2022

Investigating Anxiety-Like Behavior as a Non-Motor Side Effect of Deep Brain Stimulation of the Subthalamic Nucleus in a Parkinsonian Rat Model

Carter Mulder
Minnesota State University, Mankato

Investigating Anxiety-Like Behavior as a Non-Motor Side Effect of Deep Brain Stimulation of the Subthalamic Nucleus in a Parkinsonian Rat Model

By

Carter Mulder

A Thesis Submitted in Partial Fulfillment of the

Requirement for the Degree of

Master of Arts

In

Clinical Psychology

Minnesota State University, Mankato

Mankato, Minnesota

May 2022

Thursday, May 5th, 2022

Investigating Anxiety-Like Behavior as a Non-Motor Side Effect of Deep Brain Stimulation of the Subthalamic Nucleus in a Parkinsonian Rat Model Carter Mulder

This thesis has been examined and approved by the following members of the student's committee.

_____
Advisor

_____
Committee Member

_____
Committee Member

Acknowledgements

Applied Computational Neurophysiology and Neuroscience Lab, Mayo Clinic, Rochester

Dr. Suelen Lucio Boschen De Souza, Thesis Advisor, Committee Member

Sydney Hillan, Ph.D. Candidate

Dr. Luis Lujan, ACNN Principal Investigator

Department of Psychology, Minnesota State University, Mankato

Dr. Jeffrey Buchanan, MNSU Advisor

Dr. Adam Steiner, Thesis Committee Member

This thesis was made possible by the prestigious Mayo Clinic in conjunction with the Applied Computational Neurophysiology and Neuroscience Lab led by principal investigator, Dr. Luis Lujan. I would like to extend my deepest appreciation and gratitude to Dr. Suelen Lucio Boschen De Souza. Dr. Boschen is an example of a true advisor. This project would not have come to fruition without Dr. Boschen's expertise, patience, and mentorship. Ph.D. candidate Sydney Hillan also made this project possible by providing her surgical and behavioral experience, without Sydney, this project would not be available, thank you.

I also want to thank Dr. Jeffery Buchanan, Clinical Psychology Program Director and my advisor at MNSU. Dr. Buchanan helped facilitate this study between Mayo Clinic and MNSU. Finally, I would like to thank Dr. Adam Steiner, Assistant Professor at MNSU, and thesis committee member, for offering his background in neuroscience and animal research.

INVESTIGATING ANXIETY-LIKE BEHAVIOR AS A NON-MOTOR SIDE EFFECT OF DEEP BRAIN STIMULATION OF THE SUBTHALAMIC NUCLEUS IN A PARKINSONIAN RAT MODEL

Carter Mulder

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ARTS IN CLINICAL PSYCHOLOGY

MINNESOTA STATE UNIVERSITY, MANKATO

MANKATO, MINNESOTA

ABSTRACT

Parkinson's disease (PD) is estimated to impact nearly 10 million people globally and is estimated to increase in the future. PD is a progressive neurodegenerative disease that worsens through continuous cell death of dopaminergic neurons. This cell death can create motor symptoms such as bradykinesia, tremor, and muscular rigidity. Subthalamic nucleus deep brain stimulation, STN DBS, is a surgical intervention which places stimulating electrodes in the STN greatly improving motor symptoms. However, STN DBS has been reported to possibly influence non-motor symptoms such as anxiety both acutely and long-term, which decreases the quality of life for those with PD. We hypothesize that acute and chronic STN DBS will produce more anxiety-like behavior in a rat model of PD compared to PD rats that are not stimulated. Nineteen rats underwent stereotactic surgery and were bilaterally lesioned in the dorsal striatum with 6-Hydroxydopamine to create PD phenotype and neuropathology. Each rat had a stimulating electrode unilaterally implanted into the STN. All rats were recorded for 10 minutes in the open field behavior paradigm to examine anxiety-like behavior such as rearing, grooming, and time spent in by the walls and in the center, along with measures of locomotion such as total distance traveled and velocity. Statistical analysis of each measure within the initial five minutes and total ten minutes of the open field arena did not reveal any significant differences between groups. Limitations including differences between clinical and animal studies, absence of histological confirmation of lesion and electrode placement, small sample size, lack of appropriate controls, and additional behavior paradigm to measure anxiety-like behavior likely contributed to the current lack of significant results. We concluded that STN DBS does not create more anxiety-

like behavior in acutely or chronically stimulated rat models of PD compared to PD rats that were not stimulated.

Investigating Anxiety-Like Behavior as a Non-Motor Side Effect of Deep Brain Stimulation of the Subthalamic Nucleus in a Parkinsonian Rat Model

The Socio-Economic Impact of Parkinson's Disease

In 2016 the prevalence of Parkinson's disease (PD) was of approximately 700 thousand in the United States (US) and 6.1 million globally, with an increase in incidence of 22% from 1990 to 2016 (Dorsey et al., 2018, p. 939). Presently, there are 930 thousand individuals living with PD in the US and 9.4 million in the world (Maserejian & Vinikoor-Imler, 2020). Additionally, PD shows a consistent increase in premature mortality and years of life lived with disability (Feigin et al., 2017, p. 885).

