ASSESSING NON-MOTOR SYMPTOMS IN PARKINSON'S DISEASE USING A 6-HYDROXYDOPAMINE LESION IN THE SUBSTANTIA NIGRA

by

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Abstract

ASSESSING NON-MOTOR SYMPTOMS IN PARKINSON'S DISEASE USING A 6-HYDROXYDOPAMINE LESION IN THE SUBSTANTIA NIGRA

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Motor symptoms within Parkinson's disease (PD) are some of the most well-researched areas, whereas there remains an extensive gap in the literature researching non-motor symptoms, which include sleep, mood, pain and cognitive impairments to name a few. It is well-known that PD affects the brain by depleting dopamine within the nigrostriatal pathway, specifically targeting the substantia nigra, which plays a vital role in producing and controlling motor movements. However, the substantia nigra, as well as dopamine, also play a role in reward and motivation and yet there is still little research done to assess how the degeneration of dopamine within the substantia nigra could impact these cognitive aspects. Pain has also been a common complaint amongst PD patients, yet there is little knowledge of where this pain is coming from or if it is simply a byproduct of the stiffness in muscles that results from PD. Therefore, this project examines both the potential cognitive deficit in decision-making as well as pain as non-motor symptoms in PD over time.

In this study, forty Sprague-Dawley rats were subjected to learn a novel version of the Rat Gambling Task (RGT). Then, they were subjected to the Mechanical Paw Withdrawal Threshold (MPWT) test to establish no prior allodynia. After they successfully learned the RGT, they underwent stereotaxic surgery to inject either 1 μl of saline or 1 μl of 6-hydroxydopamine

(6-OHDA) (5μg/μl dissolved in 0.9% saline and 0.02% ascorbic acid). Animals were then either tested at two weeks or four weeks, as prior literature has suggested maximum dopamine depletion at four weeks. Animals were placed in one of four groups: saline 2 weeks, 6-OHDA 2 weeks, saline 4 weeks or 6-OHDA 4 weeks. After two weeks, animals in the first two groups were tested using the RGT to assess cognitive performance in decision-making. To assess pain thresholds, animals were subjected to the MPWT, as well as the open field task. At four weeks, animals in the latter two groups were assessed the same way. Results indicated a significant difference found between animals injected with 6-OHDA tested at 2 weeks in that they scored significantly lower in percent accuracy within the RGT than the other two saline groups. Surprisingly, there was no difference between the animals in the 6-OHDA 4 weeks group and any other group. Animals in the 6-OHDA 2 weeks group also showed higher omissions in the RGT when tested at 2 weeks than they had done at baseline. Regarding pain, there was no difference between any group in either paw when assessing for hypersensitivity. The results of this study indicate a potential link between cognitive impairments (decision-making) and PD, which should lead to more research being done in this field in order to fully understand the underlying mechanisms. As for pain, there is still work to be done to uncover the etiology of the pain that is experienced by PD patients. It is imperative to continue research to uncover a comprehensive understanding of these non-motor symptoms within PD.

Keywords: Parkinson's disease, non-motor symptoms, cognitive, decision-making, pain, 6-OHDA, Rat Gambling Task (RGT)

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Chapter 1

1.1 INTRODUCTION

Parkinson's disease (PD) is a debilitating disease that disrupts the dopamine neurons and receptors within the substantia nigra. The disease has detrimental effects on motor movements affecting nearly one million people in the United States. Each year, as many as 60,000 new cases are being identified (Downward & Pool, 2019) and is the second most common chronic neurodegenerative disorder (Tieu, 2011; Cunha et al., 2019). Parkinson's disease can involve many brain regions; however, it specifically targets the nigrostriatal pathway by depleting dopamine neurons. Some of the behavioral disturbances include a tremor, rigidity and slowness in movements, as well as bradykinesia, while also causing difficulty with balance and coordination.

While motor symptoms have been the focus of diagnoses, non-motor symptoms have been the subject of research in recent years. Non-motor symptoms are those that are not related to movement and include cognitive impairments, fatigue, pain and mood disorders (Chaudhuri & Schapira, 2009). Non-motor symptoms are extremely common, but highly underdiagnosed and can be just as debilitating as motor symptoms. Due to this tremendous oversight, this study will examine the non-motor symptoms related to cognitive deficits and pain within Parkinson's disease.

1.2 Cognitive Deficits and Parkinson's Disease

Cognitive deficits have been reported as a non-motor symptom from patients suffering from PD. Namely, issues with attention, planning, memory and decision-making seem to be the cognitive deficits that have decreased in functionality as the disease has progressed. Emerging

research has begun studying cognitive deficits primarily in memory capacities. Many cognitive performances have been evaluated by assessing memory utilizing predominantly the novel object recognition tasks (Kadowaki Horita et al., 2013; Matheus et al., 2016; Haghparast et al., 2018; Hsueh et al., 2018; Sampaio et al., 2020) and the Morris water maze task (Tadaiesky et al., 2008; Ma et al., 2014; Haghparast et al., 2018; Liu et al., 2021) for spatial learning. However, only one study has looked at the cognitive ability of decision-making using a risk-based decision-making paradigm while implementing a 6-hydroxydopamine lesion in both the orbitofrontal cortex (OFC) and nucleus accumbens (Mai & Hauber, 2015). They utilized a choice task with probabilistic discounting, where one lever offered a small (one-pellet) but certain reward, and the other lever offered a large (four-pellet) but risky reward, as the odds kept decreasing. To date, there are four widely used variations of the rat gambling task, all differing in equipment used, number of sessions, and the different task possibilities (de Visser, 2011). In this study, the use of a novel version of the rat gambling task will be applied where one lever is the advantageous choice because it provides a small reward 90% of the time, whereas the disadvantageous choice would offer a larger reward 40% of the time.

