Cannabinoids and Neurodegeneration: Using *C. elegans* as a Model System to Determine the Effectiveness of CBD as a Treatment for Parkinson's Disease

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Abstract

Parkinson's Disease (PD) is a neurodegenerative disease that causes a loss of control in motor function as it progresses. Our project analyzed the ability of Cannabidiol (CBD) to restore movement to or protect against neurodegeneration in *C. elegans* nematodes with chemically induced PD using 6-OHDA (6-hydroxydopamine). Experiments were conducted in liquid media and recorded under a light microscope for further analysis. The number of times that each worm "turned" was scored to determine the relative activity of each worm. Our results suggest that there may be a benefit to using CBD as both a preventative measure and a treatment for physical symptoms of PD, as there is an increase in relative turn activity seen in nematodes treated with CBD both before and after chemical induction of PD.

Acknowledgments

We would like to begin by thanking the Biology and Biotechnology Department for equipping our team with the necessary materials for our project. This could not have been made possible without them.

We would also like to extend our immense gratitude to our advisors Professor Mike Buckholt, Professor Jill Rulfs, and Professor Jagan Srinivasan for their generous support throughout the entire year. Without their expertise in this subject and their generosity, this project would not be possible. A special thanks to Professor Jagan Srinivasan for providing us with the nematodes and various husbandry methods we needed to kickstart our project. We are truly thankful to our advisors for their help along the way—we are highly appreciative of the ability to work on this project.

Table of Contents

Introduction

Impact of Neurodegenerative Diseases

Neurodegenerative diseases impact the lives of millions of people worldwide. One of the most common neurodegenerative diseases is Parkinson's Disease (PD) with 7 to 10 million people affected worldwide (Butters, 2015). "Parkinson's disease is a motor system disorder attributed to the loss of dopamine-producing brain cells" (National Academies of Sciences, Engineering, and Medicine, 2017). PD is a disease that affects physical movement causing tremors, slow movement, and difficulties with balance, speech, and coordination (National Institute of Environmental Health Sciences). In 2022, a study supported by the Parkinson's Foundation found that the number of people in the US diagnosed with PD had increased drastically. It was discovered that around 90,000 people are diagnosed with PD each year in the US. The same study found that the increased rate of PD is correlated with the increasing average age of the population since the primary risk factor for the disease is age. By the year 2030, it is predicted that 1.2 million people in the US will be living with Parkinson's disease (Parkinson's Foundation, 2023).

What's the Big Deal?

People suffering from PD are always looking for alternative methods to alleviate their symptoms. Due to CBD's anti-inflammatory and antioxidant properties, many people have turned to CBD as a potential therapeutic. Through using *C. elegans* as a model system, we hope to learn more about the effectiveness of using CBD to alleviate the symptoms of PD

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Neurodegenerative Diseases—Neuronal Degradation and Worsening Symptoms

There are several neuron types that when degraded can cause the onset of various neurodegenerative diseases, but the neuron type that plays a large role in the development of Parkinson's is dopaminergic neurons. Dopamine (DA) is a brain hormone that is synthesized by substantia nigra (SN) dopaminergic neurons which have axon projections in the striatum (Zhou et al., 2023). As a brain neurotransmitter, DA is released from the presynaptic membrane to the synaptic cleft, where it then binds and activates DA receptors on the postsynaptic membrane (Zhou et al., 2023). Progressive degeneration of dopaminergic neurons reduces DA content in the SN and striatum which ultimately triggers the onset of PD clinical symptoms such as tremor, postural instability, bradykinesia, and muscle rigidity (Zhou et al., 2023). These neurons when degraded cause a significant decrease in the dopamine uptake and signaling cascade within the brain that directly affects different neurological functions and behaviors shown above (Dias et al. 2013).

The preservation of redox homeostasis, cell cycle signaling, and hormone production which all play a role in conserving neuron health rely on maintaining controlled levels of intracellular reactive oxygen species (ROS). When uncontrolled, the result of this imbalance between oxidants and antioxidants is oxidative stress, the consequences of which are oxidative modifications of lipids, nucleic acids, and proteins (Esposito et al., 2011). The increase in ROS leads to the activation and transduction of Nuclear factor-κB (NF-κB)—transcription factor protein complexes—with the subsequent production of pro-inflammatory cytokines. These can both directly engage in the induction of inflammation and act indirectly through promoting the differentiation of inflammatory T cells (Liu et al., 2017). More specifically, these pro-inflammatory cytokines/mediators are "debris" from pathogens or damaged cells, and they

activate the resting microglia to express pro-inflammatory factors. When regulated, these complexes restrict neuroinflammatory responses shown in Figure 1, but when unregulated lead to the onset of inflammation, leading to uncontrolled levels of ROS and worsened neurodegenerative symptoms. Overall, the increased proportion of pigmented dopaminergic neurons with nuclear NF-κB suggests that over- and under-expression of the transcription factor, essential to its activation, may be related to the pathophysiology of PD (Hunot et al., 1997).