With the sharp increase in prevalence and disability, the economic impact of PD has grown. Kowal and colleagues (2013) estimated that PD cost individuals $14.4 million in direct medical expenses and over $6.3 million in other indirect costs due to factors such as decreased employment to adult day care expenses (p. 314-316). A more recent study conducted by Yang and colleagues in 2020, found a significant rise from the previous 2013 analysis. They reported a total economic burden of $51.9 billion, almost a 151% increase in economic burden, with an estimated future economic burden of $79.1 billion (p. 1, 5). These results illustrate the concerning and increasing trend of PD prevalence and economic burden in our society.

The Pathophysiology of Parkinson's Disease

PD is a progressive neurodegenerative disease that worsens through continuous cell death (Dauer & Przedborski, 2003; Hawley et al., 2014). PD is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNc), and the presence of abnormal alpha-synuclein (α-syn) protein that accumulate to form intracellular inclusions called Lewy bodies (Hawley et al., 2014; Jakobs et al., 2019).

A complex interplay between genes and the environment shapes the development of PD. Genetic mutations and multiplications to specific genes can cause PD, such as SNCA, which codes α-syn, and PRKN and LRRK2 that code enzymes involved in mitochondrial dysfunction and apoptosis, cell death of dopaminergic neurons (Lill & Klein, 2017). Other environmental factors have been found to cause PD, such as the groundbreaking discovery of 1-methyl 4-phenyl 1,2,3,6-tetrahydropiridinium (MPTP), a neurotoxin that causes dopaminergic cell death in the SNc (Dauer & Przedborski, 2003; Nonnekes et al., 2018). Studies also show that exposure to heavy metals, pesticides and herbicides, and other chemicals may cause similar damage and degenerations in the basal ganglia and dopaminergic neurons (Ball et al., 2019; Dauer & Przedborski, 2003).

Clinical presentations of PD principally manifest as motor deficits that include bradykinesia, or slowness of movements, resting tremor, and muscle rigidity (Pahwa & Lyons, 2012). In general, PD falls in the spectrum of hypokinetic neurological disorders because voluntary movements have a reduced velocity and amplitude, actions are slow in speed and direction and smaller in size (Bologna et al., 2016). PD is also marked by postural instability, or difficulty maintaining balance when executing movements, standing still, preparing to move, or attempting to correct oneself after losing equilibrium (Palakurthi & Burugupally, 2019). A result of both bradykinesia and postural instability are difficulties with gait, one's pattern of movement. Abnormal gait patterns seen in PD include festinating gait, small quick successive steps like running; freezing of gait, or the stopping of forward motion regardless of the desire to move, which can be seen as walking in place; and shuffling gait, small steps like festinating gait but slower (Chen et al., 2013; Mirelman et al., 2019; Nonnekes et al., 2019). Muscle rigidity is an involuntary, velocity-independent resistance to movement at the joint that can be expressed as

quick, jerky movements or a singular, snapping motion decreasing the smoothness of a movement (Armstrong & Okun, 2020; di Biase et al., 2018). Finally, the resting tremor, or often called “pill-tremor”, is a rhythmic motion where the individual rubs their thumb and pointer-finger together (Kaindlstorfer et al., 2013). These motor deficits are the core characteristics of PD and help contribute to its diagnosis and significantly reduce quality of life for the individual.