Decision-making has particularly been a choice of interest as it incorporates the use of weighing out potential rewards. Decision-making is a crucial cognitive process that encompasses reward and motivation, as well as learning and memory, as key components to making successful and advantageous decisions. It is critical that these cognitive processes remain intact, especially when diagnosed with a debilitating and life-altering disease, such as Parkinson's disease. While there are few studies that have begun looking at this connection, Cunha et al. found differences between the left and right hemispheres when injecting 6-OHDA unilaterally, noting that there were issues with impulsivity and contingency degradation (habit-based/goal directed

decision-making) in the left and right hemispheres, respectively (2017). There have been studies that used human patients suffering from Parkinson's disease to closely look at how decisions are made, specifically by utilizing the Iowa Gambling Task (Mimura et. al, 2006; Kobayakawa et al., 2007). These studies have shown a significant difference in persons who have been diagnosed with Parkinson's versus people who have not. It is evident that more studies need to be conducted in order to find a potential deficit in decision-making, as hardly any have been done in the past using rodents as subjects, as utilizing rodent models will allow us to be able to manipulate variables more easily than in human subjects.

1.3 Pain and Parkinson's Disease

Pain is a very common non-motor symptom that is continuously gaining traction in Parkinson's research (Thompson et al, 2017). Not everyone who is diagnosed with Parkinson's disease experiences pain, however about 65% to 85% of patients diagnosed with Parkinson's disease do report pain (Buhidma et al., 2020; Beiske et al., 2009). Approximately one-third of patients have reported experiencing pain before motor and cognitive symptoms (Domenici et al., 2019) The pain that accompanies Parkinson's disease tends to be overlooked and underdiagnosed, as most patients, as well as primary care physicians, tend to focus on the disease itself. The most common form of pain that is reported by patients is musculoskeletal, followed by radicular and dystonic pain (Valkovic et al., 2015; Tai & Lin, 2020). The musculoskeletal pain that is classified as moderate to severe has been compared to chronic pain and has therefore been the subject of study for assessing quality of life among patients (Thompson et al., 2017). Patients experiencing aching and cramping of muscles is thought to come from a lack of mobility, awkward postures and stiffness of the limbs (Ford, 2010). The pain that has been reported by

patients suffering from Parkinson's disease has been a reduction in thermal, electrical, and/or mechanical pain thresholds, suggesting hypersensitivity (Buhidma et al., 2020). It has been theorized that due to the neurodegenerative changes in the nigral dopaminergic pathways, this may alter the way nociceptors process pain (Rukavina et al., 2019; Gandolfi et al., 2017). While there are treatments to alleviate living with PD, there is a need to understand the underlying mechanisms of the pain that is a symptom of PD in order to improve the quality of life. One mechanism worth noting is the A11 cells located in the periventricular, posterior portion of the hypothalamus that creates the descending dopaminergic fibers within the spinal cord (Björklund & Skagerberg, 1979). The A11 area has widely been regarded as modulating nociception processes in which any dysregulation of the descending inhibitory pain modulatory pathways could possibly to be at fault for the rise of chronic pain (Puopolo, 2019). In a study done by Barraud et al. (2010), they aimed to find whether injecting a neurotoxin that depletes dopamine would have any effect on the A11 neurons in the spinal cord, in which their results confirmed their hypothesis. If this is true, it is plausible that A11 neurons affected by the depletion of dopamine caused by PD could cause a malfunction of the modulation of nociception to which PD patients would feel pain.

Only a few studies have tested for allodynia and hyperalgesia with a 6-OHDA lesion but the few that have, have done so by the use of assessing thermal thresholds (both hot and cold) by the use of the hotplate, tail flick or hot bath water test (Faivre et al., 2020; Gee et al., 2015), and acetone (Elshennawy et al., 2021). As for mechanical thresholds, von Frey filaments were used to apply a mechanical stimulus to the hind paw to assess hypersensitivity (Gee et al., 2015; Takeda et al., 2014; Cao et al., 2016) with some even using electric von Frey filaments (Zengin-Toktas et al., 2013; Charles et al., 2018).

1.4 The Role of the Substantia Nigra

As mentioned before, the substantia nigra is one of the main parts of the brain that degenerates and is associated with cell death in dopaminergic cells (Hirsch & Hunot, 2009). In a healthy brain, the substantia nigra projects to the basal ganglia and releases dopamine in order to generate smooth, persistent movements (Sonne, Reddy & Beato, 2020). When the neurons that produce and send dopamine are damaged in large quantities within the substantia nigra, the basal ganglia are no longer able to function properly, which then creates movements that are rigid, along with a tremor. However, despite the substantia nigra also well-known to play a major role in reward and motivation due to the abundance of dopamine neurons and receptors, very little research has been done to assess the potential cognitive deficits that might arise from the depletion of dopamine. Regarding pain, if targeting the substantia nigra does make subjects experience painful sensations, then this may potentially indicate that the A11 neurons might be affected. It cannot be said for certain if they should be affected, but because the substantia nigra and A11 neurons are in close proximity of each other, there could be some type of interaction happening.

1.5 6-Hydroxydopamine

In order to induce dopaminergic neuronal degeneration in rodents to mimic Parkinson's disease, this project used 6-hydroxydopamine (6-OHDA) microinjections into the substantia nigra of the rodent brain. 6-OHDA has been identified as a catecholamine selective neurotoxin, specifically regarding dopamine, and has been extensively used to lesion the nigrostriatal dopaminergic system (Ungerstedt, 1968; Tieu, 2011). Once it has entered the targeted neurons, 6-OHDA accumulates in the cytosol where it is then oxidized, and finally the neuron is

succumbed to an oxidative stress-related cytotoxicity (Tieu, 2011). After it has been injected into the substantia nigra, degeneration of dopaminergic neurons takes place as early as 12 hours, with a majority of dopamine neurons being depleted by week 4 (Blandini et al., 2007; Jeon, Jackson-Lewis & Burke, 1995).

