

CBD Water and Nicotine: The Effects on *Daphnia magna*'s Neurogenic Pacemaker Not What You May Expect

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Abstract

The experiment was based on the question--because of the neurogenic pacemaker, will the neurophysiological effects of nicotine and CBD water be the same in daphnia as in humans? The research question addresses whether the daphnia hearts are similar to human heart. Our experiment addresses the question in a unique way because nicotine and CBD are used by humans to relieve anxiety and stress. During this experiment, we tested the effects of 10mM Nicotine and .06mM CBD Water on daphnia heart rate. The results were inconclusive in showing that the 10mM Nicotine solution and the .06mM CBD Water had any effect on the heart rate of daphnia.

Introduction

Nicotine and CBD are two things that are often used to relieve stress and anxiety. In this experiment we are going to look at how nicotine and CBD water change the heartbeat of daphnia. Our experiment is unique because we are examining whether the effects the neurogenic pacemaker of the daphnia respond similarly to human hearts when introduced to nicotine and CBD water. Unlike many vertebrates, *Daphnia magna* have a neurogenic heart which requires an input from the nervous system (Bekker & Krijgsman, 1951). Even though *D. magna* have a neurogenic heart that differs from

humans, it still reacts to many toxins similarly to humans and has other similar key mechanisms (Leatherman et al., 2009). The hearts of *D. magna* differ in that they are relatively thin and in most spots are only 1 cell thick (Stein, et al., 1966). Besides having a heart similar to vertebrates, *D. magna* are also a good animal to test because they are transparent (Villegas-Navarro et al., 2003). It has been shown in other types of daphnia that nicotine raises the heart rate (Corotto et al., 2010). However, the cardiovascular effects of nicotine have been shown to mainly contribute to the stimulation of the sympathetic nervous

system. (Haass & Kubler, 1997). In human trials CBD was shown to be safe and not play a role in how the heart was beating (Martin-Santos et al., 2012). Our hypothesis is that nicotine will raise the *D. magna* heart rate while CBD water will lower the *D. magna* heart rate like these drugs do in humans because daphnia hearts have been shown to respond similarly in the presence of specific drugs. If our hypothesis is supported, the *D. magna* will respond in a similar manner to humans when introduced to nicotine and CBD. If our hypothesis is not supported, the *D. magna* will experience the opposite effects when compared to humans.

Methods

Experimental Design

In order to test the hypothesis, we conducted a series of experiments using a 10mM Nicotine solution and a .06mM CBD Water solution to determine whether the solutions will have an effect on the daphnia's heart rate. We conducted five trials to account for variation within a population. The heart rate of the daphnia was measured in beats per minute because this is a good way to visually observe the direct neurophysiological effects of nicotine and CBD water on the neurogenic pacemaker of the daphnia. We collected the heart rate before and after drug exposure. The pre-drug exposure daphnia heart rate served as the control group when being compared to the experimental groups containing the 10mM Nicotine and .06mM CBD Water.

Procedures

With a pipette, the daphnia was transferred to the concave depression slide of the depression slide set. Once the depression is full of water and one daphnia, add the smallest wisp of cotton this restricted the

movement of the daphnia, but still allowed for a clear viewing window. The slide was placed on the mechanical slide of the microscope and the lowest magnification lens was in the front position. The daphnia was recorded in slow-motion for 10 seconds and replayed to make counting the heart rate easier. The pre-drug exposure heart rate served as the control group because it provided a good baseline heart rate.

After being recorded pre-drug exposure, the water from the depression slide was removed using a paper towel. Immediately, the 10mM nicotine solution was added to the concave depression slide of the depression slide set with the cotton and daphnia still inside. The daphnia was allowed to sit in the nicotine solution for 7 minutes. After the timer went off, the daphnia was recorded in slow-motion again for 10 seconds and then replayed to allow for easier counting of the daphnia's heart rate. The heart rate was counted using a hand counter then multiplied by six to get the total beats per minute. This process was repeated using .06mM CBD Water instead of 10mM Nicotine. The heart rate post-drug exposure served as the comparison group because it showed the effects that the drug had on the daphnia's heart rate.

Data Analysis

The heart rate for the daphnia was placed into four different categories, pre-nicotine, post-nicotine, pre-CBD water, and post-CBD water. We conducted a total of five trials per category to account for variation within a population. The percent change was then calculated. The percent change for each category was then placed into a box-and-whisker graph to show the range and variability of percent change. We also conducted a Paired t-Test using the

percent change from the categories previously stated.

