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The Effect of Cannabidiol (CBD) on the Short-Term Memory of young *Drosophila melanogaster*

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Abstract

Research that is focused on memory is prominent in modern times because age-related memory loss is a growing issue throughout the world. Previous research has suggested that cannabidiol (CBD) can improve the memory of the elderly suffering from neurodegenerative diseases. However, the effect that CBD has on the memory of young people has not been extensively studied. Here we show that CBD does not improve the short-term memory of young male D. melanogaster. Our finding has contradicted the known knowledge of how CBD could potentially be used for memory loss. Our results suggest that exposure to CBD may result in impairment of the short-term memory and cause erratic behavior in young organisms. These outcomes could be a starting point for future study on the effect that CBD may have on young humans.

Introduction

Memory plays a major role in adapting to a habitat and acquiring various skills. Learning is essential for memory, and memory allows organisms to recall the information they learned and use it whenever they need to. There are two main types of memories pertaining to humans: long-term and short-term memory (Cowan, 2008). Long-term memory is a remembrance of events that have taken place at an early time of one's life and cannot be forgotten easily. Short-term memory is the remembrance of actions or events that have occurred recently and can be forgotten quickly (Cowan, 2008). There are important factors that can cause damage to short-term memory such as aging, physical injury, and substance abuse. Short-term memory impairment increases as humans age. Throughout the world, there is an estimate of 50 million people with dementia. This number is expected to grow by 10 million cases every year (World Health Organization, 2020).

Substance abuse has been a growing issue throughout the world. One of these substances is Cannabis, found in the Cannabis sativa plant, and is commonly known as Marijuana. Two major components found in Cannabis are Cannabidiol (CBD), which is not a mind-altering component and Tetrahydrocannabinol (THC), which is a mind-altering component (Schoeler and Bhattacharyya, 2013). CBD has several positive effects on the human body, such as reducing neuroinflammation, reducing brain damage caused by neurodegenerative diseases, promoting the production of new neurons in the brain, and raising levels of synaptic plasticity in the brain (Maroon and Bost, 2018). However, there are negative effects of CBD which include irritability, extreme tiredness, and nausea (Grinspoon, 2018). Previous studies have stated that CBD can improve the memory of people over the age of 65 with neurodegenerative diseases (McGuire et al., 2017). Currently, there is not a large amount of research on how CBD affects the memory of people under the age of 25 years old.

The current study aims to test if CBD improves the short-term memory of young male Drosophila melanogaster, commonly known as fruit flies. This organism is ideal for this study as it is easy to maintain, and it reproduces fairly quickly. Drosophila also have a short life span, which allows us to study short-term memory in a limited period of time. We hypothesized that exposure to CBD may improve the short-term memory of the male D. melanogaster when the aversive phototaxic suppression assay (APSA) is performed.

Materials & Methods

Fly stock and rearing conditions

A wildtype D. melanogaster Oregon R is used in this study. Flies were reared in tubes containing cornmeal media. They were flipped into fresh media every three weeks and were kept in a 20oC chamber. For this study, we used flies that were up to two weeks old.

Pilot Study: Testing the Amount of CBD To Use

Prior to performing the experiment, the amount of CBD oil (Fisher Scientific, 1 mg/mL CBD in 1 mL ethanol or methanol) that the flies were exposed to was determined by exposing the flies to fly food that contained differing amounts of CBD. The flies consumed food that contained 0.4 mL, 0.2 mL, 0.1 mL, 0.075 mL, or 0.050 mL of CBD. The CBD was mixed into the food along with 2 mL of water. Based on our data, we decided

that 0.050 mL of CBD (0.025 M CBD solution) was appropriate for our experiment. The control was the same amount of ethanol without CBD.

Pilot Study: Testing the Experimental Apparatus

Figure 1. This apparatus used for the phototaxis test and APSA test. Quinine hydrochloride solution was applied on the inside of the lighted tube (left). It was used to test whether the fly was sighted or not, was used for the learning and short-term memory tests.

The efficiency of the experimental apparatus we made for the current study was tested (Fig.1). Tube 1 contained 1.8 g of fly food, 2 mL of 1MΩ water, and 0. 05 mL of CBD solution. Tube 2 contained 1.8 g of fly food, 2 mL of 1MΩ water, and 0.05 mL of 95% ethanol as the control. The fly food containing ethanol was used as our control because the CBD was dissolved in ethanol. The students transferred 3 young, sighted flies into each tube, so they were exposed to these food conditions for 24 hours, and then the Aversive Phototaxic Suppression Assay was performed on each fly for 6 trials.