1.6 Purpose

As stated before, very little research has been conducted in order to look at the effects that Parkinson's might have after depleting dopamine neurons from the substantia nigra. The goal of this project is two-fold. First, to explore if injecting 6-OHDA, a neurotoxin that depletes dopamine neurons, into the substantia nigra would have a negative effect on decision-making, which would be assessed by utilizing a novel version of the Rat Gambling Task. The second goal is to see if there is any pain that can be detected within the rodent once subjected to the Parkinson's model, which would be measured by the Mechanical Paw Withdrawal Threshold, by measuring hypersensitivity. Despite dopamine playing a large role in reward and motivational behaviors, it is possible that it also engages in the pain symptoms that have been reported by Parkinson's patients. Common aspects of using this model would include animals circling behavior dependent on the side of the brain injected with 6-OHDA, as well as the impairment in gait due to the hind paw contralateral to the side of the lesion lagging in step size (Iverson. 2010). This project is also looking at differences between time, specifically at 2 weeks and 4 weeks, in order to see if there are any differences in cognitive deficits or pain hypersensitivity over time. It is hypothesized that depleting dopamine in the substantia nigra will render the rodents to perform worse on the RGT when compared to vehicle animals. Specifically, over time, the 6-OHDA lesioned animals tested at 4 weeks will perform the worst. With the little research

done in humans and rodents, the findings that they have denoted is an indication that the issue at hand should and is worth exploring regarding rodent models. The etiology of Parkinson's disease has remained to elude scientists for years, but perhaps by taking a closer look at the lack of dopaminergic projections within the nigrostriatal pathway by targeting the substantia nigra, we can begin to piece the information together to potentially better understand the mechanism of this disease.

Chapter 2

METHODS

2.1 Subjects

Forty Sprague Dawley rats (n = 10 per group) were purchased from Charles River and used in this study. Animals were maintained on a 12:12 light/dark cycle and single-housed to maintain a food-controlled diet until 85% of their original weight was attained while also receiving unlimited water throughout the study. To ensure animals maintained the optimal weight to encourage reward-seeking motivated behavior, animals were weighed daily. All procedures and protocol were reviewed and approved by the University of Texas at Arlington Institutional Animal Care and Use Committee.

2.2 Stereotaxic Procedure

The animal was anesthetized with isoflurane and secured in a stereotaxic frame. The head of the animal was shaved, and 70% alcohol was applied in a circular motion from center to periphery. Then, betadine was applied in a similar motion, and finally, 70% alcohol was applied once more. A scalp incision of about one inch was made and the skull was cleaned and

dried to expose bregma and lambda. Using the coordinates from the Paxinos and Watson stereotaxic atlas of the rat brain (2006), a small hole was made over the region of interest (AP: -5.2, ML: 2.2, DV: -7.7). The microinjection needle was lowered within the region of the substantia nigra and the 1 µl of 6-OHDA (5µg/µl dissolved in 0.9% saline and 0.02% ascorbic acid), or saline, was administered slowly at the rate of .5 microliter per minute. Once finished, the needle was removed, skin stapled and antibiotic ointment was applied. The rat was placed in a clean cage on a heating pad during recovery.

2.3 Procedure

All animals were on a food-controlled diet until 85% of their original weight was reached. Then, they were trained to press a single lever for a grain pellet reward that was distributed via a food dispenser, then moved on to learning how to dual lever press. This training was done once daily for 30 minutes until animals successfully associated the lever press with appetitive reward. Animals then underwent the Mechanical Paw Withdrawal Threshold (MPWT) test to establish a baseline for possible examination of pain sensory. Animals were then subjected to a novel rat version of the Iowa Gambling Task (RIGT) in which they had the ability to choose a reward based on weighing out both long term (how many grain pellets the animal receives) and short-term consequences (how quickly the animal can obtain the reward), which occurred over a 30-minute session. They learned this paradigm for three days in order to establish a baseline. Forty animals received microinjections of 6-OHDA or saline into the substantia nigra using standard stereotaxic procedures. Two weeks later, 20 animals (ten from the experimental group and ten from the control group) were tested using the RGT. Animals were also tested using the MPWT to confirm mechanical hypersensitivity due to pain being a possible non-motor symptom

of PD. Animals were then subjected to the Open Field Task which assesses exploration and general locomotor abilities. The remaining 10 animals that were injected with 6-OHDA, as well as the 10 that were injected with saline, were then subject to the same procedure as listed previously, but at 4 weeks after the 6-OHDA, or saline, injection.

2.4 Mechanical Paw Withdrawal Threshold Testing

Chambers used for Mechanical Paw Withdrawal Testing (MPWT) consisted of Plexiglass chambers on top of mesh screen for easier access to hind paws for stimulation. For habituation purposes, subjects were left in the chamber for ten minutes. To measure possible hypersensitivity, an up/down method to the plantar portion of the hind paws was completed using a set of von Frey monofilaments (3.85, 5.68, 9.74, 18.39, 39.42, 77.3, 135.3, and 251.34 mN). At the beginning of each trial, a 9.74 mN von Frey filament were delivered to the right hind paw for about 1 second, then to the left paw. If a withdrawal response was detected (i.e. paw withdrawal or licking of the paw), the next lowest force was used, whereas the next highest force was delivered if a response was not observed. This procedure was repeated until no response had been made at the highest force (251.34 mN) or until five total stimuli are given. This test was conducted three times and scores were averaged to determine the mean threshold to tactile stimulation for the right and left paws for each animal (Dixon, 1960).

2.5 Operant Training

Animals were then assigned to learn how to lever press a single lever, each being assigned either the right or the left lever randomly. In the first phase of training, a light above one of the levers was lit for one second before the assigned lever would come forward and dispense a

pellet. The rodent should then associate the light and lever with the dispense of a pellet. Once the rodent has moved on to phase two, the light shone and the lever came forward for an indefinite amount of time until the rodent pressed the lever, which would then dispense a pellet. In order to successfully complete phase two, a rodent must press the lever 80 times in one thirty-minute session. Once it has moved on to phase three, the light and lever came forward for ten seconds before retracting. If the lever was pressed within the ten second trial, the rodent was rewarded with a single pellet, the light turned off and the lever retracted for ten seconds until the next trial began. If the lever was not pressed within ten seconds, then the light went out, the lever retracted, there was a time out for ten seconds, and no reward was dispensed. Once these three phases had been completed for one side, phases two and three were repeated but for the opposite side. Once those two phases had been completed for the opposite side, the rodent was subjected to dual lever pressing, where both lights were turned on while both levers came forward for ten seconds. The goal of this is to eliminate a bias for either side, where the animal needed to have pressed a lever 80% of the time, but less than 60% of a bias on any side. Once this had been completed, the animal was then ready to learn the Rat Gambling Task. All operant procedures were similar to the ones used in Salcido's thesis (2017).

