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CBD, the misleading stimulant: No effect on Daphnia magna heart rate

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Abstract

Quantum Cannabidiol Water (CBD) has become a popular substance in the treatment of anxiety, pain, and other medical conditions. Several studies have been conducted in order to prove CBD's effects on multiple health conditions, specifically human heart rate. These studies have shown that CBD, a stimulant, increases human heart rate at low and high doses. We proposed that testing the effect of CBD on *Daphnia magna's* heart rate would give us intel on how it affects organisms at a smaller dosage and therefore how it would affect the human heart. We conducted five trials and tested the heart rate of each Daphnia with and without being exposed to CBD. Our statistical analysis showed no significant difference in the Daphnia's heart rate before and after it was exposed to CBD. We expect this study to be of interest to those wishing to know how Quantum Cannabidiol Water directly affects the heart rate of humans.

Introduction

The human body has a massive series of complex pathways that help translate external sensations into chemical information processed by receptors and sent to the brain as electrical signals. The entire world is simply a translation of chemical gradients and energy pulses. The smallest sensation, an ant crawling across a hand for instance, initiates a cascade of chemical events within the body.

The ant crawling across the hand is first sensed by mechanoreceptors found on the skin and triggers the release of sodium ions inside a neuron. This specialized sensor is responsible for sensing touch and pressure. As the stimuli increases in intensity, more sodium ions are allowed to enter the neuron. As the sodium ions diffuse across the cell body, they build up at the axon hillock. An electrical pulse is released once the charge within the neuron reaches a threshold potential. This signal, referred to as an action potential, is carried across the network of neurons from your hand to your brain (Hoefnagels, 2018). The sequence of converting a stimuli to an electrical pulse fired by a neuron happens numerous times each second.

The neuron network and action potentials created can be affected by various chemicals. Stimulants and depressants can inhibit or promote the creation of action potentials or the efficiency in transportation. A study was conducted that tested the effects of certain stimulants (ouabain, verapamil, metaproterenol and metoprolol) on the heart rate of Daphnia. The study showed that ouabain and verapamil lead to an increase in heart rate, while metaproterenol only lead to an increase in heart rate at high concentrations. Metoprolol was unique and increased the heart rate at low concentrations and decreased the heart rate at high concentrations (Navarro et al., 2003).

To study the effects of cannabidiol water on heart rate and action potentials, we will observe the heart rate of Daphnia. *Daphnia magna* are small organisms that belong to the order Cladocera. They have a single chamber heart that can be seen through their transparent exterior. Despite being classified as an invertebrate, their heart has a self regulatory system similar to those of vertebrates, making them well suited for comparison to the human heart (Shaw and French, 2018). Studies have shown that CBD/ marijuana increases the heart rate of humans at both low and high doses (Dornbush et al., 1971). We hypothesize that CBD will lead to an increase in the Daphnia's heart rate because it is a stimulant and will increase the number of action potentials fired. CBD (cannabidiol) is a non-psychoactive component of marijuana that inhibits the uptake of neurotransmitters such as 3H-labelled norepinephrine, dopamine, γ-aminobutyric acid (GABA) and serotonin (Hershkowitz et al., 1977). The increase of neurotransmitters within the synaptic cleft increases the number of action potentials generated.

Our hypothesis will be supported if the Daphnia's heart rate significantly increases after being exposed to CBD. Our hypothesis will not be supported if the Daphnia's heart rate decreases or stays the same after being exposed to CBD.

Methods

For this experiment, we are testing the effects of Quantum Cannabidiol Water (CBD) on the heart rate of Daphnia. Five trials were conducted. Each trial compared the non-manipulated (Daphnia in normal water) heart rate with the manipulated heart rate (Daphnia in CBD solution) of the same Daphnia. A new Daphnia was selected for each trial. This allowed us to specifically measure the CBD's effect on the heart rate and eliminate the possibility of heart rate differences between individual Daphnia. We used a concentration of .06 mM of CBD which contained 20 mg of CBD per Liter of water. A Daphnia was extracted and transferred to a concave depression on a microscope slide. In order to keep the Daphnia in place when observing, a wisp of cotton was placed over the water to trap the Daphnia. A 10 second video was recorded and played back in slow motion to count the heart beats per minute using a hand counter. This measurement served as the baseline heart rate. A baseline measurement was obtained for each trial. The Daphnia was transferred to a new slide with a solution of 0.06 mM of CBD. After 7 minutes of the Daphnia being in the concentration, the heart rate was measured using the same method as before. This process was repeated in order to produce five replicates. Once the data was collected, it was entered into Past3 and tested for normality to determine the appropriate statistical analysis. The data was normally distributed, therefore we conducted a paired t-test. The data was then organized into a box and whisker plot to compare the average heart rate of the Daphnia before and after being exposed to CBD.

Results

The average heart rate for all five trials in the absence of CBD was 559.2 bpm. Trial 4 in the absence of CBD resulted in the highest heart rate at 630 bpm. Trial 5 in the absence of CBD resulted in the lowest heart rate at 456 bpm. The average heart rate for all 5 trials in the presence of CBD was 526.8 bpm. Trial 2 in the presence of CBD resulted in the highest heart rate at 594 bpm. Trial 5 resulted in the lowest heart rate at 378 bpm.

