Exploring the effect of CBD on *C. elegans* with 6-OHDA induced Parkinson's Disease to determine potential therapeutic implications

A Major Qualifying Project Report

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Abstract

The purpose of this project was to determine if Cannabidiol could be used to reduce the behavioral effects of 6-OHDA induced Parkinson's Disease in *C. elegans*. Behavioral assays measuring thrashes, Omega-, and Delta-turns in liquid media were used to analyze restorative abilities CBD had on the movement of the worms. It was found that CBD was able to increase thrashing behavior from 20/minute to 40/minute in PD worms, and was able to decrease time spent in turns by half. Although conclusions were heavily limited by sample size of the data collected, this project provides preliminary evidence in support of CBD restoring normal movement behaviors for PD-model worms.

Acknowledgements

We would like to first and foremost thank our primary MQP advisors, Professors Jill Rulfs and Michael Buckholt, for their invaluable guidance and patience, as well as their humor and ability to lift our spirits after weeks of failed experiments. We would also like to extend gratitude to Professor Jagan Srinivasan for his guidance in our methods and protocols, as well as his infectious enthusiasm for worms. We are also grateful to Professors Jim Doyle and Suzanne Scarlata for ensuring that our project was meeting the many different major requirements for both of us. A special thanks goes to Hannah and Alexia for allowing us to bounce ideas off of them, bringing our worms back from the dead, and being the best worm girls we could ask for. Finally, we would like to thank our friends and family for their continuous encouragement throughout our time at WPI.

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Introduction

Parkinson's Disease (PD) is the second most common neurodegenerative disorder in the world. The disease is characterized by muscle tremors and rigidity, as well as other symptoms including depression, dementia, disrupted sleep, and anxiety that all have a distinct impacts on the patient's quality of life (Cooper, 2018). PD is caused by a loss of dopaminergic neurons, and its occurrence is frequently sporadic for most cases (Surmeir 2010). Currently treatments for PD include medications such as dopamine agonists and surgical interventions such as Deep Brain Stimulation or focused Ultrasound Thalamotomy. While these medications can treat symptoms, they can not slow or stop the progression of neuron degeneration, and they have significant side effects that prevent them from working for certain patients (Rao, 2006; Dallapiazza, 2018). This current state of PD treatment necessitates exploring other therapeutic options for symptom management. In this study we sought to specifically explore the therapeutic potential of Cannabidiol (CBD) for symptom management in PD. *C. elegans* was used as a model for PD to determine if symptomatic improvement was present with CBD using behavioral measurements. We assessed symptoms by quantifying the turning and thrashing behavior of the *C. elegans*. We observed that in the Parkinson's Disease Model thrashing behavior was decreased and the duration of Omega and Delta Turns increased when compared to standard controls. When CBD solution was used with disease state *C. elegans*, the number of thrashes was higher and the duration of each turn was lower, indicating potential therapeutic ability of CBD to restore normal movement behaviors in PD-model worms. While this evidence is preliminary, it supports further research into CBD and its use in the management of Parkinson's Disease.

Background

An Overview of Parkinson's Disease

Parkinson's disease (PD) is the result of profound degeneration of dopaminergic neurons in the substantia nigra in humans, and it is the second most common neurodegenerative disorder in the world impacting 10 million people (Przedborski, 2003; Cooper, 2018). It is a progressive disease with a mean age of onset of 55 years. It is characterized by symptoms of tremors that occur at rest and then decrease with voluntary movement (Przedborski, 2003). There are a variety of forms in which tremors associated with PD can present in such as stiffness, slowness of movement, reduction of movement amplitude, impaired balance, and absence of typical unconscious movements (Przedborski, 2003; Cooper, 2018). Some other specific PD symptoms and associated behaviors can include decreased volume of speaking, drooling, handwriting difficulty, and a smaller stride (Przedborski, 2003). Symptoms can have a wide range of impact on quality of life depending on the severity and presentation (Przedborski, 2003). Patients may experience decreased quality of life when everyday tasks take significantly more time, experience falls and freezing, and lose initiative or cognitive speed (Przedborski, 2003). Additionally, depression, anxiety, dementia, and atypical sleep occur at a higher frequency in patients with PD when compared to those without (Przedborski, 2003; Cooper, 2018). Currently the diagnostic criteria requires the presence of a distal resting tremor of 3-6 Hz, rigidity, bradykinesia, and asymmetrical onset (Rao, 2006).

Dopamine is a neurotransmitter which affects many different regions of the brain. Dopamine can increase or decrease excitability of the postsynaptic neurons depending on which receptors receive it. This occurs through modulating the release of adenylyl cyclase (Fellous 2002). Some regions of the brain, including the substantia nigra, contain a specific type of neuron called dopaminergic neurons, whose main purpose is to synthesize and release dopamine for distribution among the brain (Chinta 2004). Loss of these neurons is considered to be responsible for PD. In recent years, there has been speculation as to why dopaminergic neurons specifically in the substantia nigra are targeted. One leading theory is that it is due to stressors on the neurons due to their large axonal size (Surmeir 2010). Because each axon is supporting so many synapses, there is a much higher likelihood of dopamine accidentally being oxidated,

which is damaging to the cells. This is also reinforced by the fact that age is the largest contributing factor to developing the disease (Surmeir 2010).

Current Treatments for Parkinson's Disease

The overall goal of treatment of Parkinson's disease is to reduce symptoms while maximizing function and quality of life. Medications currently available are able to treat symptoms, however they are unable to stop or slow the progression of dopaminergic neuron degeneration (Dauer, 2003). This therapy limitation is due to the lack of understanding in regards to the molecular events and pathways that initiate and sustain neurodegeneration (Dauer, 2003).

Currently, Levodopa is the primary treatment method for Parkinson's disease (Rao, 2006). This mediation is used when dopaminergic treatment is needed and motor abilities need to be improved (Rao, 2006). Levodopa works by being converted into dopamine in the brain, therefore providing the neurons with the transmitters that are needed (Rao, 2006). Generally, this medication is used with patients who are older and have more severe symptoms (Rao, 2006). A drawback of this medication is that long-term use can be limited because Levodopa can cause motor complications through drug-induced dyskinesia (Rao, 2006). Due to this complication, dopamine agonists are used in treatment of patients who are younger with milder symptoms in an attempt to limit long term use of Levodopa while still providing treatment for motor abilities (Rao, 2006). Dopamine agonists work by stimulating dopamine receptors in the brain (Choi, 2022).

There are options for surgical treatments offered for PD that are typically reserved for later stages of the disease (Dallapiazza, 2018). Deep Brain Stimulation (DBS) is the most common of the surgical procedures used (Darrin, 2018). For this treatment electrodes are implanted in specific areas of the brain which produce electrical impulses in order to regulate or affect specific impulses or chemical signals in the brain (Dallapiazza, 2018). The goal of this treatment is to target specific areas of the brain including the subthalamic nucleus or globus pallidus pars interna, in order to improve movement symptoms such as instability and freezing (Dallapiazza, 2018). In general this treatment is used when a patient is no longer responding to medication well and is having tremors or other symptoms significantly impacting quality of life (Darrin, 2018).Another surgical type of treatment is focused ultrasound thalamotomy (FUS) which involve radio waves being targeted at specific area of the brain to destroy the tissue

without affecting other regions, and does not require physical penetration of the brain (Dallapiazza, 2018). This treatment is mainly reserved for patients with severe tremor symptoms that are not good candidates for DBS treatment (Dallapiazza, 2018). Future treatments for PD are being researched including gene therapy, immunotherapy, and cell transplantation, however these strategies remain very new and in the research phase of development (Darrin, 2018)

An overview of C. elegans

C. elegans is a microscopic, free-living, roundworm of the nematode phylum. This organism grows to be about 1-2mm in length and is transparent in color (Cooper, 2018). The adult *C. elegans* hermaphrodite has 959 somatic cells, and of those 302 are neurons (Dexter, 2012). The nervous system of C. elegans is the most complete nervous system to be mapped, as all connections between the 302 neurons have been identified (Dexter, 2012). This organism is ideal because the simplicity of its nervous system has allowed for it to be well defined. Additionally, the nervous system includes many of the molecular pathways and complex behaviors that are shared with higher level organisms such as humans (Corsi, 2015). This allows for comparisons to be drawn between *C. elegan*s and mammals as they share many of the same neurotransmitters, ion channels, receptors, transporters, and other cellular processes (Dexter, 2012). Along with the similarities in the biochemical mechanisms, at least 50% of the *C. elegans* genes have human homologs along with orthologs for 70% of human disease related genes (Dexter, 2012). These similarities allow for the study of human disease in *C. elegans* as a model organism to be relevant and applicable for the functions and interactions of both genes and cellular processes. Other benefits to studying genetic conditions with this organism include that the primary form of this model organism is a hermaphrodite, which is self fertilizing, and therefore the progeny are all genetically identical, allowing for the organism to remain genetically predictable and consistent (Cooper, 2018).

C. elegans lifespan is 2-3 weeks, and they mature within 2 days in a laboratory setting at 20°C (Cooper, 2018). They produce eggs continuously after maturation, which results in around 300 progeny per roundworm (Cooper, 2018). This allows for the maintenance of the C. elegans to be relatively simple as thousands of worms can be kept on a single Petri dish, and they do not need expensive or complicated propagation methods that cells may require (Cooper, 2018).