2.6 Rat Gambling Task (RGT)

The main method to assessing decision-making in rats was to utilize a novel version of the rat gambling task. Which was slightly modified from the one used in Salcido's thesis (2017). While there are currently five widely used versions of the Rat Gambling Task, this project aimed to use a similar but novel version that used both rewards and punishments in order to increase the complexity of the task. Within this version, it utilized lever pressing in operant chambers in order

to make a decision between two choices, one being a more advantageous choice than the other. Within the advantageous choice, if pressed, one pellet was dispensed 80% of the time, while the other 20% of the time, a 10-second time out was issued. However, if the disadvantageous choice were to be pressed, then three pellets were dispensed 40% of the time, while the other 60% of the time, a 30-second time out was issued. While the latter choice dispenses more pellets, it would ultimately be disadvantageous in the long run as within a thirty-minute session, the advantageous choice ultimately dispenses more pellets and also provides a more secure and consistent reward.

2.7 Open Field Task

This task assesses the rat's willingness to explore and is a test for general locomotor abilities. They were placed in a circular arena and monitored for 5 minutes. The total distance they travelled, mean velocity and rearing behavior was quantified using Ethovision, a software used to record and analyze the behavior, movement and activity of an animal by use of video recording. Methods used here were similar to the ones used by Ferro et al (2005).

2.8 Immunohistochemistry

To fix the brain tissue, animals were subjected to a transcardial perfusion. To anesthetize, animals were given an intraperitoneal injection of 150 mg/kg Euthasol (euthanasia solution including pentobarbital and phenytoin). Once anesthetized, animals were transcardially perfused with 200 ml of phosphate-buffered saline (PBS), and then with 200 ml of 4% paraformaldehyde (PFA). Brain tissue were then extracted, placed in PFA for post-fixture and stored at 4°C. After 24 hours, brains were moved to 20% glycerol. To slice, the stage of the microtome was frozen with dry ice and the brain placed on top was frozen. The brain was sliced

down the coronal plane and placed in .01% sodium azide in 1x PBS. After slicing, immunohistochemistry was used for staining of tyrosine hydroxylase (TH). Slices were washed three times with PBS for 10 mins each, then brain sections were incubated in 3% H_2O_2 in 1x PBS for 15 minutes. Brain sections were washed in 1 x PBS again three times for ten minutes each. Then, brain sections were blocked for 45 minutes at room temperature in 0.3% Triton X and 3% serum in 1x PBS. After, brain sections incubated with rabbit anti-tyrosine polyclonal hydroxylase primary antibody (item #AB152, Sigma Aldrich, United States, 1:5000) over night at room temperature. Sections were then washed again with 1x PBS three times at ten minutes each. Brain sections were then incubated with AffiniPure Goat Anti-Rabbit IgG secondary antibody (Item # 111-065-003 JacksonImmuno, United States, 1:1000) for 1.5 hours. Brain sections were washed with 1x PBS three times for ten minutes each. Avidin-Biotin-Complex (ABC) kit (Item # PK6100, Vector, United States) was added to brain sections and incubated at room temperature for 1.5 hours. Brains sections were washed with 1x PBS 2 times for ten minutes each, then washed with Tris (0.1M, pH 7.4) two times for ten minutes each. Then brain sections received 4 drops of buffer & H_2O_2 and then 8 drops of 3,3'-diaminobenzidine (DAB) substrate (Item # SK-4100, Vector, United States). Finally, brain sections were washed with 1x PBS three times for ten minutes. Brain sections were then placed on a slide, dehydrated and cover slipped. The methods used for immunohistochemistry were similar to the methods used by Lima et al (2021).

Chapter 3

RESULTS

3.1 Immunohistochemistry

After slices were stained for tyrosine hydroxylase, they were viewed under a microscope to identify if there was a lesion for the 6-OHDA animals, as well as to confirm there was no lesion for the saline animals. Upon review, it was determined five animals from the 6-OHDA week 4 group had to be omitted due to no lesion found, one animal from the 6-OHDA week 2 group due to no lesion found, and one animal from the saline week 4 group due to a lesion being identified. A lesion was identified if one side of the substantia nigra pars compacta had staining of tyrosine hydroxylase and the other side was clear of staining. In the end, the total $N = 33$, where the total number of animals in the saline week 2 group was $n = 10$, 6-OHDA week 2 group was $n = 9$, saline week 4 group was $n = 9$, and 6-OHDA week 4 group was $n = 5$.