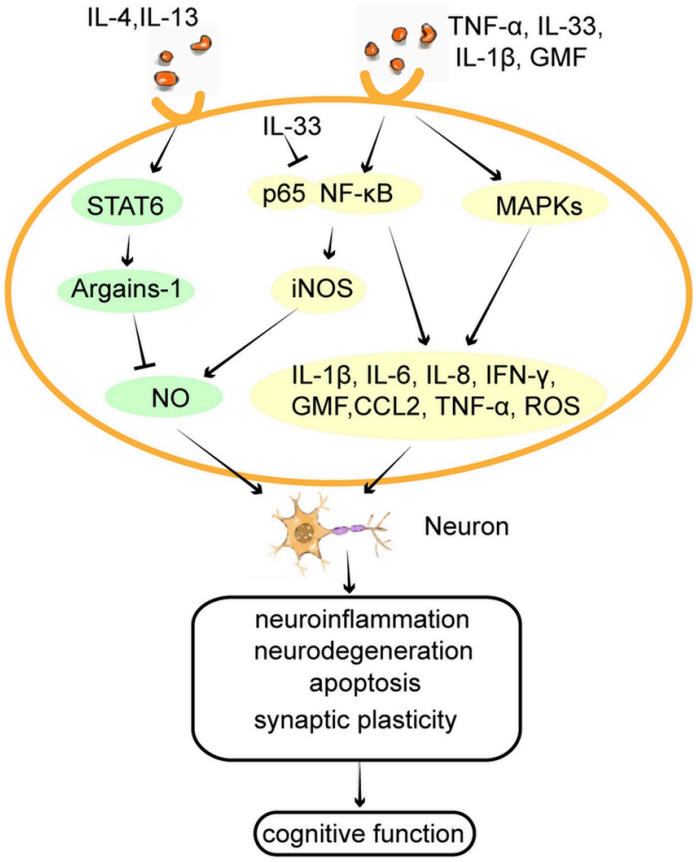

Figure 1: Inflammatory cytokines intracellular pathways when properly regulated. (1) IL-4 and IL-13 induce arginase I expression (2) High levels of IL-33 and other pro-inflammatory molecules activate the NF-κB and MAPKs pathways (induces pro-inflammatory cytokines, chemokines, and neurotoxic mediators (IL-1β, IL-6, IL-8, IFN-γ, TNF-α…and ROS in astrocytes and microglia)). (3) IL-33 binds to p65 in the nucleus as a transcription factor where it directly inhibits the nuclear translocation of NF-κB. This then inhibits the NF-κB downstream pro-inflammatory signaling pathway and suppresses the inflammatory response. (4) These IL-33-induced inflammatory mediators act on neurons and modulate neuroinflammation, neurodegeneration, apoptosis, synaptic plasticity, and cognitive function (Rao et al., 2022).

The brain utilizes 20% of the body's oxygen supply with a significant amount of this oxygen used being converted to ROS, and with these elevated levels of ROS in the brain it is important to maintain a controlled system to allow the brain to regulate ROS (Dias et al. 2013). The neurotoxin 6-hydroxydopamine (6-OHDA) is an agent that induces oxidative stress and encourages dopaminergic neurodegeneration in *C. elegans* and other model organisms (Offenburger et al., 2018). 6-OHDA is translocated into dopaminergic neurons by the dopamine transporter and this leads to oxidative stress by blocking complex I of the respiratory chain—highly important for apoptosis cell proliferation with the various activated stress response pathways (Sharma et al., 2009). When *C. elegans* are exposed to 6-OHDA, dopaminergic neurons gradually die. The decrease in these dopaminergic neurons leads to the increase of ROS, causing the onset of neurodegenerative disease-like symptoms in the *C. elegans*.

Potential Therapeutics

Existing Treatments for Parkinson's

There are currently no finite cures for PD, but there are several pharmaceutical therapies that have started clinical trials for PD treatment. The main types of therapeutic strategies used in these clinical trials include gene, cell, and plasma therapies, monoclonal antibody or vaccine therapies, herbal extract therapy, and the largest proportion of therapeutic strategies in clinical trials are small molecule therapies. Of these small molecule therapies dopamine receptor agonists are the main class that mimic normal dopamine function within PD patients (Prasad & Hung 2021). This therapeutic strategy is approached by having the patient take an oral dose of

the dopamine receptor agonist to increase the number of molecules in the brain that will bind to the dopamine receptors and kickstart normal signaling pathway function in patients with PD.

Plasma therapy has also emerged as a highly specific therapeutic due to the infusion of young plasma successfully targeting α-synuclein, a neuronal protein that regulates synaptic vesicle transport and subsequent neurotransmitter release (Stefanis 2012). Modulating neurotransmitter release aims to make up for the loss of function in affected neurons. While the reduction of α-synuclein levels did allow improvement of physical symptoms of the disease in PD patients, aggregation of this protein is not the sole cause of PD in humans and this therapy does not account for the several other potential causes of PD, so it is not the best option for the general population of those with PD.

More therapies include gene and cell therapies, with the purpose being to target many more causes of PD at once. Cell-based therapeutic strategies involve transplanting new dopamine-producing cells into the brain to reduce neurological inflammation. The introduction of embryonic dopamine cells was found to have more of a benefit in reducing physical symptoms of PD in younger patients but not as significantly in older patients (Prasad & Hung 2021). While cell therapy was found to have success in multiple phases of clinical trials, this type of therapy was likely to cause immunosuppression and potential genetic changes that could lead to the development of cancer.

Lastly, gene therapy has been used for PD treatment through genetic engineering of the genome of PD patients essentially to have these new genes knockout, replace, or positively alter defective genes in patients with the disease (Prasad & Hung 2021). The overall goal for introducing these engineered genes is to decrease the death rate of dopaminergic neurons and increase the stimulation of neurotropic action, which in both cases would improve the motor

function that is negatively affected in PD patients. The main concern with this method of therapy is the potential for further impairment to occur to one's motor function, damage to the dorsal root ganglia, and further loss of coordination and voluntary muscle movement. Overexpression of these engineered genes can also lead to severe toxicity in the organ or tissue of the transgene targets (Prasad & Hung 2021).