Using *C. elegans* in modeling PD is beneficial because the worms are genetically predictable, have a manageable lifespan, and their nervous system is well defined (Cooper, 2018). Their transparent color is especially of interest as it makes using green fluorescent protein possible, alloweding for neurons to be directly analyzed while the model is still living (Corsi, 2015). Additionally, Based on their similarity with mammals in regards to cellular and molecular processes, *C. elegans* are sensitive to toxins such as 6-hydroxydopamine, which are used to model neurodegeneration in mammals as well (Dexter, 2012).

Modeling Parkinson's Disease in C. elegans

PD can be modeled in *C. elegans* in both genetic and toxicant models. To determine which mode of disease generation is to be used, the desired research goal needs to be established. If a genetic link needs to be generated, then using a genetically tied model of PD will be important. However, if no genetic link needs to be established, such as research into symptoms management rather than disease cures, than a toxicant model can be beneficial to easily induce the disease (Corsi, 2015).

There are a variety of genetic models that can be used, but a majority of them include a transgenic genotype that has been developed to induce the expression of α-synuclein or LRRK2 (Cooper, 2018). Other examples of genetic manipulation to express α-synuclein include deletions of *PRKN/pdr-1, PINK1/pink-1, DJ-1/djr-1.1/djr-1.2* or *ATP13A2/catp-6* (Cooper, 2018). All of these variations result in a phenotype of worm which exhibits PD categorized symptoms such as dopamine neuron loss, disruption of dopamine-dependent behaviors, increased stress sensitivity and response, and movement deficits (Cooper, 2018). The goal of using these genetic models is to develop therapeutic treatment options that can address the genetic cause and resulting symptoms of PD.

Another way to model PD and movement disorders in *C. elegans* is through adding toxins to their environment and systems to induce neuron degeneration in wild type roundworms. One of the most frequent methods of doing this is using the chemical 6-hydroxydopamine (6-OHDA). In mammals who contain a blood-brain barrier, the neurotoxin must be injected

directly into their brain. With *C. elegans*, however, the procedure is even easier because it does not have a blood-brain barrier, so it will absorb 6-OHDA from its environment (Simola 2007). 6-OHDA is recognized and taken up exclusively by dopaminergic and noradrenergic neurons (Simola 2007). Once inside the cell, it is oxidized by monoamine oxidase and releases hydrogen peroxide, therefore causing the production of dangerous oxygen radicals (Simola 2007). These radicals damage the proteins and DNA within the cell and overall result in death of the dopaminergic neurons.

In PD models of *C. elegans*, behavioral changes are visually evident. The two main behaviors observed in *C. elegans* during liquid media assays are thrashing, a directional movement of the head from side-to-side, and Omega- and Delta-turns, a reorienting movement used to change the direction the worm is heading which involves clapping of the head and tail together (Hart 2006). In PD models, thrashes are significantly decreased in number, and turns take a longer duration of time to be completed (Simola 2007).

An Overview of the Cannabinoid System

Cannabinoids are a family of lipids that bind to cannabinoid receptors in the brain and perform depolarization-induced suppression of inhibition (DSI) (Wilson and Nicoll 2001). DSI is a form of retrograde signaling that sends inhibitory signals from postsynaptic neurons back to the cells they are innervated by and suppresses their signaling temporarily (Narushima 2006). There are two types of receptors that cannabinoids can bind to - CB1 and CB2. Both are G-protein coupled receptors, so their activation is able to have many consequences within cells, including inhibiting adenylyl cyclase and voltage-gated Ca^{2+} channels (Lu 2016). CB1 receptors are present in high amounts in the central nervous system, including the basal ganglia and substantia nigra, two of the most frequently implicated brain regions for movement disorders (Lu 2016). For this reason, cannabinoids that bind to this receptor are particularly of interest for potential PD therapy.

Cannabinoids can either be taken in from the environment (exocannabinoids) or synthesized by the body (endocannabinoids). The two endocannabinoids which have been discovered in humans are anandamide and 2-arachidonoyl glycerol (2-AG). Some endogenous molecules that can bind to the cannabinoid receptors but do not seem to have an effect on them have been identified, although these are not of focus for therapeutic purposes (Mechoulam

2013). Unlike many neurotransmitters, endocannabinoids are not readily available in the cell. Instead, they are synthesized as needed by enzymes within the cell (van der Stelt 2003). When endocannabinoids need to be degraded, they are hydrolyzed by fatty acid amid hydrolase and broken down into constituents of arachidonic acid and other small molecules (Dainese 2020).

Many exocannabinoids have been isolated from the plant *Cannabis sativa*. However, the two of most interest are Δ^9-tetrahydrocannabidiol (THC) and cannabidiol. Δ^9-tetrahydrocannabidiol is responsible for the psychoactive effects that are widely associated with cannabis. Cannabidiol does not have these effects, and in fact may compensate for some of the psychoactivity of Δ^9-tetrahydrocannabidiol, as well as serving as an anti-inflammatory agent (Mechoulam 2013). Unlike the rapid hydrolysis of endocannabinoids, THC and cannabidiol take many hours to be fully metabolized.

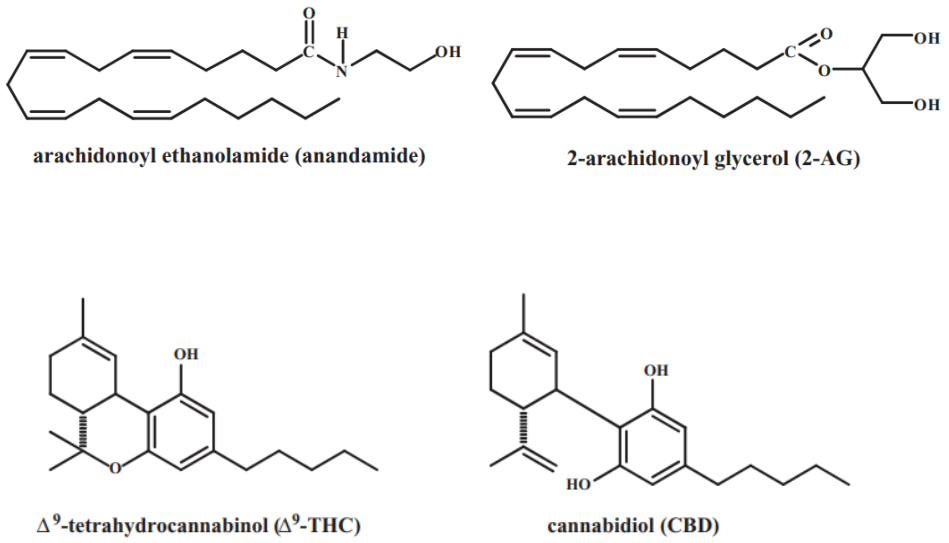


Figure 1. Structures for the endocannabinoids anandamide and 2-AG, as well as the common exocannabinoids Δ^9-tetrahydrocannabidiol and cannabidiol, isolated from the plant *Cannabis sativa*. Image adapted from Mechoulam 2013.

Cannabinoids in C. elegans

C. elegans does not possess a direct ortholog for the CB1 receptor. However, it has been demonstrated that cannabinoids are still able to be metabolized in the receptor's absence due to presence of FAAH-1, the ortholog of FAAH that is found in *C. elegans* (Estrada-Valencia 2021).

Endocannabinoids are also able to be synthesized using the same precursor used in humans, with the enzymes NAPE-1 and NAPE-2 serving as the orthologs for NAPE-PLD, the biosynthetic enzyme used in humans (Estrada-Valencia 2021).

Although there is no direct ortholog for CB1 in *C. elegans,* studies have been done recently to find receptors that may serve a similar purpose. One has been identified - NPR-19. This receptor, thought containing only a 23% sequence identity with CB1, has many important residues conserved in the cannabinoid binding pocket. The pocket is largely hydrophobic, to allow the hydrophobic lipid cannabinoids to bind, and it shows the residues F189, L193, F379, and S383 as being conserved across both CB1 and NPR-19. The F and L residues contain large, nonpolar side chains that help to create the necessary hydrophobic environment of the binding pocket, and the serine residue forms a hydrogen bond with the amide oxygen found in many cannabinoids (Oakes 2017).

Currently, the only studies that have been done using exocannabinoids in *C. elegans* used cannabidiol and cannabidivarin, a non-psychoactive derivative of the latter. One prominent study explored the impacts of cannabidiol on the dopamine signaling system, which is analogous to that in humans. It found that cannabidiol was binding to the same receptor as dopamine and amplifying its presence, which is similar to the effects it has in humans, making *C. elegans* a viable model system for studying effects of CBD on neurological disorders (Shrader 2020).