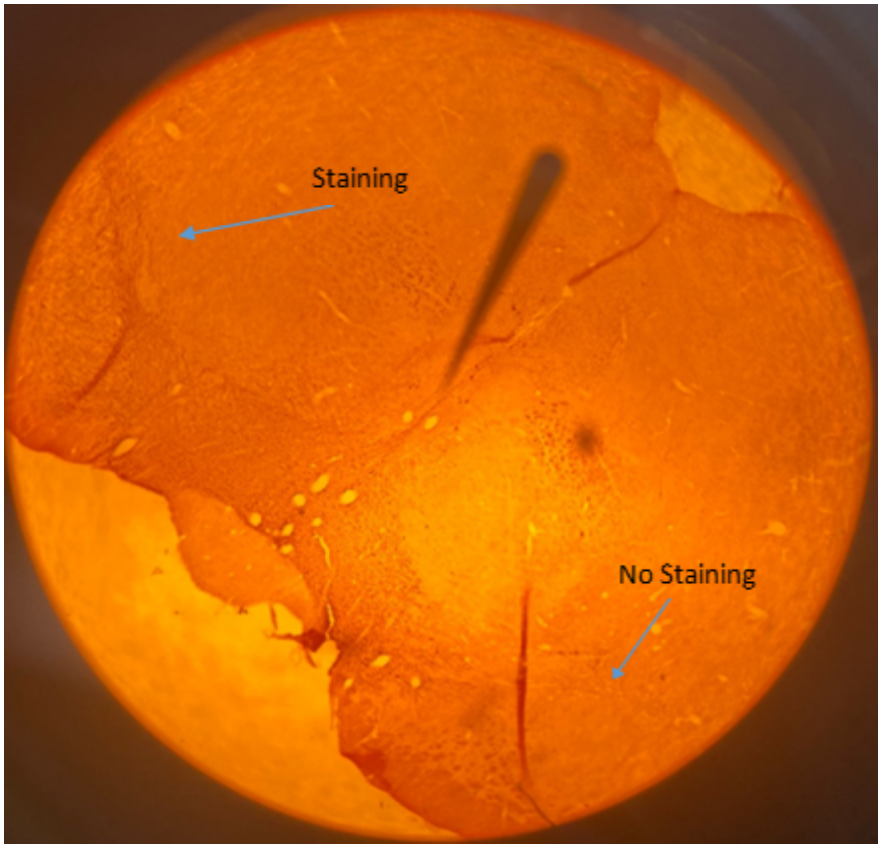

Figure 1: Photo of tyrosine hydroxylase staining in animal subject EL31 (6-OHDA week 2) indicating lesion on left side of substantia nigra.

3.2 Rat Gambling Task (RGT)

3.2.1 Percent Accuracy by Condition

A univariate Analysis of Variance (ANOVA) was used to assess group difference in percent accuracy for advantageous outcomes. Significant differences were revealed, $F(3, 29) = 4.40$, $MSE = .04$, $p = .011$, $\eta_p^2 = .31$. Bonferroni post-hoc tests showed specifically those tested at 2 weeks after injection of 6-OHDA had significantly lower scores of accuracy ($M = .46$) than both groups of animals injected with saline that were tested at 2 and 4 weeks ($M = .77$ and $M = .75$, respectively). Surprisingly, those tested at 4 weeks after injection of 6-OHDA showed no difference with any other groups ($M = .61$)

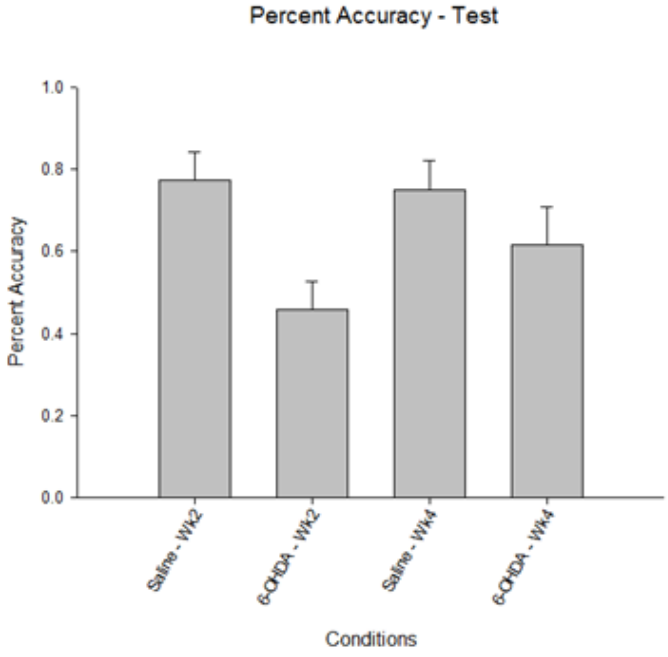

Figure 2-1: Percent Accuracy across groups. Percent accuracy was calculated by dividing the number of advantageous lever presses by the total number of lever presses in the testing trial.

A 4(condition) x 2(time) mixed model repeated measures ANOVA was used to assess group differences in percent accuracy over time. There was no significant main effect for condition, $F(3, 29) = 1.85$, $MSE = .06$, $p = .16$, $\eta_p^2 = .16$. There was also no significant main effect for time, $F(1, 29) = 1.41$, $MSE = .03$, $p = .25$, $\eta_p^2 = .05$. There was, however, a significant interaction between condition and time. $F(3, 29) = 3.16$, $MSE = .03$, $p = .039$, $\eta_p^2 = .25$. Post hoc test show the significance to be in time 2, between animals tested at two weeks after injection of 6-OHDA ($M = .46$) and animals both tested after two ($M = .77$) and four ($M = .75$) weeks after injection of saline, as iterated above.

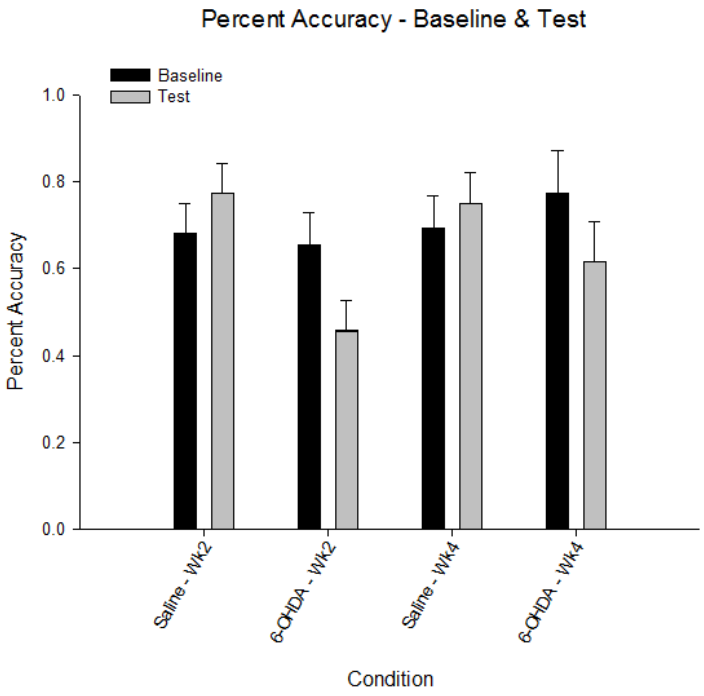

Figure 2-2: Percent Accuracy over time, including baseline data and test data. Percent accuracy was calculated by dividing the number of advantageous lever presses by the total number of lever presses