Therapeutic use of CBD

The CBD product industry has experienced tremendous growth, mostly because CBD is widely advertised as an effective therapeutic for a wide range of health conditions. More specifically, CBD has been used as a therapeutic to accommodate a multitude of medical conditions that include seizures, anxiety, pain/inflammation, schizophrenia, substance use disorders, and PD. In an open-label trial, 13 Parkinson's patients received CBD (Epidiolex®) titrated from 5 to 20 to 25 mg/kg/day, for 10–15 days (Sholler et al., 2020). The ten participants who completed the study experienced improvements in total and motor scores on the Unified Parkinson's Disease Rating Scale (UPDRS) and self-reported emotional/behavioral dyscontrol scores (Sholler et al., 2020). With very few clinical trials, it is hard to formulate conclusions on the efficacy of CBD in treating Parkinson's Disease. Still, there is promising and preliminary evidence that it can, which fuels the need for more extensive research. Overall, advanced and controlled evidence for the therapeutic efficacy of CBD is significantly lacking for PD, which is why we hope that our project can add to the lack of data on utilizing CBD as a therapeutic to control the symptoms of PD.

Use of CBD for Neurodegenerative Diseases

It is well known that neurodegenerative diseases are severely lacking treatment options. However, in recent studies, several researchers have introduced CBD as having a fundamental and promising neuroprotective effect with antioxidant and anti-inflammatory properties in neurological disorders (see Figure 2) by the targeting of neuroinflammation, reducing some proinflammatory cytokines, reactive oxygen species as well other neurotoxic factors (Pagano et al., 2023). CBD's proposed interactions are seen in the endocannabinoid system and the one-carbon metabolism (OCM) pathway. Within this pathway, CBD has been observed to act as a regulator by interacting with methionine levels, an integral molecule to OCM and its interactions with CBD. It has been found that CBD reduces methionine levels in a glycine cleavage system-dependent manner and it has been observed that methionine restriction reduces oxidative stress, which this project will aim to follow.

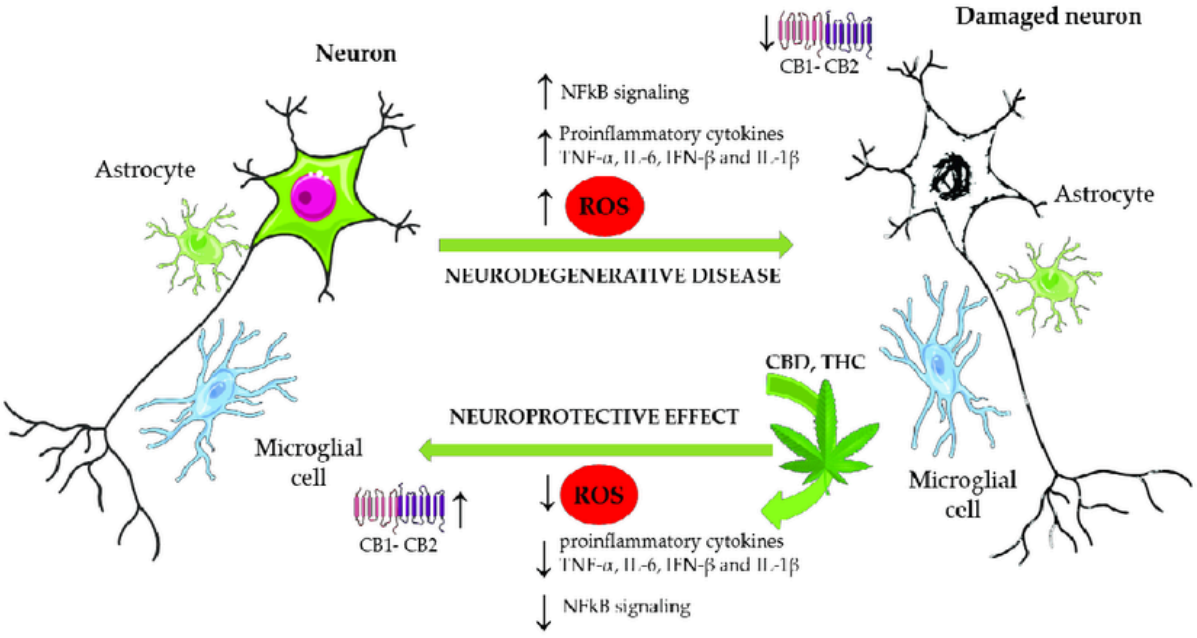

Figure 2: Flowchart diagram of the effects of neurodegenerative disease on a healthy neuron (left to right) in comparison to the neuroprotective effects of CBD and THC on a damaged neuron (right to left) (Pagano et al., 2023b)

Symptoms of neurological and inflammatory diseases such as PD worsen and are enhanced when levels of ROS within the cell elevate beyond typical amounts with damage in lipid, protein, and DNA molecules. Many researchers agree that CBD is an appealing therapeutic agent for neuroimmune disorders, as it can affect the redox state in many ways (Pereira et al., 2021). In a study analyzing how ROS plays an essential role in dopaminergic neurodegeneration and dysregulation in PD, researchers found that after 24 hours, CBD-treated N2 worms were found to have less ROS than the control group (Guedes et al., 2023). We will also utilize the *C. elegans* model to determine whether or not ROS can be stabilized to improve symptoms related to PD. CBD modulation of oxidative stress is the basis of its effectiveness in alleviating the symptoms of disease. Research has also shown that CBD may account for a significant reduction of neuronal cell death, due to its ability to scavenge reactive oxygen species and reduce lipid peroxidation (Iuvone et al., 2004). With the decrease in ROS, we hope that the effects of neuroinflammation and neurodegeneration in Parkinson's can prove to be inhibited with our model.

Indeed, cannabinoids can mitigate inflammation, reduce CNS spasticity, and alleviate neuropathic pain Pereira et al., 2021). Cannabinoids have shown therapeutic value against inflammatory and neuropathic pains by providing neuroprotection following injury or inflammation in the CNS (Manzanares et al., 2006). With the increase in neuroinflammation that results from these neurodegenerative diseases, we will test to see if there is a possibility that some effects of Parkinson's could be reversed or alleviated.