The Cortico-Basal Ganglia Motor Loop and its Importance in Parkinson’s Disease

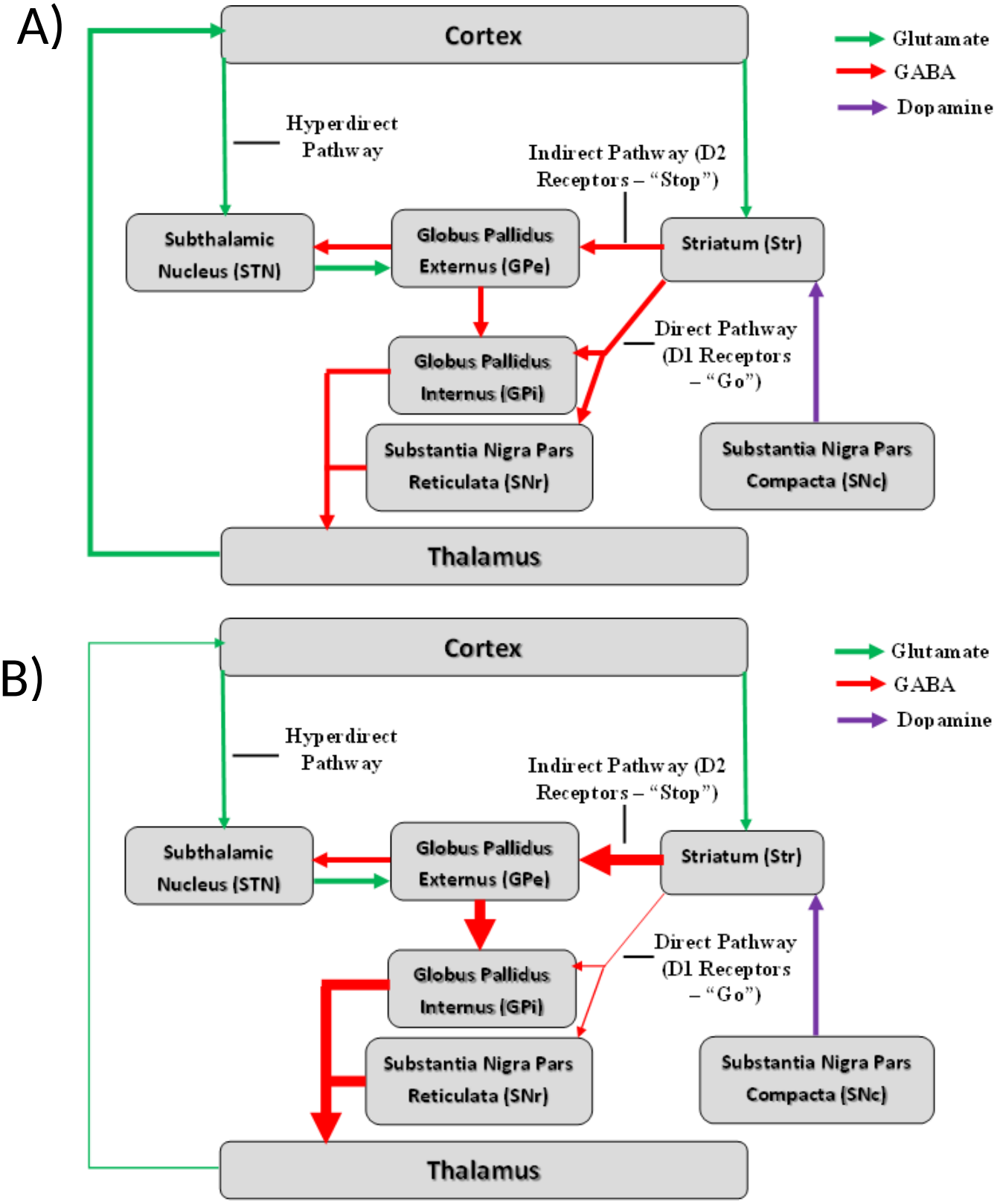
The basal ganglia (BG) comprise a group of interconnected subcortical nuclei that regulate functions including voluntary movement, cognitive planning, motivation, and limbic processing (Sonne et al., 2021, par. 1). Specifically, action-selection, the main functional component that governs voluntary movement, is under control of the cortico-BG motor loop (Frank and Claus, 2006; Frank, 2011; Isoda and Hikosaka, 2011; Mink, 1996; Mogenson et al., 1980). Figure 1A below illustrates how the cortico-BG motor loop regulates normal motor function. In this circuitry, information from nearly all cortical and limbic subcortical areas flow into the BG input stations, including the dorsal striatum (DS) and the subthalamic nucleus (STN). The DS is composed of inhibitory GABAergic projection neurons named medium spiny neurons (MSNs) that form projections to the main output stations of the BG: the substantia nigra pars reticulata (SNr) and the globus pallidus internus (GPi). MSNs also form distinct indirect projections to the BG output nuclei via the globus pallidus externus (GPe). The SNr/GPi project mostly to motor areas of the thalamus, which in turn, project to motor areas of the neocortex. Activation of MSNs of the direct pathway are responsible for inhibiting inhibition, removing inhibition of neurons for excitation to occur, resulting in a ‘go’ signal to initiate movement. Activation of MSNs of the indirect pathway are responsible for inhibition disinhibition, reinstating inhibition of these neurons, which serves as ‘stop’ signal to inhibit movement.

Additionally, the inhibitory control of the BG over the thalamocortical neurons can be increased by the hyperdirect pathway formed by cortical projections that bypass the DS by sending "hyperdirect" excitatory projections to the STN that stimulate the SNr/GPi input onto the thalamus. The hyperdirect pathway of cortical projections to the STN, which stimulate the SNr and GPi provide overall inhibition of unnecessary movement (Da Cunha et al., 2015). Finally, the DS MSNs are differentially regulated by dopaminergic (DA) efferents originating from the SNc via activation of stimulatory D1 receptors (D1R), preferentially expressed in MSNs of the direct pathway, and activation of inhibitory D2 receptors (D2R), preferentially expressed in MSNs of the indirect pathway. Thus, activation of both D1R and D2R in the direct pathway and indirect pathways culminates in the facilitation of movement by increasing the 'go' signal and decreasing the 'stop' signal, respectively (Da Cunha et al., 2015).

Figure 1B below shows the changes that occur to the cortico-BG motor loop in PD. The reduction in DA transmission onto DS MSNs prevents activation of the direct pathway and prevents inhibition of the indirect pathway. Therefore, the go signal cannot be activated, and the stop signal is enhanced ultimately causing inhibition of motor nuclei in the thalamus. The result is the inhibition of movement initiation (akinesia) and execution (bradykinesia), and increased inhibition of ongoing movements (muscular rigidity) (Okun, 2012).

Figure 1

Proposed Functioning of the Cortico-BG Motor Loop in Healthy Individuals (A) vs. PD (B)



Note. Image A) depicts normal functioning in the cortico-BG motor loop. Image B) depicts the hypokinetic cortico-BG motor loop in PD.

What is Deep Brain Stimulation?

Therapeutic Process of Deep Brain Stimulation

Deep brain stimulation (DBS) is a well-established surgical intervention to treat the motor symptoms of PD (Aviles-Olmos et al., 2014; Goetz et al., 2005; Obeso et al., 2001). DBS entails surgically implanting a stimulating electrode into certain brain regions so that delivery of continuous high frequency electrical stimulation modulates the neuronal activity of interconnected circuitry. The stimulating electrode is subcutaneously connected to an implantable pulse generator that provides power to generate and maintain electrical stimulation. In PD, the electrode is placed into specific regions within the cortico-BG motor loop, namely the STN and the GPi. It is suggested that STN and GPi DBS inhibit pathological patterns of neuronal firing in the cortico-BG motor loop resulted from poor dopaminergic transmission; however, the therapeutic process is not completely understood (Hamani et al., 2017; Jakobs et al., 2019). Current suggests that STN DBS may overwrite tonic inhibition on the thalamus by stimulating the STN and its efferents, removing the inhibition on the GPi and SNr, allowing the thalamus to once again send "go" signals to the motor cortices, thus regulating the cortico-BG motor loop (Da Cunha et al., 2015).