3.2.2 Percent Omission by Condition

A univariate ANOVA was used to assess group difference in percent omission during testing. No significant differences were found, $F(3, 29) = 2.18$, $MSE = .01$, $p = .11$, $\eta_p^2 = .18$. A 4(condition) x 2(time) mixed model repeated measures ANOVA was used to assess group differences in omission over time. There was no significant main effect for condition, $F(3,29) = .98$, $MSE = .01$, $p = .42$, $\eta_p^2 = .09$. There was also no significant main effect for time, $F(1, 29) = 3.14$, $MSE = .01$, $p = .087$, $\eta_p^2 = .10$. There was, however, a significant interaction between condition and time, $F(3, 29) = 3.35$, $MSE = .01$, $p = .033$, $\eta_p^2 = .26$. Post Hoc tests showed the difference between the animals tested at two weeks after injection of 6-OHDA, in which they omitted choices significantly more in time 2 ($M = .19$) than in time 1 ($M = .06$).

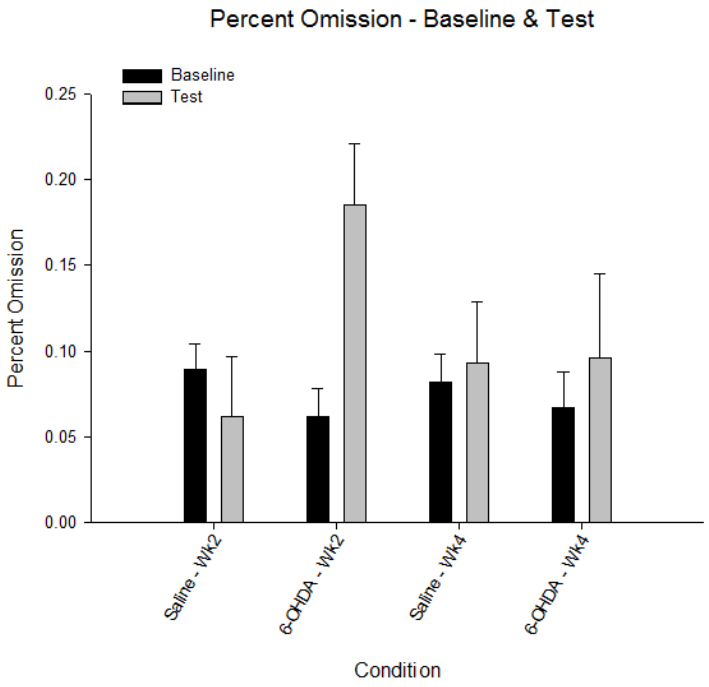

Figure 2-3: Percent Omission over time. Percent omission was calculated by dividing the number of omissions by the total number of lever presses.

3.2.3 Left Latency

A 4(condition) x 2(time) mixed model repeated measures ANOVA was used to assess group differences in latency in left lever pressing over time. There was no significant main effect for condition, $F(3, 29) = 1.07$, $MSE = .76$, $p = .379$, $\eta_p^2 = .10$. There was no significant main effect for time, $F(1, 29) = 1.18$, $MSE = .24$, $p = .286$, $\eta p^2 = .04$. There also no significant interaction between condition and time, $F(3, 29) = .98$, $MSE = .24$, $p = .416$, $\eta_p^2 = .09$.

3.2.4 Right Latency

A 4(condition) x 2(time) mixed model repeated measures ANOVA was used to assess group differences in latency in right lever pressing over time. There was no significant main effect for condition, $F(3,29) = 1.06$, $MSE = 1.58$, $p = .381$ $\eta_p^2 = .10$. There was no significant main effect for time, $F(1, 29) = .06$, $MSE = .57$, $p = .813$, $\eta_p^2 = .002$. There was also no significant interaction between condition and time, $F(3, 29) = 1.05$, $MSE = .57$, $p = .385$. $\eta_p^2 = .10$.

3.2.5 Latency for Choosing Correct Choice

A 4(condition) x 2(time) mixed model repeated measures ANOVA was used to assess group differences in latency in lever pressing of the correct choice over time. There was no significant main effect in condition, $F(3,29) = .65$, $MSE = 1.00$, $p = .589$, $\eta_p^2 = .06$. There was no significant main effect for time, $F(1,29) = .01$, $MSE = .38$, $p = .918$, $\eta_p^2 = .00$. There was also no significant interaction between condition and time, $F(3,29) = 1.67$, $MSE = .38$, $p = .19$, $\eta_p^2 = .15$.

3.2.6 Latency for Choosing Incorrect Choice

A 4(condition) x 2(time) mixed model repeated measures ANOVA was used to assess group differences in latency in lever pressing of the incorrect choice over time. There was no significant main effect for condition, $F(3,29) = .40$, $MSE = 1.73$, $p = .756$, $\eta_p^2 = .04$. There was no significant main effect for time, $F(1,29) = .001$, $MSE = .39$, $p = .977$. $\eta_p^2 = .00$. There was also no significant interaction between condition and time, $F(3,29) = 2.59$, $MSE = .39$, $p = .072$, $\eta_p^2 = .21$.

3.3 Mechanical Paw Withdrawal Threshold (MPWT)

3.3.1 MPWT Left Paw

A univariate ANOVA was used to assess group differences indicating potential pain in the left paw due to the degeneration of dopamine. There was no significant difference between groups, $F(3,29) = 1.96$, $MSE = 8192.70$, $p = .142$, $\eta_p^2 = .17$.

A 4(condition) x 2(time) mixed model repeated measures ANOVA was used to assess group differences indicating potential pain in the left paw due to the degeneration of dopamine over time. There was no significant main effect for condition, $F(3,29) = 1.19$, $MSE = 4446.36$, $p = .332$, $\eta_p^2 = .11$. There was no significant main effect for time, $F(1,29) = 3.94$, $MSE = 4587.28$, $p = .057$, $\eta_p^2 = .12$. There was also no significant interaction between condition and time, $F(3,29) = 2.49$, $MSE = 4587.28$, $p = .08$, $\eta_p^2 = .21$.