Results

The data we collected was inconclusive in determining whether nicotine or CBD water had an effect overall. A Paired t-Test was conducted to compare the effect of Nicotine and CBD Water on daphnia heart rate. There not a statistically significant difference between the conditions: two-tailed P value equals 0.3165. In Figure 1, the largest percentage change was found in the CBD daphnia: -30.7%. The smallest percentage change was found in the nicotine daphnia: -1.45%. Overall, the average change in both categories could not be calculated because the data range was too

great. Outliers occurred in both CBD Water and Nicotine trials making it hard to see any trends.

Discussion

We tested the hypothesis the nicotine will raise the daphnia heart rate while CBD water will lower the daphnia heart rate like these drugs do in humans because daphnia hearts have been shown to respond similarly in the presence of specific drugs. Our results could not support or contradict our original hypothesis. Our results couldn't indicate any relationship is occurring between nicotine and CBD water and heart rate. This result is likely because of the age range of daphnia available, and possible unknown medical conditions. Based on our results and outside

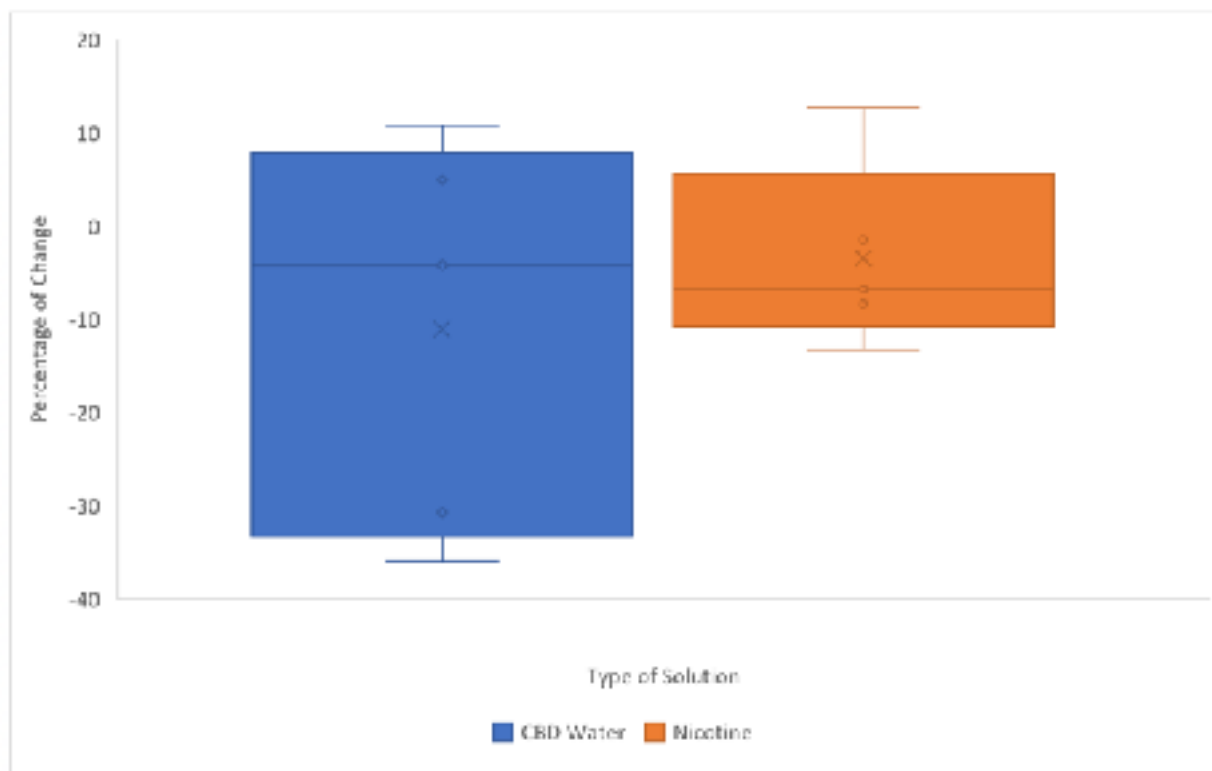


Figure 1. The percentage change for heart rates of daphnia before and after exposure to nicotine and CBD water after five trials. There is no trend concerning increasing or decreasing heart rate across the daphnia that were exposed to nicotine and CBD water.

sources, we cannot infer that the neurogenic pacemaker of daphnia reacts in any way when compared to humans after being introduced to nicotine and CBD water.

While we were observing the relationship between nicotine, CBD water, and heart rate, there could have been other factors that effected the daphnia's heart rate that we did not observe such as the neuropsychological effects that occur within the brain which must be viewed at a magnification level, we do not have access to. The daphnia were also being exposed to multiple chemicals prior to our experiment that could have damaged the neuroreceptors for nicotine and CBD water.

For future experiments, we suggest conducting more trials to allow for a broader view of the population of daphnia available. We also suggest reviewing the video as soon as it is taken to prevent conducting multiple trials that the heart is not seeable.

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