Side Effects of Deep Brain Stimulation

The benefits of STN DBS in reducing the motor symptoms in parkinsonian patients are well documented (Aviles-Olmos et al., 2014; Obeso et al., 2001; Ramirez-Zamora & Ostrem, 2018; Wong et al., 2020). STN DBS, but not GPi DBS, also has shown an ability to reduce

antiparkinsonian drug treatment (Couto et al., 2014; Okun et al., 2009; Peng et al., 2018). For this reason, STN DBS has become a preferred target in the treatment of the motor symptoms of PD. There is, however, increasing evidence that STN DBS may be associated with higher incidence of psychiatric side effects such as anxiety, depression, suicidal ideation, mania, and others. These psychiatric side effects are often transient and treatable, but can be long-term, especially when unaddressed (Chaudhuri & Schapira, 2009; Voon et al., 2006; Voon et al., 2008).

Anxiety has been reported as a transient symptom during STN DBS parameter setting, shortly following surgery, and during long-term stimulation (Abulseoud et al., 2016; Anderson et al., 2005; Couto et al., 2014; Houeto et al., 2002; Temel et al., 2005; Voon et al., 2006). Contrastingly, studies have also found no differences and even contradictory results reporting positive changes in short- and long-term mood from baseline to post-operative assessments (Couto et al., 2014; Kaiser et al., 2008; Lopiano et al., 2001; Rothlind et al., 2007; Wang et al., 2016; York et al., 2008).

Moreover, Chang and colleagues (2012) used bilateral STN DBS while controlling for DA medication found that anxiety-related PD was "influenced by the severity of the motor symptoms and the level of life quality" as levels of anxiety mirrored increases and decreases in motor function over time (p. 320, 322). The researchers also noted a correlation between changes in the pulse-width (area of the stimulation) and duration (chronicity) of stimulation and anxiety. Higher pulse-width and duration stimulation produced greater anxiety symptoms. On a similar note, previous research by Kalteis and investigators (2006) reported that during individual assessment of anxiety and other psychiatric non-motor side effects, these symptoms worsened regardless of motor improvement. These results are of interest because of the anatomical structure of the STN.

The STN has been found to be a key regulatory region of the cortico-BG motor loop. However, the STN is also associated with cognitive and affective regulation because of its overlapping topography with its cortical connections which determines three functional subregions: a motor, an associative, and a limbic subregion (Temel et al., 2005). The STN also has projections into other brain regions that regulate emotional processing such as the nucleus accumbens, anterior cingulate, ventral pallidum, orbitofrontal cortex, ventral tegmental area, and the amygdala (Péron et al., 2013; Temel et al., 2005). Research has shown that STN DBS can influence an individual's emotional state, more so when the ventral portion of the STN is stimulated (Castrioto et al., 2014). Despite current evidence, no agreement or substantial conclusion has been made in the divisions of the STN and their location, understanding of neuronal connections to and from the STN, and why changes in anxiety occur. Hence, continued investigation is needed as to what factors related to STN DBS may contribute or cause anxiety.

Animal Models

Subthalamic nucleus research with rodents has also implicated the STN in regulating anxiogenic-like behavior, similar to our understanding of how STN DBS stimulation may elicit anxiety in human patients as described above (Badstuebner et al., 2017; Reymann et al., 2013). Animal models, particularly rodent models such as rats, have been increasingly incorporated into neuroscientific research because of their ability to serve as accurate preclinical models of disease and illness allowing results from rats to translate to humans. An apt explanation of understanding animals in research was given by Dr. Michael Rand, DVM, in his chapter "Selection of Biomedical Animal Models". Dr. Rand writes, "...the term "animal model" is actually studying human conditions. In other words, it is not the image of the preferred animal that is the focus of

research but the analogy of the physiological behavior of this animal to our own (or another) species" (2008, p. 10).

We utilize animal models of rats because of their many advantages. An important advantage is that brain regions and circuitry are anatomically consistent between rats and humans. Rats also provide an advantage because their brains are larger than other animal models and rodents (i.e., mice, pigeons) allowing for easier stereotactic targeting when conducting surgery for precise implantation of DBS or injection of neurotoxic chemicals to induce Parkinson's-like degeneration, and reducing the damage caused to surrounding tissue when conducted (Bryda, 2013; Ellenbroek & Youn, 2016; Jonsson, 1983).

Behaviorally, rats can be trained with greater ease and exhibit behaviors analogous to humans making them highly desirable when undergoing behavioral tests. Also, rats take less time to train, to habituate to their surroundings, perform better over time, and are less affected by external distractions than other models such as mice, a benefit when studying anxiety (Ellenbroek & Youn, 2016). Moreover, routine handling of rats can further reduce their anxiety prior to behavioral testing (Costa et al., 2012). Other important factors for the use of rats include fewer expenses to acquire and maintain, and ease of training researchers to work with rodents compared to non-human primates, which are considered the "gold standard" of PD DBS translational research but are harder in each regard of the aforementioned information (Chia et al., 2020; Pereira & Aziz, 2006, pp. 293, 295-296; Tieu, 2011). These factors together make rats an important and accessible model within neuroscience research.