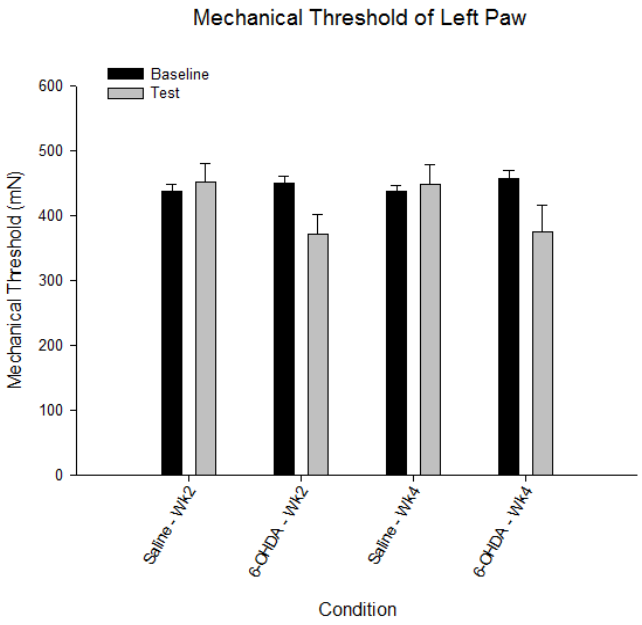

Figure 2-4: Mechanical paw withdrawal thresholds over time between conditions in the left hind paw.

3.3.2 MPWT Right Paw

A univariate ANOVA was used to assess group differences indicating potential pain in the right paw to the degeneration of dopamine. There was no significant difference between groups, $F(3,29) = 1.05$, $MSE = 1114.06$, $p = .384$, $\eta_p^2 = .10$.

A 4(condition) x 2(time) mixed model repeated measures ANOVA was used to assess group differences indicating potential pain in the right paw due to the degeneration of dopamine over time. There was no significant main effect for condition, $F(3,29) = .54$, $MSE = 956.30$, $p = .656$, $\eta_p^2 = .05$. There was no significant main effect for time, $F(1,29) = .004$, $MSE = 1031.15$, p

= .953, η_p^2 = .00. There was also no significant interaction between condition and time, $F(3,29)$ = 1.24, MSE = 1031.15, p = .314, η_p^2 = .11.

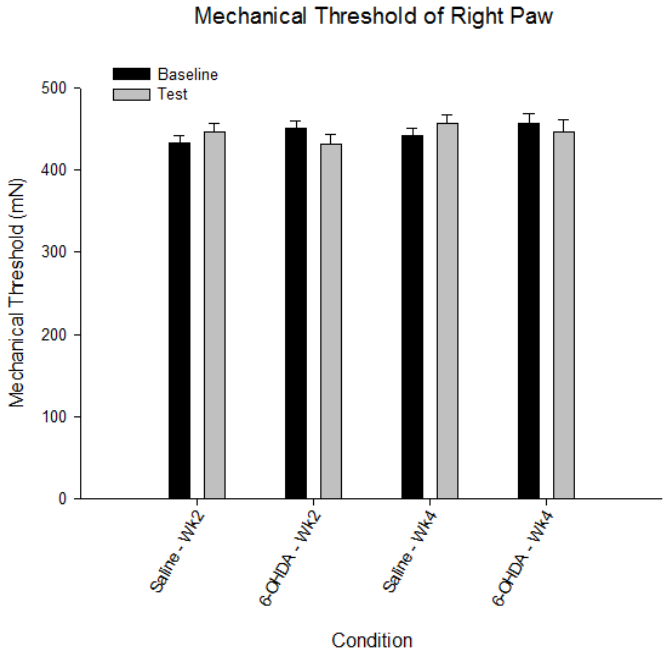

Figure 2-5: Mechanical paw withdrawal thresholds over time between conditions in the right hind paw.

3.4 Open Field Task

A univariate ANOVA was used to assess group difference in distance moved using the open field task. There was no significant difference between groups, $F(3,29)$ = .05, p = .984, η_p^2 = .005. A univariate ANOVA was used to assess group difference in velocity and no significant difference was found, $F(3,29)$ = .05, p = .986, η_p^2 = .005. A univariate ANOVA was used to assess group difference in turn angle and no significant difference was found, $F(3,29)$ = .59, p = .626, η_p^2 = .06.

Chapter 4

DISCUSSION

4.1 Cognitive Impairments in Decision-Making

4.1.1 Percent Accuracy

It was hypothesized that animals injected with 6-OHDA would score lower on percent accuracy than the control animals; specifically, animals tested at week 4 would score the lowest, as they should have reached maximum dopamine depletion. Animals that were tested at 2 weeks after injection of 6-OHDA showed significantly lower accuracy scores than the other two saline groups. This is congruent with the hypothesis previously proposed in that dopamine depletion in the substantia nigra will cause cognitive deficits in decision-making. Surprisingly, the group that should have been exposed to maximum depletion in dopamine showed no significant difference between any other groups; however, they did indicate lower scores when compared to their baseline scores, as well as the other saline groups during test time, though not enough to be considered significant. This is an important finding as it might suggest a type of compensatory effect mitigated by other areas of the brain, such as the ventral tegmental area (VTA) or the nucleus accumbens, both of which are associated with reward, which would then let the mesolimbic or mesocortical pathways perhaps help out (Smith & Masilamoni, 2017). The VTA emits dopamine to the ventral striatum and the cerebral cortex, so it is possible that with these areas still intact, decision-making could have been restored. These results are contradicting to what Mai & Hauber (2015) concluded, however, they injected 6-OHDA into the orbitofrontal cortex and nucleus accumbens, which could account for the difference in results. Our results are consistent with results found by Mimura et al. (2006) and Kobayakawa et al. (2008) in which

they assessed decision-making utilizing the Iowa Gambling Task between PD patients and healthy controls in which both studies concluded that PD patients scored significantly lower than the healthy controls. Though there are not many studies using pre-clinical data examining decision-making using animal models, it is evident that is it essential to continue exploring in this direction.

