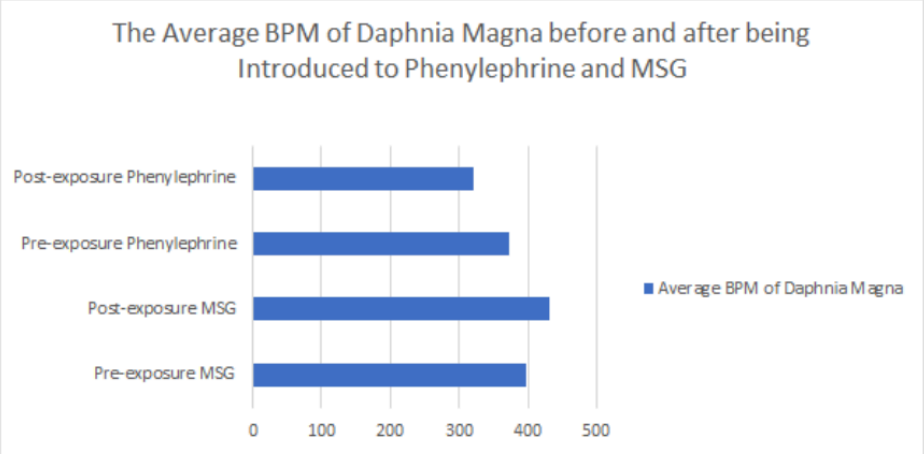



**Figure 2**: The graph above shows the average bpm of a *Daphnia magna*'s heart in pre-exposure and post-exposure of MSG and phenylephrine solutions among three trials. The average bpm of the *Daphnia magna* increased when it was exposed to the MSG solution by a value of 25 beats. The average bpm of the *Daphnia magna* decreased when it was exposed to the phenylephrine solution by a value of 53 beats.

# Discussion

Based on the data in Figure 3 and Table 1, our hypothesis was supported. This can be stated because generally, when the *Daphnia magna* were introduced to the MSG solution, an acyclic molecule, the heart's bpm increased on an average of 9.54% (See Table 1). However, when the *Daphnia magna* were introduced to the phenylephrine solution, a cyclic molecule, the heart's bpm decreased on an average of 17% (See Table 1). This allows us to assume that cyclic molecules cause higher frequency of action potentials, however with fault. Another study claimed that the consumption of MSG in humans lead to no significant increase in heart rate which was interesting as this is a very controversial topic (Raif et al., 2000). There was another study that agreed with our results. It stated that after an hour of *Daphnia magna* resting in phenylephrine solution, the heart rate generally slowed along with other chronotropic effects (Postmes & Prick, 1989). This leaves our hypothesis not yet concluded as some studies agree and some studies disagree with our results.

Based on the data collected and the Paired t-Test statistical test conducted, there is a significant difference between the average bpm of *Daphnia magna* heartbeats before and after being inflicted with both a MSG solution and a phenylephrine solution. This suggested that both of these solutions did indeed have their effects on the heart rate of *Daphnia magna.* However, this does not imply anything more than the afore mentioned. The solutions just had significant effect on the heartbeats but this may not be due to the acyclic or cyclic structures of the molecules. Our experiment can be used for further research in seeing correlations between cyclic and acyclic molecules with their effects on the body. There is a lot of research out there on the differences between acyclic and cyclic molecules so this idea may inspire other research projects down the road analyzing the effect of such molecules. It may also spur on further research on this specific

topic pertaining to these molecules' effects on the heart. Although this experiment was not conclusive in its hypothesis, the idea of it is much more important than its results.

Our experiment had one major error which was our limitations of variables and molecules used due to a time constraint. There could have been no correlation between the effect of the presence of aromatic rings. This can be stated with certainty because only two molecules were used and many other variables could have caused the results to be so. To prevent this mistake for further experiments, many more molecules should be used with multiple rings and of differing polarities as well just to control that variable. The correlation in our results may just be from the chemicals themselves and have absolutely nothing to do with the cyclic or acyclic structures because studies have stated that the decrease of heart rate due to phenylephrine was due to another factor in the adrenoreceptor agonists and antagonists that control these molecules (Postmes & Prick, 1989).

A very reasonable explanation for why cyclic and acyclic molecules may change the heartrate (if they do at all) lies in the crossing over membranes. Membranes functions are to separate polar and nonpolar molecules, large and small molecules, and those used within the contents of that membrane. On the polar and nonpolar topic, aromatic rings mainly consist of nonpolar carbon-hydrogen bonds since these atoms are what make up the rings. Polar molecules usually do not take the form in aromatic rings and are usually acyclic. This may be the reason that acyclic molecules act faster because the heart membrane and heart wall may be to block out nonpolar molecules however much more research should be done on this topic. This reason should also inspire further research on the topic of drugs that are polar and nonpolar or acyclic or cyclic and their activation time based on the different membranes they must cross to activate.

# Literature Cited


Geha, S., R. et al. (2000). Multicenter, double-blind, placebo-controlled, multiple-challenge evaluation of reported reactions to monosodium glutamate. Science Direct. 106 (5): 973-980.

Hammer & Harper. (2013). PAST3 (3.2) [Computer Software]. Oslo, Norway: https://folk.uio.no/ohammer/past/

Meyer, A., E. et al. (2003). Interactions with Aromatic Rings in Chemical and Biological Recognition. Angewandte Chemie Internation Edition. 42(11): 1210-1250

National Center for Biotechnology Information. (2005) Phenylephrine. https://pubchem.ncbi.nlm.nih.gov/compound/6041 (accessed on Apr. 1, 2019).

National Center for Biotechnology Information. (2008) Glutamic acid, Monosodium salt, D-. https://pubchem.ncbi.nlm.nih.gov/compound/23695358 (accessed on Apr. 1, 2019).

Newman, J., D. (2018). Bioactive cyclic molecules and drug design. Expert Opinion on Drug Discovery. 13(5): 379-385.

New World Encyclopedia. (2017, November 14). Daphnia. Retrieved April 1, 2019, from

http://www.newworldencyclopedia.org/entry/Daphnia

Postmes, Th. & Prick, I. (1989). The deceleration of the heart frequency in waterflea *Daphnia magna* by adrenoreceptor agonist and antagonist. Hydrobiologia. 171(2): 141-148.

Shaw, T. & French, D. (2018). Authentic Research in Introductory Biology, 2018 Ed. Fountainhead, Fort Worth.

Stein, J., R. et al.(1966). Ultrastructural Characterization of *Daphnia* heart muscle. J Cell Biol. 29(1): 168-170.

Villegas-Navarro, A. & Rosas-L, E. & Reyes, J. (2003) The Heart of Daphnia manga: effects of four cardioactive drugs. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 136(2): 127-134