C. elegans for Modeling Neurodegeneration

C. elegans is a nematode species used to model many different biological processes and pathways found in humans and other mammals. They are widely utilized as a model system due to their basic physiology and behavior, their well-defined neuronal circuitry, and the inherent similarities in neuronal function to mammals. Within the neuronal circuitry in *C. elegans,* many major functional components of mammalian neurotransmitters are conserved. Numerous orthologs and paralogs exist between specifically humans and *C. elegans* that include 7943 genes of the *C. elegans* protein-coding genome (Caldwell et al. 2020). The *C. elegans* used in experimental settings are self-fertilizing hermaphrodites that will produce roughly 300 offspring in their lifespan. These progenies will be genetically identical to their parent organism allowing for genetic predictability and consistency throughout the experimentation process (Cooper et al. 2018). In *C. elegans*, eight dopaminergic neurons are mapped, and these alongside their endocannabinoid system are conserved in humans as well (Sulstan et. al, 1975; Harrington et. al, 2011). The endocannabinoid system present in *C. elegans* utilizes CB1 and CB2 receptors in neurons specific to cannabinoids, which allows cannabidiol (CBD) to bind. CB1 and CB2 are the most common cannabinoid receptors in the endocannabinoid system, and due to their effect on inflammation pathways, using CBD to activate these receptors could be useful in preventing inflammation. Since these receptors are conserved in both humans and *C. elegans*, nematodes have become a prime target for study regarding treating neurodegeneration in humans (Oakes et al., 2019).

In *C. elegans*, dopaminergic neurons play a large role in locomotive behaviors such as runs and turns in response to positive stimuli or negative stimuli, as well as affecting *C. elegans* behaviors and patterns surrounding their egg-laying process (Sawin 2000). The effects on

locomotion/static movement due to dopaminergic neurodegeneration have been defined by observing changes in *C. elegans* turns and runs in response to the progression of neuronal Damage (Dias et al. 2013). *C. elegans* can also model oxidative stress (Dias et al. 2013). Through chemical subjugation, oxidative stress can be induced within an organism which will eventually cause the degradation of several neuronal cell types including the dopaminergic, and the subsequent onset of Parkinson's symptoms (Dias et al. 2013).

PD is a neurodegenerative disease that causes a loss of control in motor function as it progresses. Understanding the connection between neuronal degradation, oxidative stress, and neuroinflammation is crucial for developing effective PD therapies, as well as therapies for other neurodegenerative diseases. Our team wanted to take on this project to determine a model system for analyzing the ability of Cannabidiol (CBD) to restore movement to or protect against neurodegeneration. The model system we successfully established uses the nematode species *C. elegans* with chemically induced PD using 6-OHDA (6-hydroxydopamine) and administering CBD at predetermined concentrations as the treatment or therapeutic. We hypothesized that if we use CBD as a pre-treatment and a post-treatment for 6-OHDA-induced Parkinson's Disease in *C. elegans*, we will see a more positive effect from the CBD pre-treatment through the conservation of turns. Our results suggest that there may be a benefit to using CBD more so as a treatment for physical symptoms of PD rather than as a preventative, as there is an increase in relative turn activity seen in nematodes treated with CBD after chemical induction of PD.

Methods

C. elegans Maintenance

The wild-type strain N2 worms were maintained at 20°C, and grown on NGM (nematode growth medium) plates with OP50 *E. coli* as a source of food. As *E. coli* ran out on plates, worms were transferred to additional plates spotted with *E. coli* by picking worms individually. A diagram of this process can be seen in Figure 3.

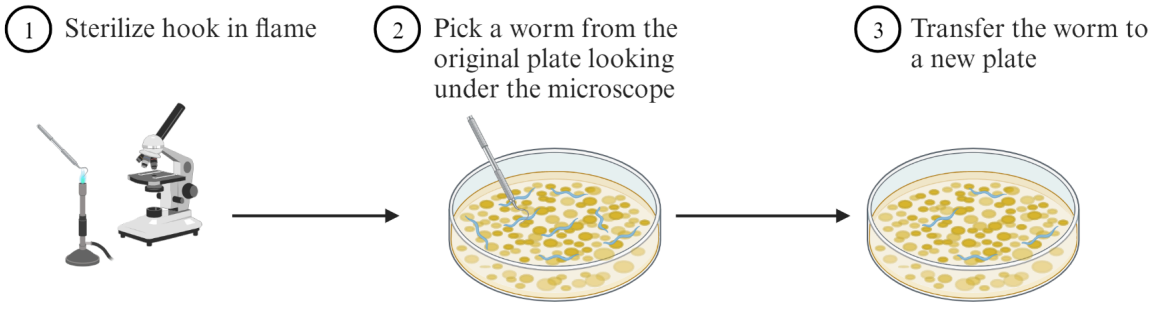

Figure 3: Diagram describing the upkeep of worm strains to be used for testing.

Strains were also maintained through two different bleaching methods which were used to collect eggs from worms to be grown on different plates. One method was a much faster process while the other was more detailed. The "quick" method involved scraping a clump of bacteria with a large number of worms and transferring them to a plate that had previously been spotted with *E. coli* in an area of the plate that had no bacteria growing. Bleach solution was then added to the clump of worms and was put back into the incubator to dry. The more involved method of worm bleaching included washing the plate with M9 to loosen the worms from the media, extracting them using a pipette, pipetting them into a 15mL conical tube, and allowing the mature worms to settle while the adolescent (non-egg carrying) worms remained on top, aspirating the adolescent worms, adding bleach to kill the mature worms while preserving the

eggs, and adding an M9 wash to dilute the bleach before replating on the NGM plates. This allows for the eggs to be removed from the mature nematodes and the generation to be synchronized as a constant variable.