Therapeutic Potential of CBD

In recent years, there has been a large interest in exploring cannabinoids as a potential therapeutic agent for a range of disorders, with a specific rise in interest surrounding its efficacy in neurological disorders beginning around 2011 (Treister-Goltzman 2019). There has especially been a rise in interest since the United States has begun legalizing recreational use of cannabis, as it makes it more widely available for research purposes as well. Currently, the US Food and Drug Administration has approved the CBD product Epidiolex, however all other non-FDA-approved products have a wide variety as far as quality and consistency which can make research difficult (White, 2019). Among research being done, the most common cannabinoid used is currently CBD. It is very promising for therapeutic purposes because it is easy to isolate from the plant *Cannabis sativa* and it does not have psychoactive effects (Morales, 2019). There has already been a significant amount of research done to explore the

therapeutic qualities and properties of CBD including potential anti-inflammatory, anticonvulsant, anxiolytic, antiepileptic, neuroprotective, and antitumor properties, as well as many others (Morales, 2019).

Examples of studies that have been conducted to understand the role of CBD in symptom treatment and management include studies for seizure disorders, anxiety, pain, schizophrenia, and Parkinson's Disease, among many others. In one study, research into CBD has shown that it may be a potentially effective additional treatment option for refractory seizures in Dravet syndrome and Lennox-Gastaut syndrome by reducing seizure frequency, however the overall treatment role of CBD still remains unclear (White, 2019). In another study, three randomized trials were conducted to assess how moderate length CBD therapy affected patients with schizophrenia, and all showed a reduction of symptoms (White, 2019). However, there was not consistency to the types of symptoms that decrease or the duration of time (White, 2019). Additionally, studies that were conducted to determine the effect of CBD on patients with anxiety or chronic pain were all inconclusive as their results were inconsistent on the effectiveness and significance of their data (White, 2019). So far, there have been very few trials that analyze the effect of CBD on patients with Parkinson's. However, those that do exist have not found that CBD improves symptoms associated with movement, but they did determine that it impacted sleep (White, 2019). With our project, we hope to provide greater evidence in favor of CBD providing symptom reduction for Parkinson's Disease using a 6-OHDA model in *C. elegans.* Due to cannabinoids' ability to amplify dopamine production, we hypothesize that CBD can alleviate the movement symptoms caused by the loss of dopaminergic neurons that is observed in PD.

Methodology

C. elegans Maintenance

Strains were maintained at 20 °C, on NG agar with OP50 *E. coli* as a food source. NG agar plates consisted of Agar, NaCL, Peptone, 5mg.ml cholesterol in ethanol, 1 M KPO_4 buffer, and 1M $MgSO_4$ components. The wild-type strain N2 was used and maintained through chunking (Hart 2006).

6-hydroxydopamine Behavioral Assay

6-OHDA powder was diluted in M9 to make a liquid solution with a concentration of 50 mM. The M9 solute consisted of KH_2PO_4, Na_2HPO_4, NaCl, $MgSO_4$, and ddH_2O. Serial dilutions using M9 were performed to make 6-OHDA solutions of the following concentrations: 0mM, 10mM, 20mM, 30mM, 40mM, and 50mM. 100µL of each concentration was added to a 96-well plate, and 5 worms were allowed to equilibrate at each concentration for 30 minutes. Omega-turns and thrashes for each worm were counted over the span of a minute using a cell counter and timer (Hart 2006).

Cannabidiol Behavioral Assay

CBD was diluted in 100% DMSO to a final concentration of 1 mM. Serial dilutions using M9 were performed to make CBD solutions of the following concentrations: 0µM, 4µM, 8µM, 12µM, 16µM, 20µM, 24µM, 28µM, 32µM, 50µM, 75µM, and 100µM. 100µL of each concentration was added to a 96-well plate, and 3 worms were allowed to equilibrate at each concentration for 30 minutes. Omega-turns and thrashes for each worm were counted over the span of a minute using a cell counter and timer (Hart 2006).

6-Hydroxydopamine/Cannabidiol Joint Treatment Assay

6-OHDA was diluted in M9 to a concentration of 50 mM. A dilution of 10 mM 6-OHDA was made using M9. 100µL was added to 8 wells and 8 worms were allowed to equilibrate at each concentration for 30 minutes. CBD was diluted in 100% DMSO to a final concentration of 1 mM. A 28 µM dilution was made using M9. 100µL were added to a 96-well plate, and 8 worms were allowed to equilibrate at each concentration for 30 minutes. Finally, a combined

well was generated using 100µL of 28 CBD and 100µL of 10mM 6-OHDA. Omega-turns and thrashes for each worm were counted over the span of a minute (Hart 2006).

Analysis

Statistical analysis was completed using Single Factor ANOVA for each data set. Using Excel, the Variation, SS, df, MS, F, P-value, and F-critical were found for each behavioral assay. These statistics were used to determine the distribution and significance of the data.

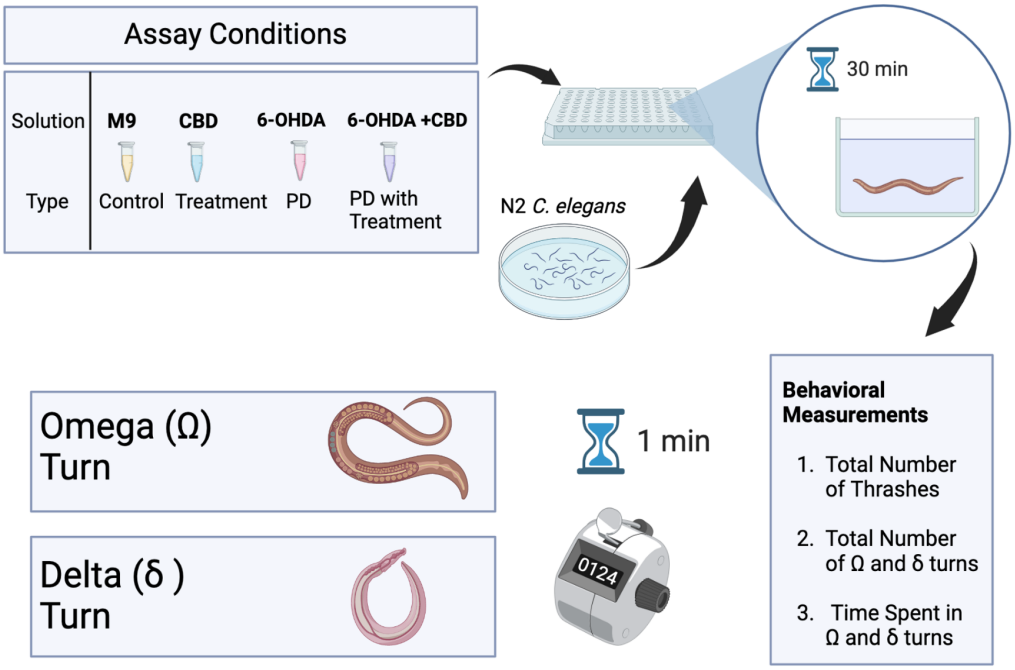


Figure 1. Methodology Flow and Set up of Assay conditions, Timing, and Behavioral Measurement

Results

A concentration of 30mM 6-OHDA with exposure time of 30 minutes maximizes behavioral symptoms while minimizing deaths

An assay was run with 5 worms at each of the following 6-OHDA concentrations: 0mM, 10mM, 20mM, 30mM, 40mM, and 50mM. For one minute, each worm's number of Omega/Delta Turns performed and Duration of each Omega/Delta Turn were counted (see **Table 1** and **Figures 2, 3, 4**).

Table 1. Mean data for each behavior measured for the 6-OHDA Dose Response Curve

Concentration of 6-OHDA	Mean Quantity of Omega/Delta Turns	Mean Duration of Omega/Delta Turns	Total Number Dead
0 mM	3	0.42	0
10 mM	3.5	1.1	0
20 mM	1.83	1.33	0
30 mM	7.6	2.18	1
40 mM	4.5	2.26	2
50 mM	2.5	1.94	3

A range of concentrations between 0 mM to 50 mM were used to generate a dose response curve to determine the concentration of 6-OHDA that would maximize behavioral symptoms and minimize death of *C. elegans*. The mean time per turn at 0 mM was 0.42 seconds, 1.1 seconds at 10 mM, 20 1.33 at 20 mM, 2.18 seconds at 30 mM, 2.26 seconds at 40 mM, and 1.94 seconds at 50 mM. This was recorded at the same time at the Mean Quantity of Omega and Delta turns that were completed at each concentration. At 0 mM 6-OHDA the Mean Quantity of turns was 3 turns, 3.5 turns at 10 mM, 1.83 turns at 20 mM, 7.6 turns at 30 mM, 4.5 turns at 40 mM, and 2.5 turns at 50 mM. (Table 2, Figure 2) In addition to the Mean Quantity of Turns, the Mean Time per Turn was recorded. This was measured as the time from when the Omega or Delta Turn was initiated through the time it took for the turn to be completed. The Mean Time

per Turn at 0 mM was 0.4 seconds, 1.1 seconds at 10 mM, 20 1.3 at 20 mM, 2.2 seconds at 30 mM, 2.3 seconds at 40 mM, and 1.9 seconds at 50 mM. (Table 2, Figure 2)