Phototaxis Test

The phototaxis test examines an organism's innate ability to move towards light (Nakamura and Yamashita, 1997). Each fly was transferred into a dark tube, which was covered in aluminum foil, and then the room was made dark. The fly was allowed to acclimate to the dark for 1 minute. Another tube was then connected to the dark tube, and a light was flashed from above on to the tube that was not covered in aluminum foil (lighted tube). The fly was given the option to move to the lighted tube or stay in the dark tube. The fly that was positively phototaxic moved towards the light (Nakamura and Yamashita, 1997) if they were sighted within 30 seconds. We performed the phototaxis test to eliminate the blind flies (or those with abnormal visual function) for the APSA.

Aversive Phototaxic Suppression Assay (APSA)

This test trained the fly to remain in the dark side of the apparatus (Seugnet et al., 2009). Prior to the Aversive Phototaxic Suppression Assay (APSA), the flies were exposed to either diets (CBD-infused food or control food) for 24 hours. Then they were starved for 6 hours before starting the Aversive Phototaxic Suppression Assay. In this assay, two plastic tubes were used, one was covered in aluminum foil to create darkness and the other one was left uncovered. The uncovered tube was coated with a 1M solution of quinine hydrochloride, a bitter substance that repels the flies (Hayes et al., 2015). Each fly was transferred to the dark tube, the room was made dark, and the fly was allowed to acclimate to the dark for 1 minute. The uncovered tube containing quinine was then connected to the dark tube. A white light from a smartphone device was flashed on the uncovered tube and immediately a timer was started when the two tubes were connected. The timer was stopped once the fly touched the quinine on the lighted side of the tube. The students performed 10 trials and after each trial, the fly was tapped back into the dark tube and was allowed to rest for 30 seconds and re-acclimate to the darkness. After the learning test was performed, the flies were starved again for 6 hours so that their short-term memory could be tested. The memory test was just one trial. It is the same procedure as the learning test to determine if the flies remember what they had learned 6 hours ago.

Results

First, we measured how many trials it took for the young male flies to learn. To do this, we performed the APSA (Materials and Methods). During the 10 trials, the flies exposed to the control food did not show a significant change until the 7th trial (Fig. 2b). There was a significant difference after the 8th trial. We interpreted this as the flies in the control group learned at the 8th trial. In the CBD treatment group, we found that the flies did not show a significant change throughout all 10 trials (Fig. 2c). We interpreted this as the flies in the CBD group did not learn at all.

We found that there was a wide variation of average avoidance times for each trial. (Fig. 2c) This could be the result of the CBD effect on the flies. We observed that some of the young male flies that were exposed to the CBD-infused food had very erratic behavior, which is described by abnormal movements of the flies, while others had sluggish or normal behavior. This could explain why the average avoidance times varied.

Second, we tested the effect of CBD on their short-term memory. To do this, the flies were starved for 6 hours and then the APSA was repeated once (Materials and Methods). We found that the flies exposed to the control food did not surpass the threshold avoidance time (Table 1). We interpreted this as the flies not remembering what they had learned. In the CBD treatment group, we could not calculate a threshold avoidance time because the flies did not learn throughout the 10 trials of the learning test. Therefore, since the flies did not learn, it is impossible for them to have remembered.

Fly	*Treatment*	*Sex*	*Avoidance time*
Fly 1	Control	Male	14.01
Fly 2	Control	Male	70
Fly 3	Control	Male	55.3
Fly 1	CBD	Male	300
Fly 2	CBD	Male	247
Fly 3	CBD	Male	4.49
Fly 4	CBD	Male	21.31

Table 1: Table shows the results of the memory test for flies exposed to control and CBD-infused food. The threshold avoidance time for the control group was 112.9 s. The threshold avoidance time was calculated by taking the average of the averaged trials that the flies learned in. (Threshold for control flies: average of the mean trials 8, 9, and 10). The flies whose avoidance times passed this threshold remembered what they learned. According to the learning test results, none of the control flies remembered what they learned. The flies exposed to CBD-infused food did not learn, so they do not have a threshold avoidance time. Since the flies did not learn, they did not remember.

Discussion

This study focuses on the effect of CBD on young male D. melanogaster's short-term memory. In order to do this, APSA was performed to observe whether a CBD-infused diet improved their learning or not. Using the APSA, 10 trials were conducted for the learning test, and then a 6-hour starvation gap was given before performing only one trial of the APSA again to test their memory. Figure 2 shows the results of the APSA tests for the control and CBD treated flies. We hypothesized that the CBD group would learn quicker than the control group. However, our results showed that the flies in the CBD group did not learn at all. Fig.1b shows a graph of the 10 trials of the learning test for the flies exposed to control food. The results of the Kruskal-Wallis test showed that trials 1-7 showed no significant learning in the flies ($p>0.05$), and trials 8-10 showed no significant learning in the flies ($p>0.05$). A Mann-Whitney test was done comparing trials 1-7 to trials 8-10 ($p<0.05$). This shows that the flies did not learn at the 8th trial as the control flies did. Also, since the results of the learning test in the CBD treated flies was erratic, we concluded that the flies did not learn at all during the 10 trials.