6-Hydroxydopamine Rat Model of Parkinson's Disease

PD has become a focus in animal research to answer questions not readily understood in current human studies, which could not be feasibly or ethically implemented in human patients

(Chia et al., 2020; Dauer & Przedborski, 2003, pp. 894-895; Pereira & Aziz, 2006). Animal modeling for PD began in earnest after Carlsson et al. (1957) discovered that haloperidol and reserpine created an acute Parkinsonian phenotype in rodent and rabbit models. Subsequently, many drugs and neurotoxins have been used to create PD phenotypes and neuropathology in rats (Blum et al., 2001; Tieu et al., 2011; Ungerstedt, 1968).

A common neurotoxin used to induce PD in rats is 6-Hydroxydopamine (6-OHDA) (Blum et al., 2001, p. 141; Chia et al., 2020). 6-OHDA has an added hydroxyl group making it chemically similar to the catecholamines, noradrenaline, dopamine, and adrenalin (National Center for Biotechnology Information, 2022). 6-OHDA was originally discovered to decrease noradrenaline in the heart by Porter et al. (1965, 1963). In the same decade, Tranzer and Thoenen (1968, 1973) demonstrated 6-OHDA could be used to cause select depletion of adrenergic neuron terminals. Following the discovery of 6-OHDA's functions, Ungerstedt (1968) established its utility in producing nigrostriatal dopaminergic degeneration through direct injection into the SN.

While there are many neurotoxins used to model PD in animals, 6-OHDA is more frequently is used (Chia et al., 2020) because of its consistent reproduction of parkinsonian phenotype and neuropathology and its selectivity to noradrenergic and dopaminergic neurons (Tieu, 2011). Greater specificity is gained when desipramine, a noradrenergic reuptake blocker, is systemically administered to the animal. Desipramine prevents 6-OHDA reuptake at noradrenergic neuron terminals protecting noradrenergic neurons from going into apoptosis (Lin et al., 2012; Linnoila et al., 1982). Therefore, 6-OHDA can be taken up into dopaminergic neurons by the same transporter for DA reuptake and causes cell death specifically in DA neurons (Deumens et al., 2001; Blum et al., 2001; Sauer & Ortel, 1994, p. 413; Tieu, 2011).

In contrast, MPTP has been found to be less effective in rats, the animal model of interest in this study (Giovanni et al., 1994). Paraquat, another environmental neurotoxin that is used for PD models, has age-dependent toxicity, nigrostriatal dopaminergic cell death is not consistently observed, and can cause possible pulmonary harm inducing motor deficits making it unsuitable for the current model. Similarly, rotenone, is also an environmental toxin. However, rotenone has not been reliable in producing PD in animal models both in phenotype and neuropathology (Tieu, 2011). Tieu (2011) and Chia et al. (2020) provide further comparison between other neurotoxins.

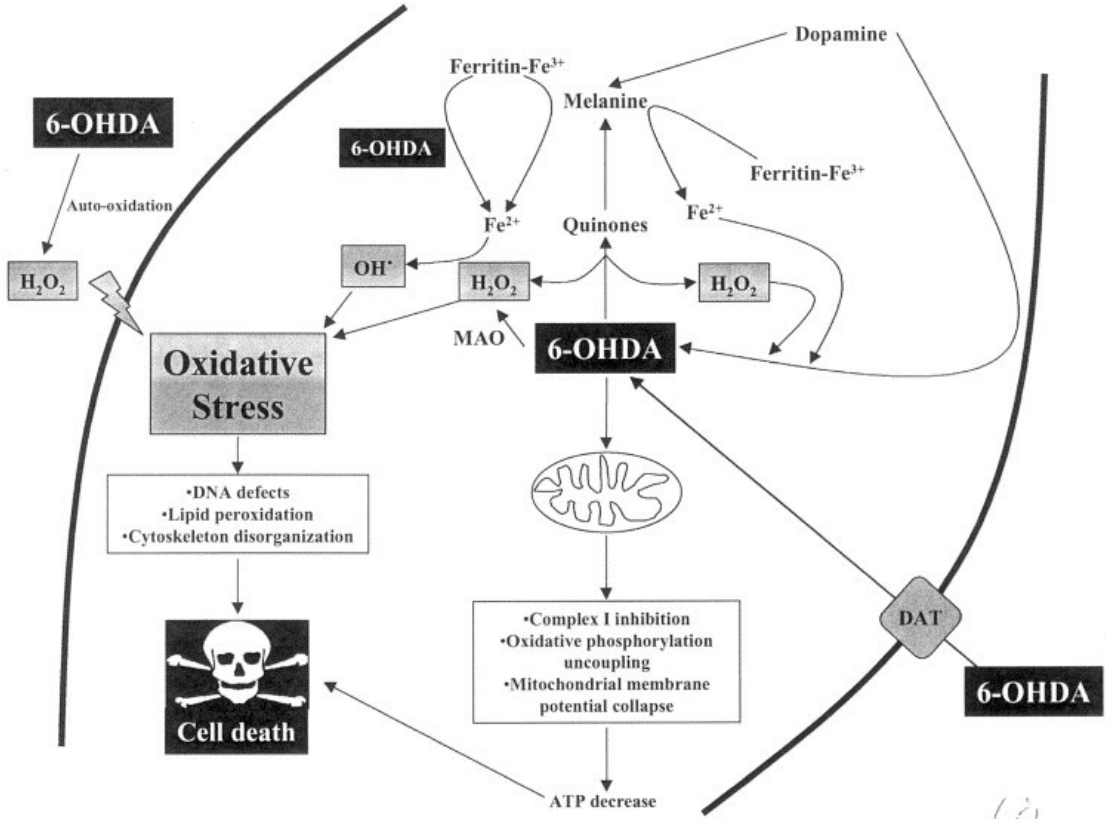
As mentioned, the striatum, specifically the DS, is involved in voluntary movement through dopaminergic activation of D1R of the direct pathway, and D2R of the indirect pathway. To model PD in animals, neurotoxins such as 6-OHDA work to decrease and eliminate DA by destroying the nigrostriatal pathway, which mimics motor deficits and neuronal degeneration. Multiple studies have been conducted to best understand the mechanisms of action of 6-OHDA (Blum et al., 2001; Deumens et al., 2001; Dauer & Przedborski, 2003; Przedborski et al., 1995; Sauer & Ortel, 1994).