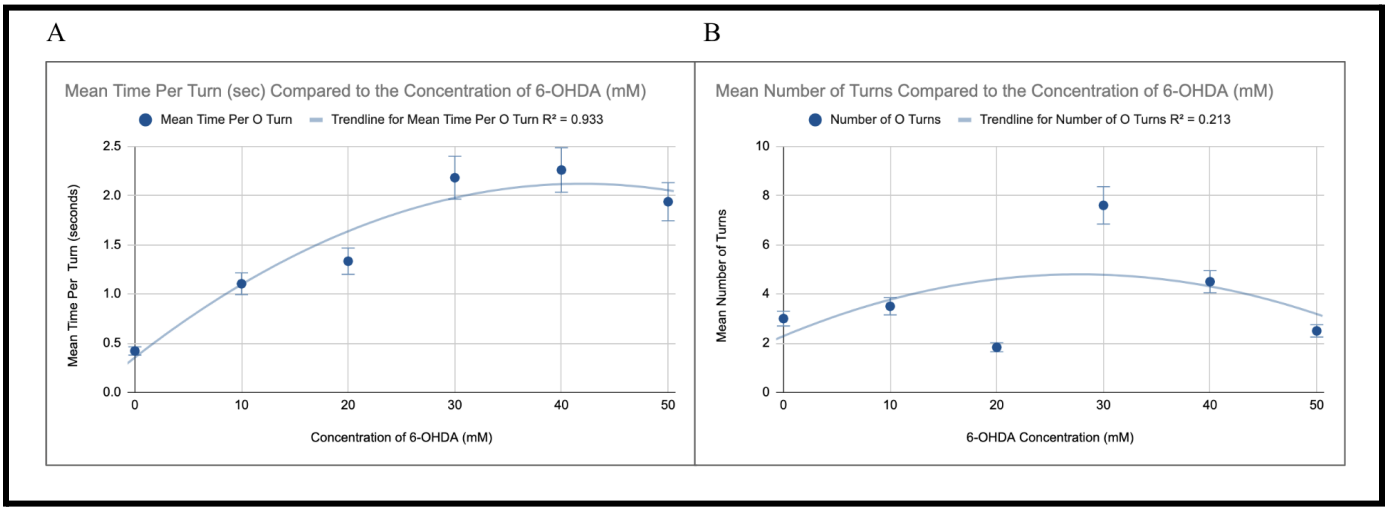


Figure 2. (A) Mean time *C. elegans* at each concentration spent in each Omega/Delta turn over the course of a minute. Each data point is representative of the mean data from five worms. (B) Average number of Omega/Delta turns at each concentration. Each data point is representative of the mean data from five worms.

The total number of *C. elegans* that were dead after 30 minutes of treatment with 6-OHDA were recorded and analyzed as overall totals. At 0 mM there were 0 *C. elegans* that were dead, at 10 mM 0 were dead, at 20 mM 0 were dead, at 30 mM 1 *C. elegans* was dead, at 40 mM 2 *C. elegans* were dead, and at 50 mM 3 *C. elegans* were dead (Figure 3).

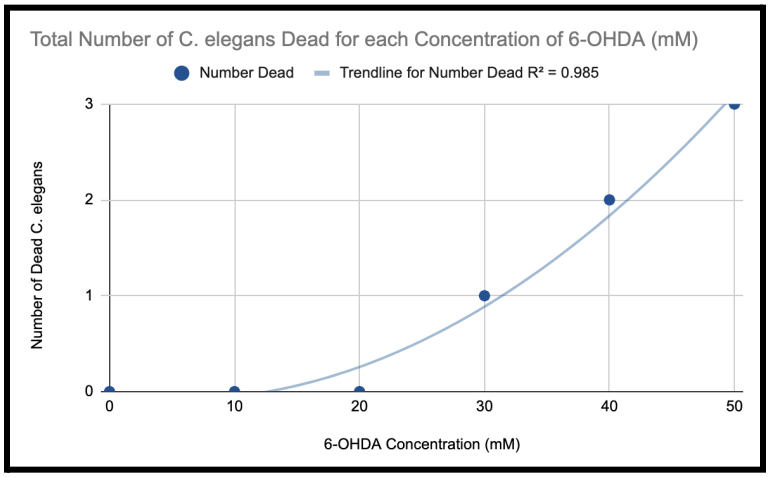
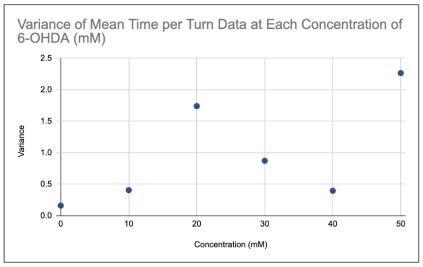


Figure 3. The total number of C.elegans that were dead after 30 minutes of treatment with 6-OHDA. Data points are representative of gross totals, not mean.

An ANOVA was run to generate the F value, F-critical value, and p-value. For the Mean Time Per Turn Behavioral Assay, when comparing F values and F-Critical Values, if the F value is numerically larger than the F critical value, then the data is normally distributed and the p-value can be used. The F value for the 6-OHDA Mean Time per Turn Behavior had an F value of 2.91, which is larger than the F-Critical Value which is 2.60 (Figure 4). This indicates that the data is normally distributed and the p-value is valid. The p-value was 0.033. The ANOVA was single factor and set to measure based on a $p < 0.05$. Because the p-value for the Mean Time Per Turn was less than 0.05, it indicates that the variance falls below a level that suggests the data is statistically significant. The variance in the Mean Time per Turn data was highest at 20 mM and 50 mM, with variance of 1.74 and 2.27 respectively. The variance was lowest for this behavioral assay at 0 mM with a 0.16. The other variance levels were 10 mM at 0.41, 30 mM at 0.87, and 30 mM at 0.39 (Figure 4). This analysis shows that 6-OHDA affects the duration of Omega and Delta turns, which is consistent with literature expectations.

For the Mean Number of Turns Behavioral Assay, the F value for the Mean Number of Turns in the 6-OHDA Assay was 1.89 and the F-Critical value was 2.60 (Figure 4). Because the F value was not larger than the F-Critical value, the data is not normally distributed, and therefore the p-value can not be considered valid. Additionally, the p-value was 0.13 (Figure 4). This value is larger than 0.05, indicating that the data is not statistically significant. The variance in the Mean Number of Turns data was highest at 40 mM, with variance of 32.333. The variance was lowest for this behavioral assay was 20 mM at 3.37. The other variance levels were 0 mM at 11.2, 20 mM at 10.3, 20 mM at 6.3, and 50 mM at 8.92 (Figure 4). This analysis shows that 6-OHDA does not affect the number of turns, which is consistent with literature expectations.

A



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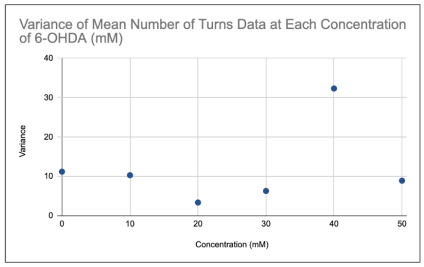


B

6-OHDA Dose Response Mean Time per Turn ANOVA			
Source of Variation	F	P-Value	F crit
Between Groups	2.91	0.033	2.60

D

6-OHDA Dose Response Mean Number of Turns ANOVA			
Source of Variation	F	P-Value	F crit
Between Groups	1.89	0.13	2.60

Figure 4. Variance Visualization and ANOVA Statistics Results for 6-OHDA Dose Response Curve Behavioral Measurements

A concentration of 28 uM CBD provides optimal behavioral symptoms while minimizing death

An assay was run with 3 worms at each of the following CBD concentrations: 0 uM, 5 uM, 10 uM, 15 uM, 20 uM, 25 uM, 28uM, 32 uM, 50 uM, 75 uM, 100 uM. For one minute, each worm's number of Omega/Delta Turns performed and time spent per Omega/Delta Turn were counted, as well as the total number of thrashes (see **Table 2** and **Figures 5, 6, 7, 8, 9**).

Table 2. Mean data for each behavior measured for the CBD Dose Response Curve

Concentration of CBD	Mean Quantity of Omega/Delta Turns	Mean Duration of Omega/Delta Turns	Mean Quantity of Thrashes	Total Number Dead
0 uM	2.4	1.56	94.6	0
5 uM	3.5	0.88	80.25	1
10 uM	3.66	1.42	78.33	2
15 uM	2.33	0.79	63	2
20 uM	4.75	0.59	78	0
25 uM	3	0.59	58.2	0
28 uM	3.8	0.35	20	0
32 uM	9.2	0.53	32.8	0
50 uM	7.25	0.12	42.25	2
75 uM	4	0.083	56.66	2
100 uM	10	0.25	72.5	3

A range of concentrations between 0 mM to 50 uM were used to generate a dose response curve to determine the concentration of CBD that would maximize behavioral symptoms and minimize death of *C. elegans*. The mean time per turn at 0 uM was 1.56 seconds, 0.88 seconds at 5 uM, 1.42 seconds at 10 uM, 0.79 seconds at 15 uM, 0.59 seconds at 20 uM, 0.59 seconds at 25uM, 0.35 seconds at 28 uM, 0.53 seconds at 32 uM, 0.12 seconds at 50 uM, 0.083 seconds at 75 uM, and 0.25 seconds at 100 uM (Table 2, Figure 2). This was recorded at the same time at the mean number of Omega and Delta turns that were completed at each concentration. At 0 mM CBD the mean number of turns was 3 turns, 3.5 turns at 10 mM, 1.833 turns at 20 mM, 7.6 turns at 30 mM, 4.5 turns at 40 mM, and 2.5 turns at 50 mM. (Figure 2) At 0 uM the mean number of turns was , 3.5 turns at 5 uM, 3.66 turns at 10 uM, 2.33 turns at 15 uM, 4.75 turns at 20 uM, 3 turns at 25uM, 3.8 turns 28 uM, 9.2 turns at 32 uM, 7.25 turns at 50 uM, 4 turns at 75 uM, and 10 turns at 100 uM (Table 2, Figure 2).