Fig.1c shows a graph of the 10 trials of the learning test for the flies exposed to CBD-infused food. The results of the Kruskal-Wallis test showed that trials 1-7 had no significant learning in the flies ($p>0.05$), and trials 8-10 showed no significant learning in the flies ($p>0.05$). A Mann-Whitney test was done comparing trials 1-7 to trials 8-10 ($p>0.05$). This shows that the flies did not learn at the 8th trial as the control flies did. Also, since the results of the learning test in the CBD treated flies was erratic, we concluded that the flies did not learn at all during the 10 trials.

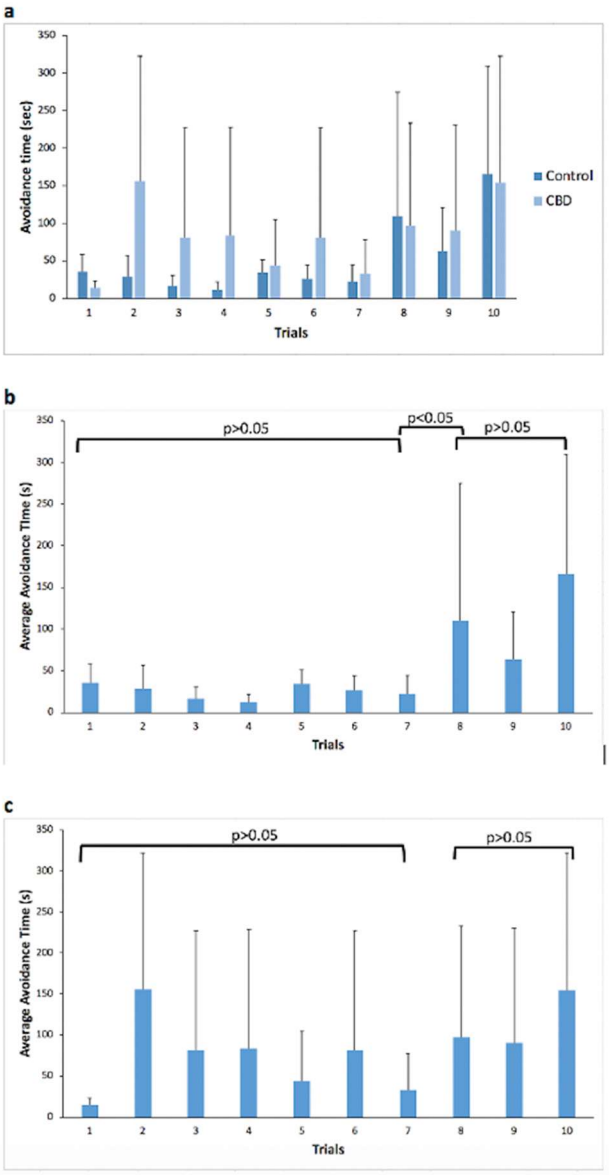

Figure 2: Results of the Phototaxic Suppression Assay. a) Graph comparing the average avoidance times for each trial in the learning tests for male D. melanogaster in the control and CBD diets. b) Graph showing the 10 trials of the learning test for the flies exposed to control food (n=3). The p-values show that the flies learned at trial 8 ($p<0.05$). c) Graph showing the 10 trials of the learning test for the flies exposed to CBD-

infused food (n=4). The p-values show that the flies did not learn at any trial.

Finally, we repeated one trial of the APSA 6 hours after the learning tests to determine if the flies remembered what they had learned. The threshold avoidance time for the flies exposed to the control food was 112.9 seconds (Table 1). Since none of the flies exposed to the control food surpassed the threshold avoidance time during the memory test, we conclude that none of them remembered what they had learned. A possible reason for this could be that the ethanol had a psychiatric effect on the flies that impaired their short-term memory. For the CBD group, it was impossible to calculate a threshold avoidance time because they did not learn. As a result, we cannot make conclusions about the CBD treated flies' memory since they did not learn. However, it is interesting to note that fly 1 and fly 2 had a higher avoidance time compared to fly 3 and fly 4 (Table 1). Here, 2 groups can be seen, one group where there is high avoidance time and one group where the avoidance time is low. We assume there may be a psychiatric effect on each fly causing each one to behave differently compared to another fly. The CBD may not be 100% pure and may contain another major component of cannabis called tetrahydrocannabinol (THC) which has a mind-altering effect. This may be a reason why the flies behaved in a contrasting manner. Another factor could be that the flies may have had a genetic variation that was contributing to affecting each fly differently.

We conclude that CBD has an inhibitory effect on the short-term memory of male Drosophila melanogaster as they did not learn during the learning test nor did they remember during the memory test. We found that time was an essential component to perform this experiment. We were also researching other variables such as sex difference and age difference, but our sample size was too small due to the death of many flies.

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