Mortality Testing

Various mortality tests were performed to determine the ideal concentrations of different solutions to use during behavioral assays. Mortality tests were performed with the M9 solution, CBD, and 6-OHDA, as well as combinations of each of these to ensure that they did not interact to affect worm lifespan negatively. For the M9 mortality test, a series of dilutions were performed with distilled water. 100uL of each concentration was added to a 96-well plate and 3 worms were added to each well and given 30 minutes to equilibrate. A diagram showing the setup of mortality testing can be seen in Figure 4. Additionally, a map of the concentrations for the mortality test is given in Table 1.

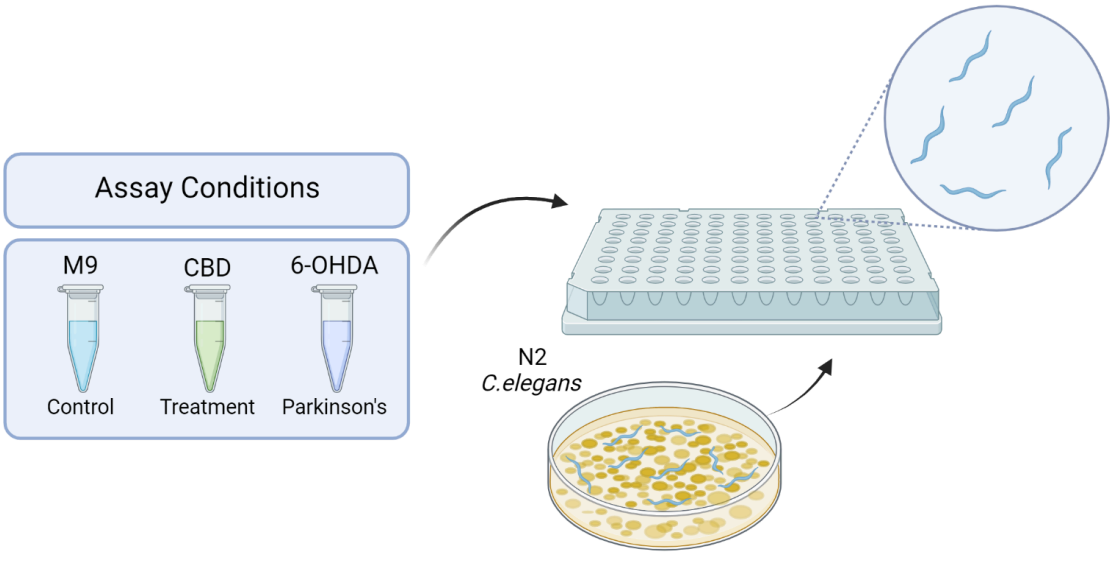

Figure 4: Schematic showing the general method of administration of 6-OHDA and CBD.

Table 1: M9 Mortality Test Dilutions

	1	2	3
A	Distilled Water (Control)	Distilled Water (Control)	Distilled Water (Control)
B	1:1000	1:1000	1:1000
C	1:100	1:100	1:100
D	1:10	1:10	1:10
E	1:1	1:1	1:1

Behavioral Assays

Following the determination of the proper concentrations of CBD and 6-OHDA to use throughout our experiments from performing several mortality tests, we dosed the nematodes and performed a behavioral assay before 6-OHDA administration as a control, a behavioral assay 30 minutes after 6-OHDA administration, and a final behavioral assay 30 minutes after subsequent CBD administration. The parameters of the behavioral assays were set to count the number of thrashes each worm performed during the 30 second video recording. Figure 5 demonstrates what was considered a thrash while counting was performed. For each well of the 96-well plate that we used for the experiment, we calculated the total number of thrashes between all worms in each well and took the average of this value to find the mean number of thrashes observed pre- and post-dose of the different varied concentrations of 6-OHDA and CBD throughout our experiments. With this, we could also find the difference between the total number of turns in each well before and after CBD treatment, and the difference between the turns on average in each well per worm before and after CBD treatment.

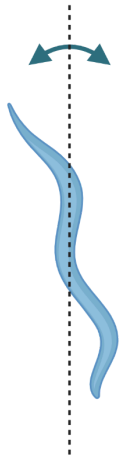

Figure 5: This diagram shows what was considered a thrash while we were counting. As long as the worm moved at least 90 degrees, the turn was counted.

CBD/6-OHDA Combined Treatment Assay

Before beginning the combined treatment assay, different concentrations of 6-OHDA and CBD were prepared. Two different methods were used for making our 6-OHDA solution. For freshly made solution, 500uM 6-OHDA solution was made by combining 0.0038g of 6-OHDA and 1.5 mL of M9. Different dilutions were made from this solution by adding different amounts of M9. Aliquots were also made to prevent the 6-OHDA solution from being exposed to air or light which could cause the 6-OHDA to oxidize. The aliquots were used by adding different volumes of M9 into wells and adding the 6-OHDA to create different concentrations to be used. 100uM CBD solution was made by dissolving CBD powder into DMSO. This solution was then transferred to M9 to make different concentrations.

The method used for the combined treatment assay consisted of transferring worms to wells with varying concentrations of 6-OHDA and adding varying concentrations of CBD after 30 minutes. A diagram showing this process can be seen in Figure 6. Thirty-second long videos

were recorded using a microscope camera before adding CBD and at the end of the 30 minutes, the amount of turns each worm completed were then counted from these recordings. Figure 5 above shows a diagram of what was counted as a turn. An example of the table used to keep track of time points during tests can be seen in Table 2. The total number of thrashes for all the worms in each well was counted and the average number of thrashes per well was calculated. These averages were then compared pre and post-treatment. These averages were used to help us determine the best concentration of OHDA to use for further experiments. Once the best concentration for OHDA was determined, we could keep the concentration constant for future experiments and vary the CBD concentrations being used. After testing a variety of different methods, we determined that using an OHDA concentration of 50uM would be the most efficient.