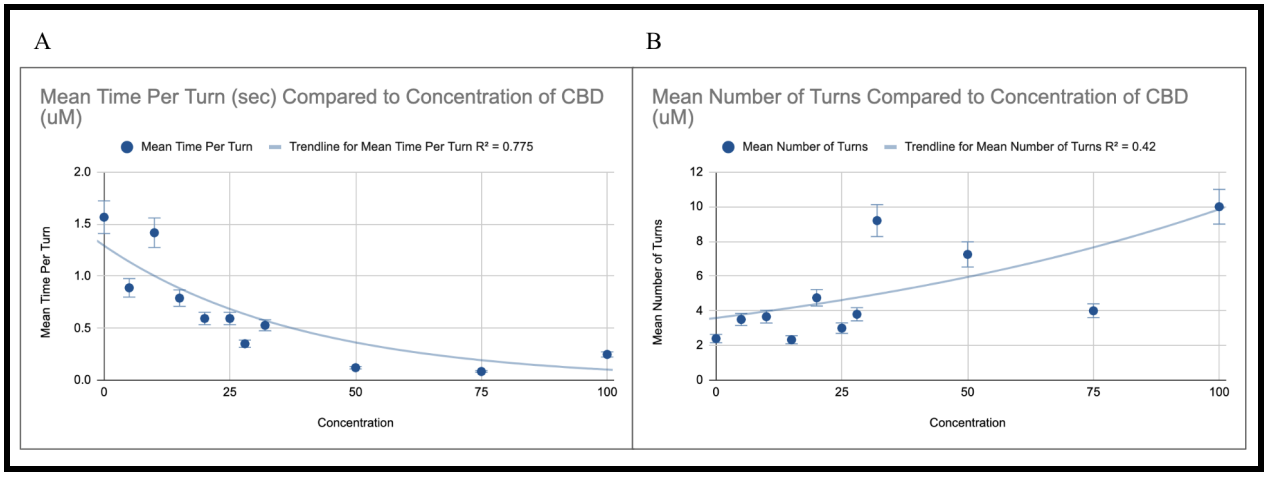


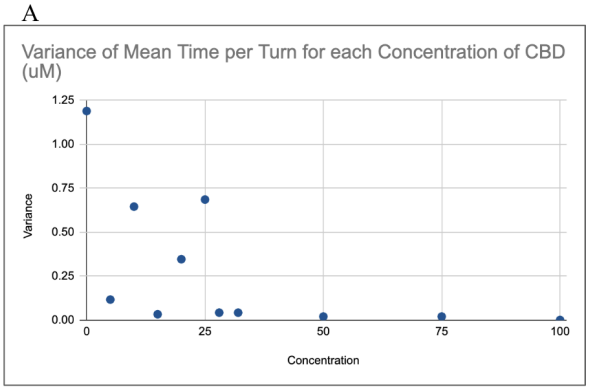
Figure 5. (A) Mean time *C. elegans* at each CBD concentration spent in each Omega/Delta turn over the course of a minute. Each data point is representative of the mean data from five worms. (B) Average number of Omega/Delta turns at each CBD concentration. Each data point is representative of the mean data from five worms.

For the Mean Time Per Turn Behavioral Assay, the F value was 2.54, which is larger than the F-Critical Value which is 2.15 (Figure 6). This indicates that the data is normally distributed and the p value can be used. The p-value was 0.023, which is below 0.05, indicating that the variance falls below a level that suggests the data is statistically significant (Figure 6). The variance in the Mean Time per Turn data was highest at 0 uM with a variance of 1.19. All other variance values were between 0.68 and 0.0013. The variance for this behavioral assay was relatively small indicating that the data is overall closer to a range that can be reliably predicted. This analysis shows that CBD affects the duration of Omega and Delta turns, and there is no previous literature to suggest what the expected result of CBD may look like in *C. elegans* movement behavior to compare to this metric.

For the Mean Number of Turns Behavioral Assay, the F value for the was 1.91 and the F-Critical value was 2.15. Because the F value was not larger than the F-Critical value, the p-value can not be considered valid because the data is not normally distributed. Additionally, the p- value was 0.082. This value is larger than 0.05, indicating that the data is not statistically significant. The variance in the Mean Number of Turns data was highest at 50 uM, with variance of 70.33 and 75 uM with a variance of 48 (Figure 6). The variance was lowest for this behavioral assay was 15 mM at 0.333. The other variance levels were between 2-22.2. Because the variance had such a wide range for many of the data points, it indicates that the data is inconsistent and is

more difficult to reliably predict. This analysis shows that CBD does not affect the number of Omega and Delta turns, and there is no previous literature to suggest what the expected result of CBD may look like in *C. elegans* movement behavior to compare to this metric.

A



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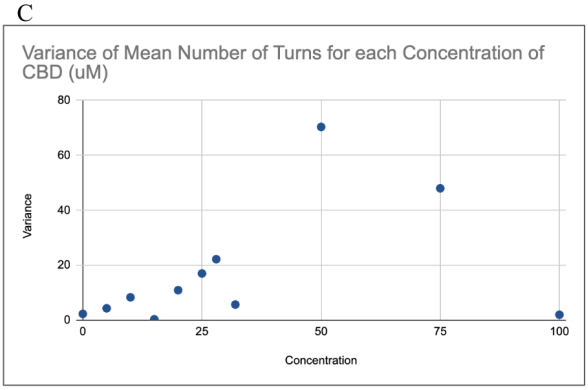


B

CBD Dose Response Mean Time per Turn ANOVA			
Source of Variation	F	P-Value	F crit
Between Groups	2.54	0.023	2.15

D

CBD Dose Response Mean Number of Turns ANOVA			
Source of Variation	F	P-Value	F crit
Between Groups	1.91	0.082	2.15

Figure 6. Variance Visualization and ANOVA Statistics Results for CBD Dose Response Curve Turning Behavioral Measurements

The Mean Number of Thrashes completed was recorded for each concentration in the Dose Response Curve for CBD. This was measured as a movement of lateral swimming motion. The mean number of thrashes at 0 uM was 95.6, 80.25 thrashes at 5 uM, 78.33 thrashes at 10 uM, 63 thrashes at 15 uM, 78 thrashes at 20 uM, 58.2 thrashes at 25 uM, 20 thrashes at 28 uM, 42.25 thrashes at 50 uM, 56.66 thrashes at 75 uM, and 72.5 thrashes at 100 uM. (Figure 7)

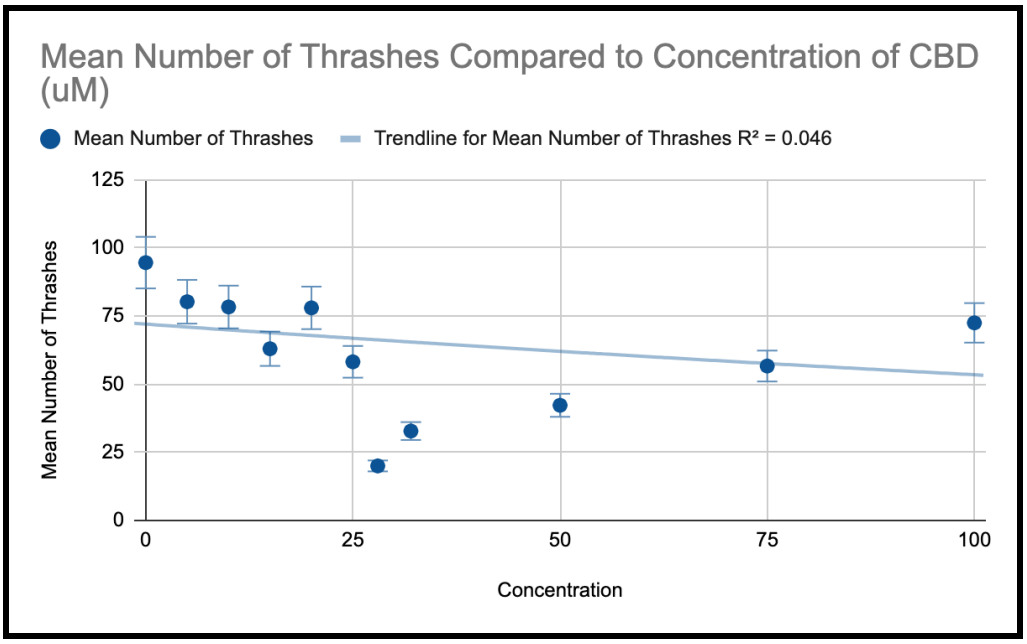
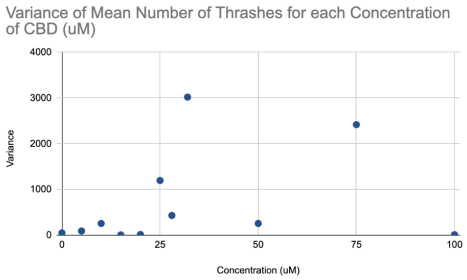


Figure 7. Mean Number of turns at each CBD concentration over the course of a minute. Each data point is representative of the mean data from five worms.