4.1.2 Omission

There was significance found within the group of animals tested at 2 weeks after injection of 6-OHDA in which those animals omitted more trials in time 2 than they did in time 1. This is interesting coupled with the fact that it was the same group that consistently chose the choice with larger quantity but less consistency, therefore it seems unlikely to be attributed to satiation. One possible explanation would be that, on average, they took longer to make a decision, as latency was slightly longer for animals in this group, though not enough to be significant.

4.2 Pain and Parkinson's Disease

4.2.1 MPWT Left and Right Hind Paw

It was hypothesized that there would be lower mechanical thresholds thus indicating hypersensitivity at two and four weeks after animals were injected with 6-OHDA. Though there was no significance difference found between any group over time in either paw, it is interesting to note the slight decrease in sensitivity threshold in the left paw for both groups of animals injected with 6-OHDA. Other studies (Gee et al., 2015; Takeda et al., 2014; Cao et al., 2016) have found allodynia using the mechanical paw withdrawal threshold test in both paws, and it is curious that we did not find anything; however, it is worth mentioning that the studies mentioned

previously injected 6-OHDA in different regions of the brain. Cao (2016) injected 6-OHDA in the caudate-putamen, while Gee (2015) and Takeda (2014) injected 6-OHDA in the medial forebrain bundle, which could account for the differences found in our results. While it is impossible to say that pain was being experienced by the animal, it is worth noting that this is the paw that is ipsilateral to the side of the substantia nigra that was injected when as early as 24 hours after injection of 6-OHDA, the right paw, which is contralateral to the lesion, was impaired; however, this is in line with what Takeda et al. (2005) found as they reported finding latency in paw withdrawal in the paw ipsilateral to the lesion. In the end, because no difference was found suggesting pain was present, it can be inferred that the A11 neurons were not affected. In the future, MPWT testing could be done 24 hours after surgery to gauge if pain levels exist during the time they are experiencing the abnormal gait.

4.3 Open Field Task

The open field task was used to assess and document distance moved, velocity and turn angle between the experimental and control animals. There were no significant differences found between any of the variables, which is not surprising as experimental animals seemed to gain back their ability to walk by two and four weeks. As early as 24 hours, experimental animals were exhibiting impaired gait, as well as walking in a circle counterclockwise. This was a daily occurrence until about the end of the first week, which might be attributed to some type of compensatory factor overridden by non-dopamine brain regions and/or pathways, as suggested previously by Iverson (2010). Perhaps in the future, open field testing could be done after 24 hours to capture a more accurate reading of their abnormal gait as well as their turning counter-clockwise.

Chapter 5

5.1 CONCLUSION

Parkinson's disease is a debilitating disease that affects the nigrostriatal pathway, mainly targeting the substantia nigra. Motor symptoms are the obvious and more prevalent symptoms being treated; however, non-motor symptoms have been gaining traction recently due to patients and health care providers recognizing and diagnosing them. The two non-motor symptoms examined were cognitive deficits, specifically in decision-making, and pain. Pain is a diverse and multidimensional experience (Melzack & Casey, 1968) and has been documented in PD patients, however, the origin is unknown. It is imperative to find the mechanism, if there is one, that is causing the pain experienced. This may prove challenging as different types of pain have been reported (dystonia, radicular, musculoskeletal and central pain), as well as the ambiguous and complex nature of the pain experience that is subjective to each person (Buhmann, Kassubek & Jost, 2020; Valkovic et al., 2015; Tai & Lin, 2020). Although there was no indication of hypersensitivity in animals injected with 6-OHDA in this study, which conflicted findings from other studies (Gee et al., 2015; Takeda et al., 2014; Cao et al., 2016), there could be other brain structures or pathways involved in the mechanism of pain in patients with PD. There could also be compensatory factors that played a role in healing or restorative aspects. Arthritis has also been named a potential culprit, as most patients with PD are of older age, which is around the same time people experience arthritis in joints (Rabin et al., 2016). Or it could be that pain is a simply a byproduct of the dystonic or musculoskeletal pain that is caused by the motor-symptoms (rigidity, stiffness and tremor).

Decision-making is a crucial skill to have in order to survive by choosing advantageous choices over disadvantageous choices that could harm or elevate risk factors. Reward and

motivation, two aspects needed in advantageous decision-making, are associated with the substantia nigra as there is an abundance of dopaminergic neurons, yet there remains a gap in the literature as to how PD could affect decision-making. Understanding the possible connection between PD and decision-making could be monumental to patients suffering from this disease. Of course, this is at the most basic, biological level of decision-making; decision-making is a highly complex and complicated mechanism that incorporates many other brain regions (such as the prefrontal cortex and hippocampus) (Bechara, 2000), as well as including several other factors like learning, memory and even emotion (Bechara, 2004). The results from this study have indicated that there may be a potential link between PD and impaired decision-making, as seen from the week 2 6-OHDA group scoring significantly lower than the other saline group. However, the question remains unknown as to why the week 4 6-OHDA group did not perform significantly worse than any of the groups. This could be due to the low sample size, as it was slightly lower than baseline and the other saline test groups, however, with maximum dopamine depletion happening at week 4 after injection of 6-OHDA, it is a wonder why it is not the lowest performing group.

Nonetheless, this study has found possible evidence linking PD and poor decision-making which could be beneficial to health-care providers, as well as patients, to educate about the effects of non-motor symptoms that are associated with PD. Perhaps by making patients and caretakers aware of this it might help them begin to understand how PD is affecting themselves. Future studies should investigate depleting dopamine in other parts of the brain that are affected by PD other than the substantia nigra to further assess potential pain hypersensitivity or even a breakdown in pain modulation as suggested before, because it is

imperative that research is ongoing to continue uncovering a comprehensive understanding of non-motor symptoms that could result from PD.

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