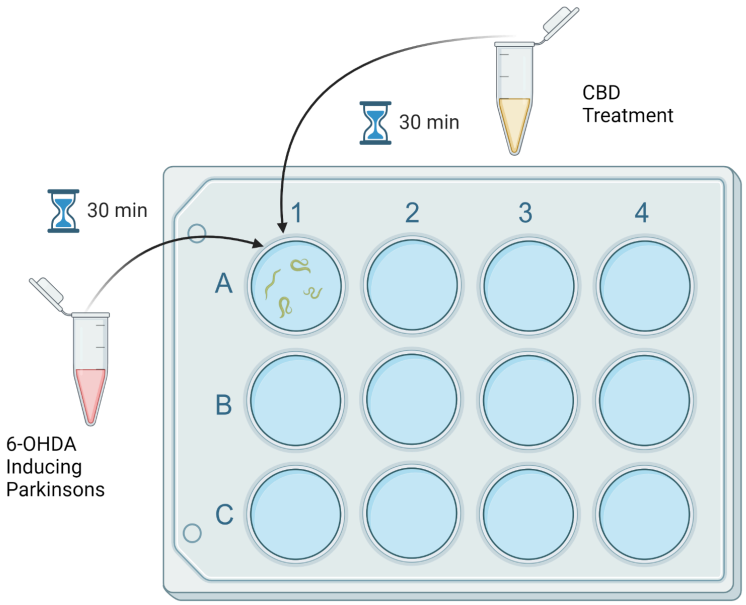

Figure 6: Diagram showing the process of the behavioral assays that were performed.

Table 2: Example Table For This Experiment

Well Row and #	_1	_2	_3	_4	_5	_6	_7	_#
Contents of control and experimental wells	CBD control (-) 1xM9	OHDA control (+) 1xM9	50uM OHDA 10uM CBD	50uM OHDA 20uM CBD	50uM OHDA 30uM CBD	50uM OHDA 40uM CBD	50uM OHDA 50uM CBD	(X)uM OHDA (X)uM CBD
	Start: Treatment: End:	S: C: E:	S: C: E:	S: C: E:	S: C: E:	S: C: E:	S: C: E:	S: C: E:
Number of Worms per well	*worms	worms	worms	worms	worms	worms	worms	worms
Number of turns counted per well**	***Turns: Turns after M9:	Turns: Turns after M9:	Turns: Turns after CBD:	Turns: Turns after CBD:	Turns: Turns after CBD:	Turns: Turns after CBD:	Turns: Turns after CBD:	Turns: Turns after CBD:

*mature worms; we are not factoring in the immature worms
**stopped counting thrashes when worms were out of frame or unable to be seen by any other reason
***total and average, respectively, over 30 seconds

Statistical Analysis

To begin the analysis, all turns for each trial of pre-treatment and post-treatment were counted and the average number of turns for each condition was calculated. After calculating the average turns for each trial of both treatment types, all averages were normalized in order to analyze only the pertinent data excluding any unstructured or redundant data points. These results were displayed through a bar graph with standard error bars. A single-factor ANOVA was then performed to obtain a p-value that would suggest whether or not our data was statistically significant.

Results and Discussion

To determine the efficacy of using CBD as a treatment for Parkinson's Disease, two separate types of tests were performed. The first was a CBD pre-treatment assessment where varying concentrations of CBD were given to worms before inducing PD with 6-OHDA. The average turns before and after inducing PD with 6-OHDA can be seen in Figure 7. The second was a CBD post-treatment assessment where varying concentrations of CBD were given to worms after inducing PD with 6-OHDA. The average turns before and after giving CBD treatment can be seen in Figure 8. Each graph also has standard error bars for each condition.