For the Mean Number of Thrashes Behavioral Assay in CBD, the F-value was 2.84, which is larger than the F-Critical Value which was 2.15 (Figure 8). Because the F-value was larger than the F-Critical Value, the data can be considered normally distributed and the p-value is valid. For this data set, the p-value was 0.013. Because the p-value for the Mean Time Per Turn was less than 0.05, it indicates that the variance falls below a level that suggests the data is statistically significant. The variance in the Mean Number of Thrashes data was highest at 75 uM, with variance of 2417.33 and 32 uM with a variance of 3020.2 (Figure 8). The variance was lowest for this behavioral assay was 15 uM at 7 (Figure 8). The other variance levels were between 12.5-1197.2 (Figure 8). Because the variance had such a wide range for many of the data points, it indicates that the data is inconsistent and is more difficult to reliably predict. This analysis shows that CBD affects the number of thrashes, and there is no previous literature to suggest what the expected result of CBD may look like in *C. elegans* movement behavior to compare to this metric.

A



B

CBD Dose Response Mean Time per Turn ANOVA			
Source of Variation	F	P-Value	F crit
Between Groups	2.84	0.013	2.15

Figure 8. Variance Visualization and ANOVA Statistics Results for CBD Dose Response Curve Thrashing Behavioral Measurements

The total number of *C. elegans* that were dead after 30 minutes of treatment with CBD were recorded and analyzed as overall totals. At 5 uM there was 1 *C. elegans* that was dead. 10 uM, 15 uM, 32 uM, and 75 uM all had 2 *C. elegans* that were dead at each concentration. At 100 uM of CBD 3 out of the 5 *C. elegans* were dead after 30 minutes of treatment (Figure 9)

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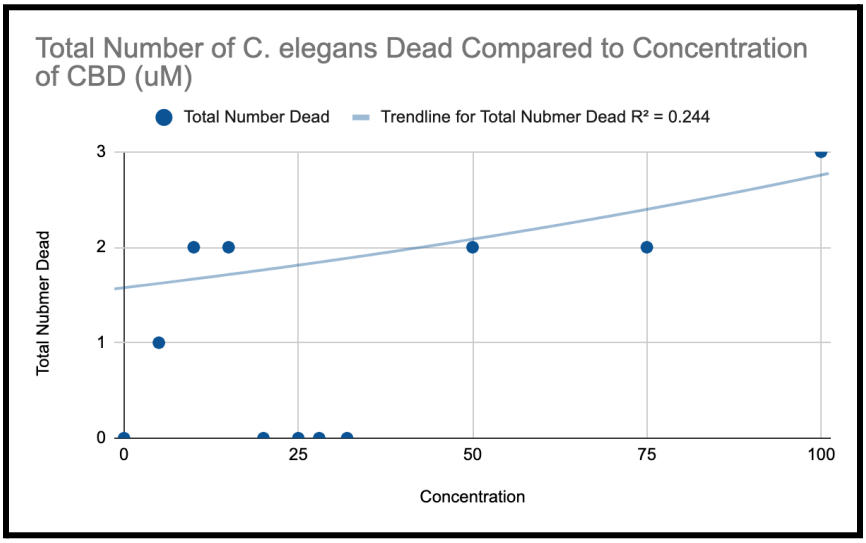


Figure 9. The total number of *C.elegans* that were dead after 30 minutes of treatment with CBD. Data points are representative of gross totals, not mean.

When treated with CBD, C. elegans with the 6-OHDA behavior phenotype exhibit increased thrashing and reduced duration in Omega/Delta turns

Table 3. Mean data for each behavior measured for the PD Treatment Assay

Treatment Condition	Mean Quantity of Omega/Delta Turns	Mean Duration of Omega/Delta Turns	Mean Quantity of Thrashes	Total Number Dead
M9 (Control)	2.83	0.56	115.17	0
CBD (Treatment)	16.16	0.93	67.67	0
6-OHDA (PD)	4.33	6.05	20	3
CBD+ 6-OHDA (Treatment and PD)	4	3.25	40.5	2

The Mean Number of Thrashes for each condition was measured and recorded. M9 had the highest mean number of thrashes 115.16 thrashes. 6-OHDA had a mean number of 20 thrashes. The CBD treatment condition had a mean number of 67.66 thrashes. Finally the Combined 6-OHDA and CBD condition had a mean number of 40.5 thrashes. (Figure 10)

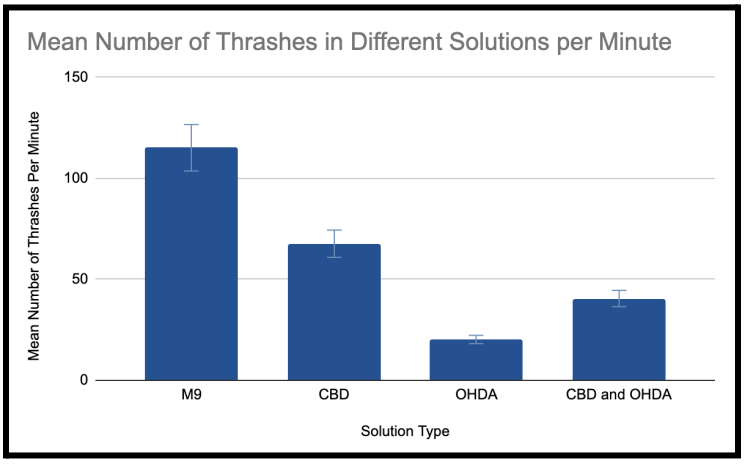


Figure 10. Comparison of Mean Number of Thrashes in Control, CBD, 6-OHDA, and CBD+6-OHDA Treatments

The Mean Number of Omega/Delta Turns for each condition was measured and recorded. CBD had the highest mean number of turns at 16.16 Turns. 6-OHDA had a mean number of 4.33 turns. The M9 control condition had a mean number of 2.83 turns. Finally the combined CBD and 6-OHDA treatment condition had a mean of 4 turns. (Figure 11)

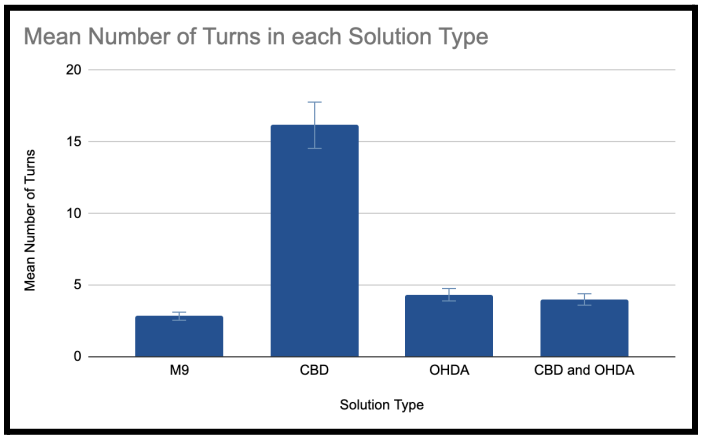


Figure 11. Comparison of Mean Number of Omega/Delta Turns in Control, CBD, 6-OHDA, and CBD+6-OHDA Treatments

The mean time in seconds per Omega/Delta Turn was measured for each treatment condition. The 6-OHDA treatment condition had the highest mean time per turn at 6 seconds. The M9 control condition had a mean time per turn of 0.56 seconds, and the CBD Treatment condition had a mean time of 0.92 seconds per turn. The CBD and OHDA condition had a mean time per turn of 3.25 seconds. (Figure 12)

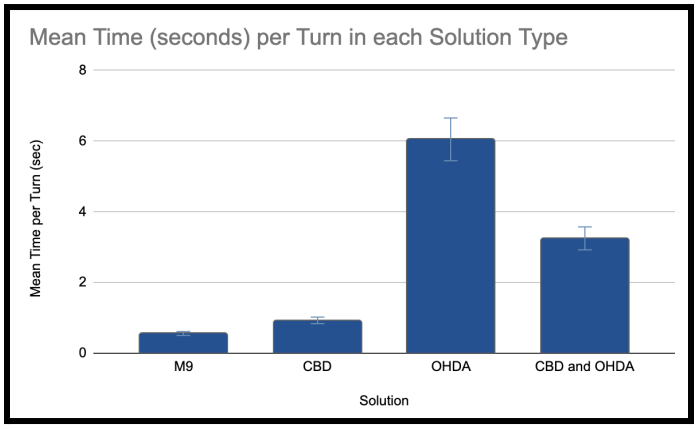


Figure 12. Comparison of Mean Time in Seconds per Omega/Delta Turn in Control, CBD, 6-OHDA, and CBD+6-OHDA Treatments

The total number of *C. elegans* that died in each treatment were measured. In both the M9 control solution and the CBD treatment, no *C. elegans* died from the 30 minutes of treatment. The 6-OHDA treatment had the highest rate of death with the total number of *C. elegans* dead being 3 out of the 8 total. In the combined CBD and 6-OHDA treatment, 2 *C.elegans* out of the 8 for the treatment condition were dead after 30 minutes of the assay (Figure 13).