A p-value of $p=0.0944$ showed the data collected was normally distributed. A Paired T-Test was conducted to compare the effect of CBD on the heart rate of Daphnia in non-manipulated and manipulated conditions. There was not a significant difference between the two conditions; $t(df) = 8.2468$, $p = 0.10824$.

Discussion

The statistical analysis of the collected data did not support our hypothesis. Figure 1 shows similar heart rates between the non-manipulated and manipulated Daphnias' heart rates. In each trial, the heart rate of the Daphnia in CBD water was lower than the comparative heart rate in normal water. The resulting p value from the paired t-test does not indicate a significant difference between

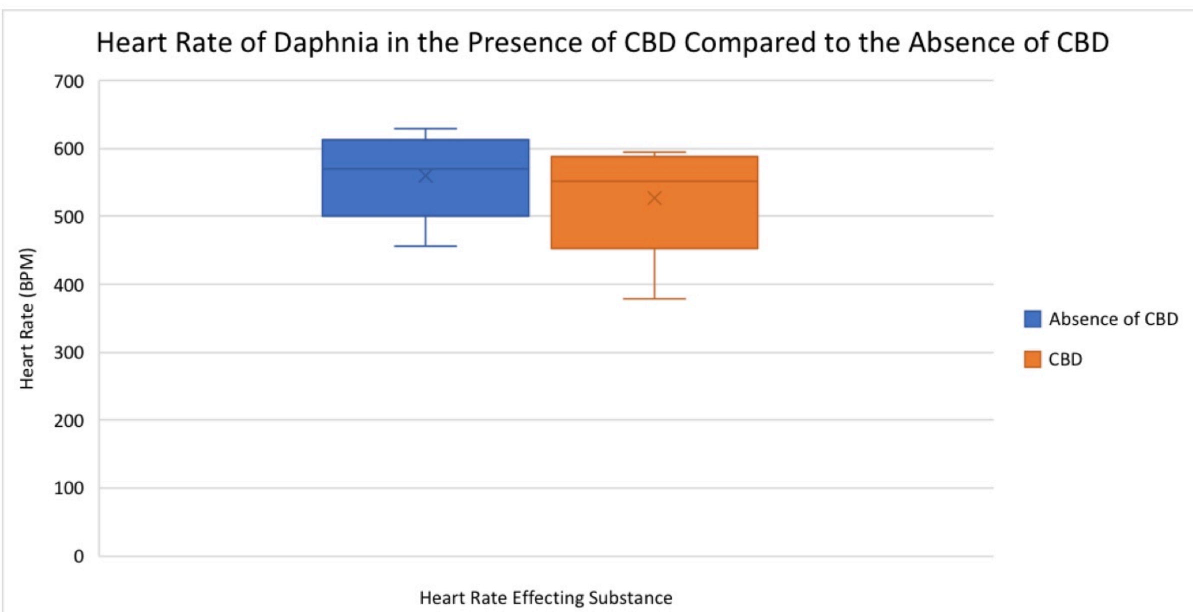

Figure 1 | Comparison of manipulated and non-manipulated Daphnia and the effect of CBD on their heart-rate. Five different Daphnia were tested producing 5 trials. Each trial had similar results on heart-rate; there was no significant difference of heart-rate when CBD was added.

the two heart rates before and after being exposed to CBD.

The molarity of the CBD solution could potentially cause damage to the synaptic terminals of neurons. CBD inhibits Na+, K+, ATPase, and Mg-ATPase activities. Electron microscopy showed highly damaged synaptosomal preparations using a 5×10^{-5} M solution. The uptake inhibition/ number of action potentials may have been the result of ATPase activity failure (Hershkowitz et al., 1977). The molarity of the CBD used in the experiment was higher than 5×10^{-5}, indicating the possibility that the number of action potentials fired were caused by the molarity of the CBD and not the CBD.

Action potentials require specific pH gradients to function properly. A study has shown that a fall in pH has the ability to activate nerves and ion channels, which promote the firing of action potentials (Bevan and Geppetti, 1994). This suggest that a more acidic solution can increase the number of action potential fired and potentially increase heart rate. This also indicates the possibility that the Daphnia's heart rate was influenced by the pH of the solution and not the CBD. For future experiments, we suggest controlling for the pH of the solution used.

For this experiment, we monitored five Daphnia. Due to natural differences in Daphnia heart rate, we suggest increasing the sample size in future studies to account for natural variations that affect heart rates. Factors such as pregnancy, age, gender, and environmental source will greatly affect the heart rate of the Daphnia. It is not uncommon for the heart rate of Daphnia to be between 120 to 450 beats per minute (Stein et al., 1966). The age and origin of the Daphnia will alter the heart rate. A population of young Daphnia will have a different heart rate than a population of older Daphnia. Additionally, the heart rate of Daphnia in fresh, unpolluted water will differ from heart rates of Daphnia found in water containing pollutants. It is also possible that CBD will affect the heart rate of a pregnant Daphnia differently than the a non-pregnant Daphnia. These attributes may change the effect of CBD on the heart rate.

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