While the mechanisms of DA neuron degeneration by 6-OHDA are not entirely understood, these studies have helped to elucidate the function of 6-OHDA. Additional understanding of how 6-OHDA is illustrated in Figure 2 below by Blum and colleagues (2001). Blum et al. (2001) details three mechanisms by which 6-OHDA is thought to work. When injected 6-OHDA is taken up retrograde, or backwards, at the neuron terminal because of its chemical similarity to dopamine. Once in the striatum, the following mechanisms are thought to occur: Mechanism (1) once inside the cell, auto-oxidation, or the interaction between 6-OHDA and intra-cellular oxygen causes oxidation producing quinones and reactive oxygen species

(ROS) such as peroxides, superoxide radicals, and hydroxyl radicals. These ROS are cytotoxic causing cell damage and disruption leading to death. Mechanism (2) in normal functioning, the enzyme monoamine oxidase (MAO), metabolizes excess dopamine. However, when 6-OHDA is present within the cell, MAO will also break it down because of its similar composition to dopamine. When metabolized, 6-OHDA turns into hydrogen peroxide an ROS, which similar to mechanism one, causes lipid peroxidization – dissolving cellular and organelle membranes and interfering with redox potential, both leading to cell death. Mechanism (3) 6-OHDA is also known to inhibit complex 1, the method by which mitochondria create adenosine triphosphate (ATP), energy for the cell to function. Without cellular energy, the cell dies. Further, interference with mitochondrial respiration includes possible uncoupling of oxidative phosphorylation also inhibiting ATP production. Finally, 6-OHDA can also cause similar oxidative stress as the first and second mechanisms, which can break down the mitochondrial membrane (Auten & Davis, 2009; Blum et al., 2001; Graves et al., 2020).

Figure 2.

Proposed Mechanisms of Action of 6-OHDA Toxicity



Note. The mechanisms of action of 6-OHDA neurotoxicity are not well understood. This model proposes three principal ways that 6-OHDA can cause cell death. First, by intra- or extra-cellular auto-oxidation from reactive oxygen species. Second, reuptake of 6-OHDA into the cell is broken down into hydrogen peroxide (H_2O_2) by MOA, dissolving the membranes and organelles. Third, H_2O_2 inhibits complex 1, the energy production process within the cell. Copied with permission from Blum et al., 2001.

The striatum is chosen as the target injection site for multiple reasons. Firstly, the rat striatum is a larger brain region than the SNc lending itself as an easier target to hit for stereotactic injection. Second, when 6-OHDA is injected into the striatum, DA degeneration (neuronal death starting in the nerve terminal at the striatum and progressing to the cell body at the SNc) occurs over a period of 1-3 weeks, leading to ~50% nigrostriatal cell death, whereas injections into the SNc or medial forebrain bundle cause rapid cell death within one day causing a severe lesion of around 90% degeneration (Dauer & Przedborski, 2003, p. 895; Przedborski et al., 1995, pp. 631-632, 644; Robinson et al., 1994, p. 2691; Sauer & Oertel, 1994, p.412; Tieu, 2011).

Unilateral injections using 6-OHDA are often a preferred method for inducing PD in rats so the contralateral side can act as a control (Dauer & Przedborski, 2003, p. 895). However, unilateral injections often require higher doses of 6-OHDA, which could create too strong of a lesion and subsequent motor deficits possibly confounding behavior in the open field test (Sauer & Oertel, 1994). Additionally, PD in clinical patients is bilateral and bilateral lesioning is more

representative of real PD progression. Moreover, behavioral (learning) or neurobiological (neurogenesis) compensatory mechanisms are less of a concern (Deumens et al., 2001, pp. 312-314). Therefore, rats in this experiment were bilaterally lesioned to produce weaker and more progressive degeneration representative of clinical PD.

Hypothesis and Goals

Based on previous research, we hypothesized that acute and chronic STN DBS would produce more anxiety-like behavior in a rat model of PD compared to PD rats that were not stimulated. Our first goal was to determine if STN DBS induces anxiety-like behavior in a PD rat model that does not display motor deficits. The second goal was to determine if acute and chronic STN DBS produce differences in anxiety-like behavior in the PD rat model.