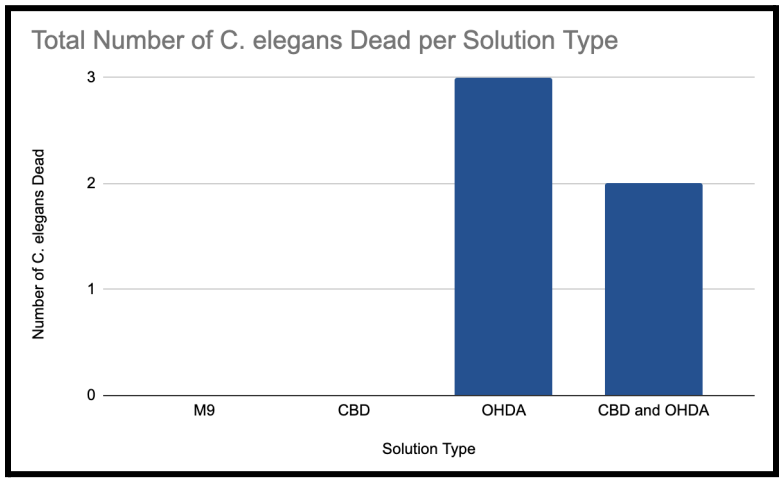
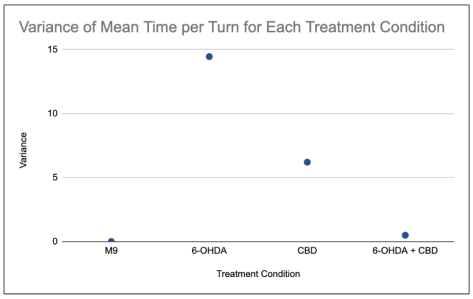


Figure 13. Comparison of Total Number of *C. elegan*s Dead in Control, CBD, 6-OHDA, and CBD+6-OHDA Treatments

For the Mean Time Per Turn Behavioral Assay for the comparison of different treatment conditions, the F-value was 10.99. This value is larger than the the F-Critical Value which was 3.29, indicating that the data is normally distributed and the p-value is valid. Additionally, the p-value was 0.00045 (Figure 14). Because the p-value for the Mean Time Per Turn was less than 0.05, it indicates that the variance falls below a level that suggests the data is statistically significant. The variance in the Mean Time per Turn data had a range between 0-15. The variance for this behavioral assay was relatively small indicating that the data is overall closer to a range that can be reliably predicted (Figure 14). This analysis shows that CBD can successfully decrease the duration of Omega and Delta turns in a PD model, and there is no previous literature to suggest what the expected result of CBD may look like in *C. elegans* movement behavior to compare to this metric.

For the Mean Number of Turns Behavioral Assay for the comparison of different treatment conditions, the F-value was 4.96, which is larger than the F-Critical value of 3.29 (Figure 14). Because the F value is larger than the F-Critical value, it means that the data is normally distributed and the p-value is valid. When assessing the p-value it was 0.014 (Figure 14). This value is larger than 0.05, indicating that the data is not statistically significant. The variance in the Mean Number of turns data had a range between 0-125. The variance for this behavioral assay was relatively small for all conditions expect for CBD, indicating that treatments including M9, 6-OHDA, and 6-OHDA+CBD can be reliably predicted. However, the results for the CBD treatment category are less reliable (Figure 14). This analysis shows that CBD can successfully decrease the number of Omega and Delta turns in a PD model, however based on the previous dose response curves this metric was not statistically significant for the conditions separately, which should be considered when viewing this data.

A



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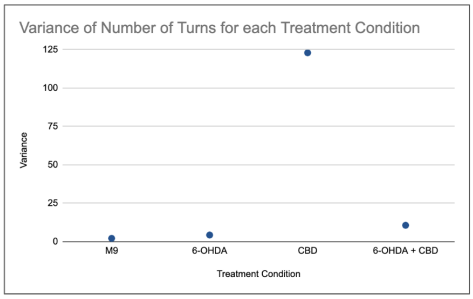

B

Mean Time per Turn for each Treatment Condition ANOVA			
Source of Variation	F	P-Value	F crit
Between Groups	10.99	0.00044	3.29

D

Mean Number of Turns for each Treatment Condition ANOVA			
Source of Variation	F	P-Value	F crit
Between Groups	4.96	0.014	3.28

Figure 14. Variance Visualization and ANOVA Statistics Results for Different Treatment Conditions and the associated Turning Behavioral Measurements

For the Mean Number of Thrashes Behavioral Assay for the comparison of different treatment conditions, the F value was 17.99, which is larger than the F-Critical Value which was 3.29 (Figure 15). Because the F-value was larger than the F-critical value, it indicates that the data was normally distributed and the p-value is valid. The p-value for this assay was 0.000031 (Figure 15). Because the p-value for the Mean Time Per Turn was less than 0.05, it indicates that

the variance falls below a level that suggests the data is statistically significant. The variance in the Mean Number of turns data had a range between 0-2000. The variance for this behavioral assay was relatively small for the M9 and 6-OHDA conditions suggesting that they can be reliably predicted. However, the results for the CBD treatment and 6-OHDA+CBD category are less reliable as they have a higher variance (Figure 15). This analysis shows that CBD can successfully increase the number of thrashes in a PD model worm.

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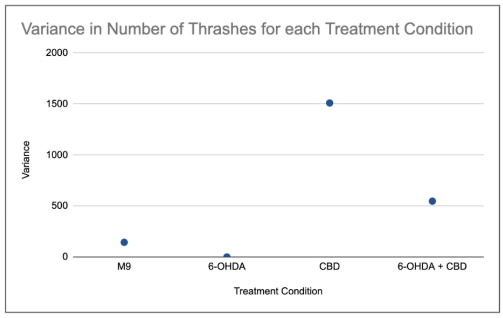

B

CBD Dose Response Mean Time per Turn ANOVA			
Source of Variation	F	P-Value	F crit
Between Groups	17.99	0.000031	3.28

Figure 15. Variance Visualization and ANOVA Statistics Results for Different Treatment Conditions and the associated Thrashing Behavioral Measurements

Discussion

CBD was able to restore some amount of abnormal thrashing behavior in PD model worms

Thrashing is an essential function of *C. elegans* in liquid media, which allows them to have directional movement throughout the well. In this project, it was found that control worms thrash at an average of 115.16 times per minute, which is in line with previously published literature. When 6-OHDA was added, this was brought down to an average of 20 thrashes per minute, which significantly impairs their ability to navigate the well.

After CBD was added to the PD worms, thrashes increased to 40.5 per minute. This is more than twice the amount of thrashes observed in untreated PD worms. Although it is still only around a third of the amount of thrashes observed in control worms, the increase from the PD worms was statistically significant based on an ANOVA analysis, and shows the ability of CBD to restore a more normal behavior pattern in *C. elegans*, which supports the original hypothesis of this project.

CBD significantly reduced the amount of time spent per turn in PD model worms

Omega- and delta- turns help the worms to reorient their direction in liquid media, which is crucial to their movement patterns. In this project, the number of turns per minute was generally the same across the control, PD, and CBD-treated PD worms, with around two to four turns per minute. This is expected, as PD does not reduce the desire to navigate a space. However, it does impair the ability to control movement as it is initiated. This is reflected in the drastic differences in average time each turn takes across the different worm samples. The average time per turn was 0.56 seconds for control worms, but this increased more than 10-fold in PD worms, for an average time of 6.00 seconds. This is a result of the lack of movement control characteristic of PD, which prevents the worms from uncontracting their muscles once the turn has been initiated.

In PD worms where CBD has been added, the average time per turn reduces to 3.25, cutting the time spent almost in half when compared to the untreated PD worms. This is a significant difference that strongly implicates CBD as able to restore normal behavior for this PD model.

Making CBD soluble

One of the largest challenges encountered in this project was determining a method for delivering CBD to *C. elegans*. Because thrashing assays are performed in liquid media, CBD needed to be dissolved. This required the use of a non-polar solvent, which poses an issue as non-polar solvents are lethal to worms in high concentrations (Ura 2002). Because of this limitation, it was necessary to dilute the non-polar solvent concentration to <1% using M9. Non-polar solvents ethanol and DMSO were both tested, and it was found the DMSO was able to dissolve more CBD in the same volume of solvent, which resulted in it being chosen for this project. It was also found that CBD at a concentration above 500µM caused precipitation when mixed with M9, which limited the range of potential concentrations being tested.