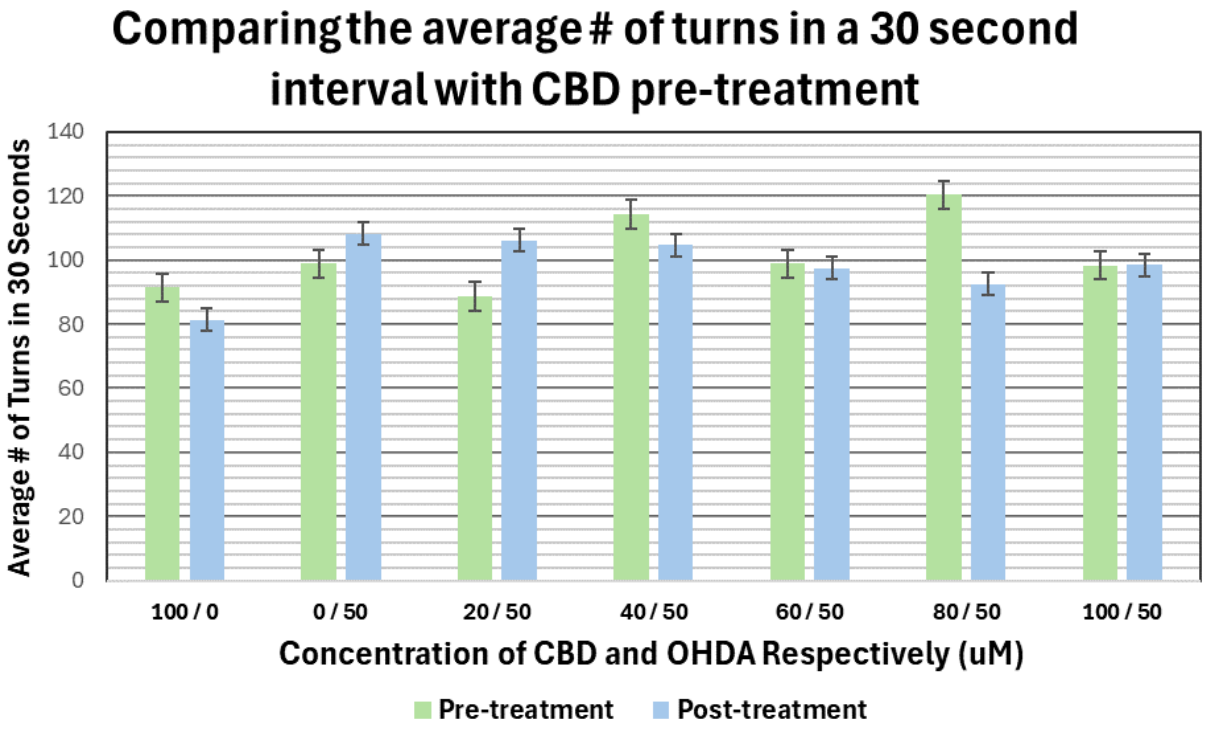

Figure 7. The average number of turns in 30 seconds using CBD as a Pre-Treatment.

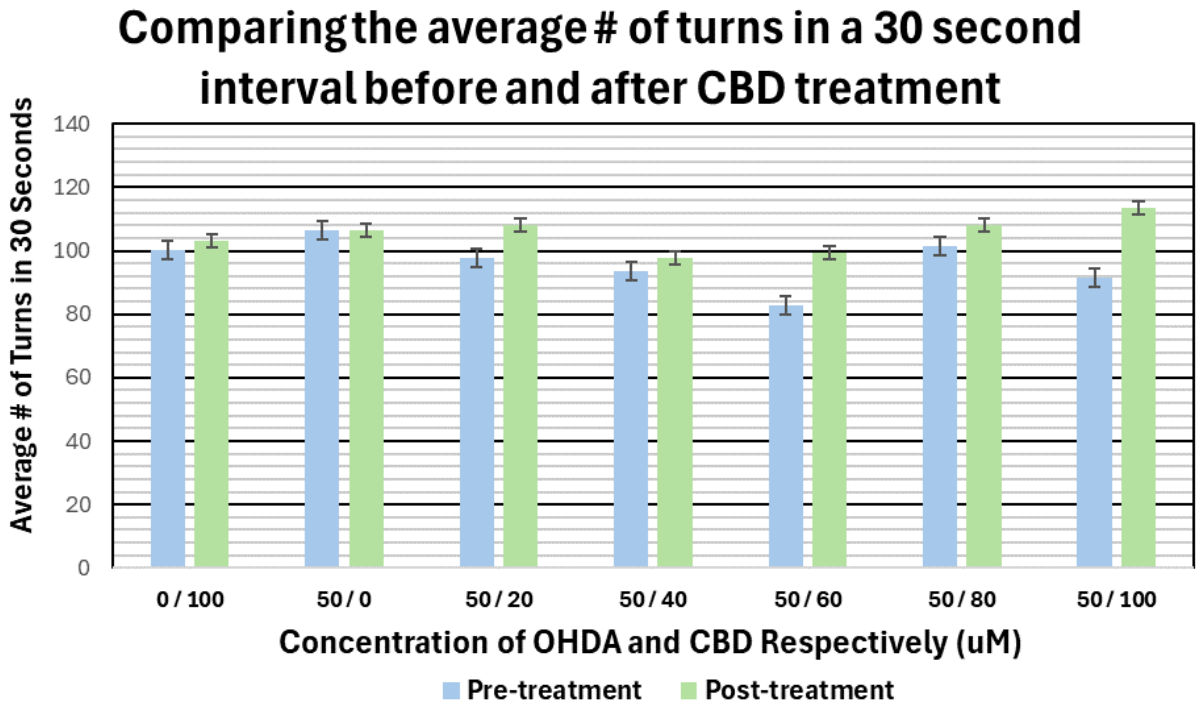

Figure 8. The average number of turns in 30 seconds using CBD as a Post-Treatment.

To confirm that the results of our experiments were statistically insignificant based on the initial results observed and to determine the ability to accept or reject the hypothesis, a single-factor ANOVA was conducted on data from both treatments following the normalization of the data. The P-values of both treatments shown in Table 3, CBD Pre-Treatment (0.59) and CBD Post-Treatment (0.78), indicate that the divergences within the data in the two treatments are likely not an outcome of the CBD dosing, but are due to other factors as well. The large variability in the data, challenged by the various limitations we faced, is likely the cause of the high P-value. Limited data was collected as we only performed three tests for each condition with five replicates. Because of the variability in our data, no concrete and definitive conclusions can be made as the lack of strong data does not allow the ability to support or refute the hypothesis. Based on the above findings, it cannot be determined, only inferred, whether or not

CBD affects the levels of oxidative stress and overall neurodegenerative symptoms in *C. elegans*. As a conclusion, our data is 'technically' insignificant based on the high P-values that were obtained. However, through the increase in relative turn activity seen in nematodes treated with CBD both before and after chemical induction of PD, the trend suggests that there may be a benefit to using CBD as both a preventative measure and a treatment for physical symptoms of PD with more research and data.