Methods

Subjects

All behavior experiments were performed with wild type Sprague Dawley rats (Envigo, Madison, WI), N=19. Both female (n=7) and male (n=12) rats were utilized and selected at random. Groups were randomly generated by computer; chronic stimulation (n=6), acute stimulation (n=6), and no stimulation (n=7). A total of 34 rats were operated on; however, eight did not respond to stimulation, four rat's headcaps detached, two could not be analyzed because of cord detection issues (software would not detect the cord attachment), and one died during surgery. Total attrition rate was 44%. The above subject information can be found in Appendix A. All laboratory procedures were reviewed and approved by the Mayo Clinic Institutional Animal Care and Use Committee (IACUC), approval number A00004425-19, and conformed to guidelines published by the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). Prior to surgery, rats were group-housed

in acrylic cages (128 in^2) with wire racks to hold feed and water. Rats had ad libitum access to food and water. Each cage had solid floors with bedding to absorb waste, provide warmth and enrichment. Additional cage enrichment was provided in the form of treats and plastic toys. At each step of the experiment, the animals were kept under standard 12-hour light/dark cycle and conditions (21°C, humidity 45%). Animals were acclimated for at least one week before use to reduce stress. Before and throughout the experiment, each rat was routinely held to familiarize the rat to the researcher reducing anxiety and discomfort during handling. All surgeries were performed on rats weighing 250-280g. Post-operation, all rats were identified via cage card and were also single housed to ensure proper recovery and reduce interference with the electrode. All efforts were made to minimize both the number of rats used and any discomfort that may be experienced. During transportation to and from the lab, the rats were kept in their home cages and covered with an opaque cloth blanket to reduce light and noise. All animals were observed by Mayo Clinic Department of Comparative Medicine (DCM) staff and researchers for any complications before and after surgery utilizing the Rat Grimace Scale, observation of coat color, rat weight, and porphyrin staining (Turner et al., 2019). Routine sanitation was conducted by DCM personnel. Clean personal protective equipment, lab coat and gloves, was always worn and surfaces used to handle rats were cleaned with Oxivir surface disinfectant (Diversey Inc., Fort Mill, SC).

Two days prior and three days after surgery, each animal received analgesia via oral Ibuprofen-infused water (15mg/kg) to minimize discomfort, improve recovery, and increase gut motility. Additional analgesia, buprenorphine HCL (.1ml/250-300g), was subcutaneously administered to each rat just before surgery and administered every 8-12 hours for 48 hours post-operation. A heating pad was used to maintain the subject's body temperature to 37.0±0.5 °C

throughout the duration of anesthesia. Animals were monitored once daily for five days following surgery for signs of distress and infection at the surgical site. If signs of infection or distress were observed, topical antibacterial ointment was applied to the surgical site, or a veterinarian was consulted for the appropriate methods of treatment.

At the termination of the study or if a humane endpoint arose (i.e., headcap comes off or inability to ambulate) subjects were euthanized. Each rodent intraperitoneally received a lethal dose of Pentobarbital (100mg/kg). Depth of anesthesia was measured with eye blink and toe pinch reflexes and respiration and heartbeat were monitored. Each rat's peritoneum was then opened, and the heart was revealed. The right atrium was clipped for blood to leave the body. Immediately, rats were transcardially perfused through a 25-gauge needle with phosphate buffered saline followed by cold 4% paraformaldehyde. To ensure death, decapitation was used as an adjunctive method using a rodent guillotine. The brains were then extracted and placed into 4% paraformaldehyde overnight and then moved to 20% sucrose solution to preserve the brain.

Surgery

A stereotaxic frame (Model 1900, David Kopf Instruments, Tujunga, CA) was used for implantation of a twisted bipolar Pt/Ir Teflon-insulated 0.127mm diameter stimulating electrode (Plastics One, Roanoke, VA) in the STN and for infusion of 6-OHDA (Sigma-Aldrich, St. Louis, MO) in the DS. All surgical instruments, electrodes, and skull screws were sterilized daily in an autoclave and in hot bead sterilization (260 °C) if multiple surgeries were conducted. Anesthesia was induced in rats with 4% isoflurane and lowered to 1-2% isoflurane for maintenance. The depth of anesthesia was monitored using toe pinch and eye blink reflexes. Then, the rats were placed in the stereotaxic apparatus and secured using ear bars. Eye lubricant was gently placed onto the eyes of each rat to prevent damage to and drying of the ocular region. Once secured,

buprenorphine was subcutaneously injected on the back of the neck. Following analgesia, an intraperitoneal injection of 25mg/kg desipramine (Sigma-Aldrich, St. Louis, MO) was administered to avoid cell death of noradrenergic neurons**.** Next, the surgical sites on rats' head were shaved and cleaned with alcohol wipes and povidone-iodine. A midline incision of approximately 1.5-2 cm was made in the skin over the skull with a scalpel to reveal bregma and lambda for stereotaxic coordination. Hydrogen peroxide, saline, and cotton swabs were used to clean the skull surface.

Coordinates for each lesion and electrode location were measured according to *The Rat Brain in Stereotaxic Coordinates* by Paxinos and Watson (2004). An electric drill was attached to the stereotactic frame; the first craniotomy bilaterally targeted the DS, and the second cranial window was created for the stimulating electrode. Next, four sterile skull screws (BASi, West Lafayette, IN) were used to provide a strong fixation of the head cap. All cranial holes were 1-2mm.