Variability in OHDA behavior results and worm viability

Another challenge that impacted results was variability in how different worms reacted to the same conditions. Across different trials, there were significant differences in survival rates for the same concentrations of 6-OHDA, which made it difficult to find a single concentration that would reliably work for both reagents. This can be attributed to several factors. First is variability of life cycle stages. Because no efforts were made to keep worms in the same life cycle stage as one another, it was difficult to ensure that all specimens were in the same stage of life when being assayed. Younger worms would be more susceptible to death than more mature ones at the same conditions. A second possible factor that was discovered late in the project was the method of moving worms into the 96-well plates for assaying. The worms were being swirled to move them from the pick to the well, which it was later found was likely harming them before the assay even began. The method was changed to just dipping them in the well, which still successfully moved them and caused less initial harm, and resulted in lower rates of death in later assays.

Variability in CBD behavior results and unexpected worm behavior

A lack of literature led to several results regarding the CBD-treated worms that currently are unaccounted for. The first is a qualitative observation in an abnormal thrashing behavior present only in the CBD-treated worms. While normal thrashing occurs side-to-side with one thrash on each side, a double thrashing behavior was observed in which worms would move their

heads to each side twice before switching. A literature review on Google Scholar provided no explanations or previous noting of this behavior, and there are currently no theories on what could cause this.

Another surprising find was that the CBD-treated worms had a significantly higher amount of observed turns than any of the other sample groups. While all other groups had an average of <5 turns per minute, the CBD group had 16.16 turns per minute. Previous literature has focused mainly on thrashing as opposed to turns, so there is also currently no accounting for this abnormal behavior.

Finally, in this project the observed lethal dosage for CBD was lower than expected. Previous literature supports a dosage of up to 100μM. However, anything beyond 28μM in these assays was lethal to more than half of the worms and was therefore deemed not worth it to use. This caused issues because it was not clear if behavioral changes could actually be noted at a concentration that low, or if this was simply a result of natural variation among organisms. All results noted were found to be statistically significant, but this could be a result of the small sample size the data represents.

Conclusion & Future Recommendations

Conclusion

The purpose of this project was to determine if CBD could be used to reduce the behavioral effects of 6-OHDA induced Parkinson's Disease in *C. elegans*. From performed assays, it was concluded that concentrations of 30mM 6-OHDA and 28µM CBD maximized PD symptoms and minimized worm death. Additionally, this project provides preliminary evidence in support of CBD restoring normal movement behaviors for PD-model worms. Although the concentration used was not able to fully restore behaviors to control levels, thrash number increased 200% and time spent per turn was reduced by half in CBD-treated worms when compared to untreated PD-model worms.

This project was limited by several factors. The first is a very small sample size for the data gathered. Each data point on the dose response curves represents only 5 worms, and the bar graph data was made up of up to 6 worms each. This sample size is very small and could be subject to a lot of random variation. Another large limitation was the length of time each assay took. Because each assay was performed by hand instead of worm behavior analysis technology, each worm took two minutes to count, which made it difficult to run larger assays. This also allowed for the potential of significant human error with all counting occurring by hand. Finally, there were no analytic methods confirming that the behavioral changes being seen were due to 6-OHDA and CBD being absorbed by the worms. It is our hope that future projects can address these limitations to build on this project's findings.

GFP Fluorescence

A potential method of ensuring 6-OHDA has successfully entered the *C. elegans* and caused neuronal degradation is using GFP fluorescence. Worm strains exist that express GFP in their neurons which can be easily ordered, which would allow for visualization of the neuronal system in both control worms and worms exposed to 6-OHDA. *C. elegans* have 8 dopaminergic neurons (Sulston, 1975) which would be absent from a fluorescence microscopy image and prove that the 6-OHDA was having the desired effect.

Comparison of Behavioral Assay of Time

During the assays performed in this project, a time of 30 minutes was allowed for each worm to equilibrate to their environment in their wells before their behavior was measured. This time was chosen arbitrarily based on previous literature. However, it would be useful to determine an appropriate length of time for the specific concentrations of CBD and 6-OHDA being used. This could be done with an assay using the specified concentration of each reagent and measuring behavior at varying time lengths from 5 minutes to 2 hours, then creating a time response curve. This would give more accurate insight into the procedure for running the assay.

Chromatography Confirmation of CBD Concentration

Due to the highly varying nature of purity in CBD and other cannabis-derived substances, it would have been useful to confirm the composition of CBD used by performing gel filtration chromatography. This could be done fairly simply using a non-polar solvent and the known molar mass for CBD of 314.47g/mol to compare against.

Increase Overall Data Set

As previously mentioned, the data used in this report consisted of either 5 or 6 worms per data point. This is an extremely small sample size that is difficult to draw conclusions from. Because of this, it would be very useful to perform the same experiments that had been done for this project in order to build up the data set and see if the observed trends still stand. This could be helped with worm behavioral analysis software, which would significantly reduce the amount of time assaying took and allow for more data to reasonably be collected per assay.

Appendix

Appendix A: ***Data collected from worms exposed to CBD at varying concentrations for 30 minutes. Data was taken over the course of a minute for each worm.***

6-OHDA Concentration (mM)	*Worm #*	*# of Omega-turns*	*Time spent per Omega-turn (seconds)*
0	1	2	1
0	2	0	0
0	3	4	0.74
0	4	9	0.44
0	5	3	0.33
10	1	7	1.86
10	2	1	1
10	3	7	1.57
10	4	1	1
10	5	5	1.2
20	1	0	0
20	2	1	2
20	3	4	1.25
20	4	4	1.25
20	5	2	3.5
30	1	10	3.8
30	2	10	1.5
30	3	7	2.14
30	4	7	1.71
30	5	0*	N/A

40	1	0*	N/A
40	2	0*	N/A
40	3	2	2
40	4	2	2.5
40	5	1	3
50	1	0*	N/A
50	2	0*	N/A
50	3	0*	N/A
50	4	2	1.5
50	5	0	0

Appendix B: ***Data collected from worms exposed to CBD at varying concentrations for 30 minutes. Data was taken over the course of a minute for each worm.***

CBD Concentration (mM)	*Worm #*	*# of Omega-turns*	*Time spent per Omega-turn (seconds)*	*# of Thrashes*
0	1	0	0	83
0	2	3	1.5	1.5
0	3	2	0.5	93
0	4	4	1.33	97
0	5	3	0.33	101
5	1	4	1	71
5	2	DEAD	DEAD	DEAD
5	3	3	0.75	75
5	4	1	0.5	93
5	5	6	1.3	82
10	1	7	1.75	95

10	2	DEAD	DEAD	DEAD
10	3	2	0.5	77
10	4	DEAD	DEAD	DEAD
10	5	2	0.5	63
15	1	2	0.666	61
15	2	DEAD	DEAD	DEAD
15	3	DEAD	DEAD	DEAD
15	4	3	0.7	66
15	5	2	1	62
20	1	7	0.5714	81
20	2	5	0.4	75
20	3	0	0	74
20	4	7	1.4	82
20	5	DEAD	DEAD	DEAD
25	1	0	0	58
25	2	0	0	89
25	3	8	1.25	0
25	4	7	1.714	78
25	5	0	0	68
28	1	12	0.4166	44
28	2	2	0.5	7
28	3	0	0	0
28	4	3	0.33	41
28	5	2	0.5	8
32	1	6	0.333	7

32	2	12	0.666	131
32	3	11	0.8181	5
32	4	8	0.375	9
32	5	9	0.44	12
50	1	0	0	26
50	2	15	0.2	58
50	3	14	0.28	44
50	4	DEAD	DEAD	DEAD
50	5	DEAD	DEAD	DEAD
75	1	0	0	0
75	2	0	0	82
75	3	12	0.25	88
75	4	DEAD	DEAD	DEAD
75	5	DEAD	DEAD	DEAD
100	1	11	0.2727	75
100	2	DEAD	DEAD	DEAD
100	3	DEAD	DEAD	DEAD
100	4	DEAD	DEAD	DEAD
100	5	9	0.222	70

Appendix C: ***Data collected from worms exposed to different treatment conditions of: Control M9, CBD, 6-OHDA, and CBD +6-OHDA for 30 minutes. Data was taken over the course of a minute for each worm***.

Experimental State	***Worm #***	***# of Omega-turns***	***Time spent per Omega-turn (seconds)***	***# of Thrashes***
M9 Control	1	4	0.75	104

M9 Control	2	1	0.5	135
M9 Control	3	2	0.5	110
M9 Control	4	2	0.5	112
M9 Control	5	5	0.6	106
M9 Control	6	3	0.66	124
10 mM OHDA	1	Dead	Dead	Dead
10 mM OHDA	2	4	4	22
10 mM OHDA	3	6	6.66	20
10 mM OHDA	4	Dead	Dead	Dead
10 mM OHDA	5	Dead	Dead	Dead
10 mM OHDA	6	2	11.5	18
28 uM CBD	1	12	1.35	106
28 uM CBD	2	10	2	75
28 uM CBD	3	0	0	0
28 uM CBD	4	29	1.03	86
28 uM CBD	5	13	0.38	93
28 uM CBD	6	28	0.79	46
10 mM OHDA and 28 uM CBD	1	Dead	Dead	Dead
10 mM OHDA and 28 uM CBD	2	4	2.75	41
10 mM OHDA and 28 uM CBD	3	8	5.75	44

10 mM OHDA and 28 uM CBD	4	Dead	Dead	Dead
10 mM OHDA and 28 uM CBD	5	0	0	37
10 mM OHDA and 28 uM CBD	6	4	4.5	40

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