Table 3: P-Values from single-factor ANOVA of Treatment Assays to Determine Significance of Data

Treatment Type	**P-Value**
CBD Pre-Treatment	0.5899443584
CBD Post-Treatment	0.783021014

Conclusions

Main Findings and Relevance

Even though the data collected is not statistically significant and does not support our hypothesis, there is a trend that suggests the potential effectiveness of using CBD as a treatment for Parkinson's Disease (PD). This was most notably seen in our Post-Treatment data, where we observed an overall increase in the average number of turns when using CBD after inducing PD using 6-OHDA. This could potentially indicate that CBD could be used as a neuro-regenerative method to restore some movement lost with the induction or progression of PD. With further research, these findings can be strengthened with significantly more experimentation and trials. Additionally, obtaining data to further explore and support the Pre-Treatment method of using CBD beforehand is essential in comparing the overall effect of using CBD as a treatment for PD.

Lastly, we believe that, through our background research and experimentation, we have successfully established *C. elegans* as a model system for discovering the effectiveness of CBD in the treatment of PD while also determining a method through which to test our hypothesis and similar hypotheses to ours. This model system and methodology can be used in future experimentation to come to a more concrete conclusion regarding the efficacy of CBD as a treatment method for PD. We would like to add that CBD's mechanism in treating PD can be shown through its interaction with the endocannabinoid system, its neuroprotective properties, and its anti-inflammatory and antioxidant properties. By protecting neurons from damage and degeneration, CBD could slow down the progression of the disease. Additionally, antioxidant activity may help mitigate the damage to dopamine-producing neurons that is seen in PD. Lastly, by lessening neuroinflammation, CBD may alleviate some symptoms of PD and possibly slow disease progression.

Limitations

Throughout this project, several limitations may have affected the validity, reliability, or generalizability of our research. The first limitation was the sample size for each experiment, where most were relatively small and consisted of only 5 worms. This may have limited the generalizability of our findings to a larger population and created variation as there was a large dependency for all 5 worms to remain countable. Additionally, the smaller sample size could have caused significant bias in our data. Another major limitation of our experiments was the variation in the timing of the treatments, where the worms may have been subjected to one treatment more than another. This could have skewed various behavioral aspects among the worms while preventing conformity of all worms used in each experiment. The accuracy of the equipment used, specifically the scale, posed a large limitation as in each experiment, the 6-OHDA was freshly made for each use causing a chance for there to be variability in the concentrations of 6-OHDA between each experimental trial.

Since counting the turns of each worm within an experiment was critical to analyzing the effect of each treatment on behavior, our main and only method of counting the turns by hand could have produced inaccuracies and variability in our data. Hand counting could have led to inaccuracies in our data because turns were counted by four people. While we decided as a group what would be considered a turn, there is still room for variability. Additionally, turns could be missed as many of the turns were fast despite decreasing the speeds of videos. This could potentially be solved by slowing down videos even more by using a different video editing software. We hope that these limitations experienced in our project can be addressed and conquered within future projects to ensure greater reliability and validity within the research.

Future Considerations and Recommendations

Well-to-plate to well transfer from 6-OHDA to CBD

To ensure that the treatments were isolated, it would have been useful for each of the worms to be moved from one treatment well to another with minimal cross-contamination. The first method through which this would be accomplished was by well-to-plate-to-well transfer. As the name suggests, the worms were to be moved from the wells and moved to a clean NGM plate. Pipetting the 6-OHDA treatment with the worms from the well onto a plate, allowing the liquid to dry, and then picking the worms off the plate and into the CBD treatment well would be the primary method. This was done for each of the 6-OHDA wells being tested into their corresponding CBD wells. If successful, the rest of the preparation and behavioral assay were to be performed with the same method as described previously.

Well-to-wash to well transfer from 6-OHDA to CBD

To ensure that the treatments were isolated, each of the worms had to be moved from one treatment well to another with minimal cross-contamination. The second method through which this could be accomplished would be through a well-to-wash-to-well transfer. A diagram of what this could potentially look like can be seen in Figure 9. This could be done by pipetting the 6-OHDA treatment with the worms in it from the well into a microcentrifuge tube using a glass pipette. Once in the microcentrifuge tube, M9 would be added to wash the worms by diluting the 6-OHDA. After the worms settled to the bottom, the excess liquid could be aspirated from the top and discarded. M9 should be added once more to ensure that the 6-OHDA in the solution was diluted, and the excess liquid would once again be aspirated. At this point, the corresponding

concentration of CBD could be added for the final wash, and the excess liquid can be aspirated and discarded. The remaining solution with the worms could then be transferred from the microcentrifuge tube to the 96-well plate. The rest of the preparation and behavioral assay would be performed with the same method as described previously.

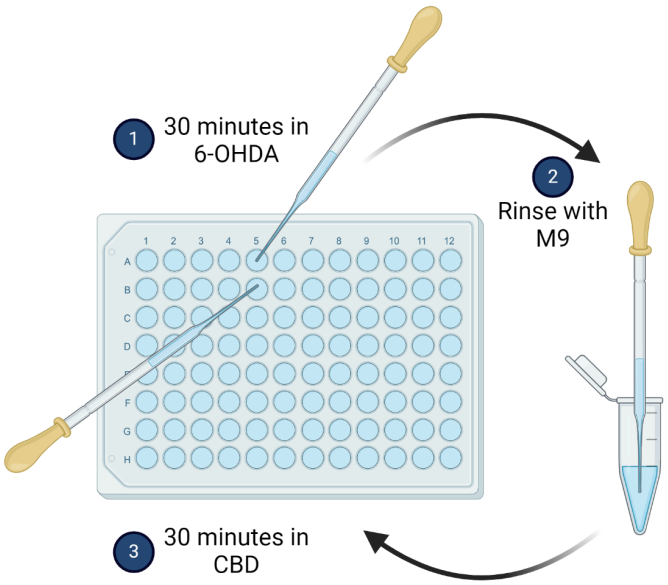

Figure 9: Diagram showing potential washing method for future tests.

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