Once all openings were created, the striatal lesions were conducted. Using a microinjector mounted to the frame, 6-OHDA (5 µg/µL) was micropipetted via Hamilton Microliter syringe (Hamilton Company, Reno, NV) and 2.5 µL per site was bilaterally injected in the DS at 0.5 µL/min according to the following coordinates: AP: +1.2 mm from bregma, ML: +/-2.7 mm, DV: −4.2 mm from dura. A five-minute waiting period elapsed prior to removal of the needle after each injection to avoid reflux and allow tissue diffusion. Finally, bone wax was placed into the lesion holes to seal them. Subsequently, the stimulating electrode was attached to the stereotactic frame and unilaterally inserted into the STN at AP: -3.8 mm from bregma, ML: +2.4 mm, and DV: −7.6 mm from dura. The electrode was fixed to the skull using Metabond (Parkell, Edgewood, NY), a quick drying adhesive, forming the headcap. Additional adhesive

was placed around the skull screws. Once dry, another layer of adhesive was applied to ensure a strong seal and headcap. Sutures were used as needed to secure excess tissue not covered by the headcap. Following the second layer drying, the ear bars and frame were removed, and Isoflurane and oxygen were stopped. Post-surgery, animals were placed back into their home cage, body heat was maintained by heating pad, and recovery was monitored until consciousness was regained. All rats received the bilateral striatal lesion and unliteral STN DBS.

Stimulation Parameters

Stimulation parameters were based on previously determined bounds used in both human and rodent STN DBS to replicate clinically relevant parameters while appropriate for rats (Jakobs et al., 2019, p. 4; Kuncel & Grill, 2004, pp. 2436, 2439; Mottaghi et al., 2020; Volkmann et al., 2006). Stimulation was provided by an external pulse generator, MINCS alongside MincsWare software (Neural Engineering Laboratory and Department of Engineering, Mayo Clinic, Rochester, MN) to define the stimulator settings. A continuous biphasic squared electric current was applied at 130 Hz and 60μs pulse-width to the STN. Current intensity was determined individually based on each rat's active motor threshold. Once the rat showed dyskinetic movements (licking, paw twitching, head, or body turn) the current intensity was set to 20% below the active motor threshold (Huotarinen et al., 2019; Ruge et al., 2011a, p. 2109; Ruge et al., 2011b, p. 1916; Xu et al., 2011, p. 295). This process was done to ensure clinically relevant amplitude was given without inducing any motor deficits that could confound the open field test.

Prior to all behavioral tests, all rats were tethered starting at 14 days post-op in their home cages to allow habituation of all animals to the cord and reduce possible anxiogenic effect from the cord swaying, pulling, or touching the rats' head. All rats were transported and tethered

in the same testing room as the open field. Rats were supervised during tethering to monitor for tangles or other safety concerns. Tethering is done by screwing the stimulating cord to the electrode attached to the headcap. Only the rats in the chronic stimulation group, received stimulation for 2 hours daily for one week prior to behavioral tests. Animals in the acute group were only stimulated during the open field test. Animals in the no stimulation group did not receive stimulation before or during the open field test.

Open Field Test

The open field test has been used to measure anxiety in rats for decades by observing patterns of locomotion and other exploratory behaviors such as rearing and grooming (Harro, 2018; Denenberg, 1969). The open field behavior paradigm followed recommendations put forth by Walsh and Cummins (1976) and previous studies using the paradigm with rodents (Kraeuter et al., 2019; Seibenhener & Wooten, 2015; Tatem et al., 2014). The open field arena consisted of a box, 60x60x40 cm, made from opaque blue polyvinyl chloride (PVC) and metal and was maintained in the same location, a quiet room with minimal outside noise. Appendix B shows the open field arena used in the experiment. The open field was illuminated from above using LED adhesive lights directly above and brightness was supplemented by LED ceiling lights. Illumination was kept at a moderate level to prevent influences on ambulation or wall hugging, thigomotaxis. Rats underwent the open field behavioral paradigm after 21 days from DA lesion induction. Once transported to the room with the open field, the rats were kept in their home cages for an hour to habituate to the room and normalize after the stress of transportation. Each rat was moved from their home cage by hand to the open field where they were tethered and placed into the center to avoid place preference, i.e., staying near the wall rats were originally placed at. Tethers were connected to external pulse generators held in baskets on the outside of

the open field. Once placed in the open field arena, the researcher would step away and monitor the rat from a stationary camera placed above the arena to minimize any possible distractions or stress from sudden movements. All rats were recorded for 10 minutes once placed in the open field. Between each recording, all surfaces of the open field were wiped down with a surface disinfectant and deodorizer and let to dry. Each rat had one trial in the open field paradigm and was placed back into its home cage after completion. EthoVision XT™ (Noldus Information Technologies©, Leesburg, VA, USA) was used to analyze each rat's behavior including velocity, total distance traveled, time spent in inner and outer zones. The software was also used to develop heat maps and timelapse tracking of the rat's path.

Measures

Locomotor activity patterns, or locomotion, is a measure that has been widely studied in the open field and is often the principal variable of interest (Walsh & Cummins, 1976). EthoVision was utilized to measure total distance (cm), velocity (cm/s), and time (seconds) spent in inner and outer zones. Total distance traveled is often the most reported measures of locomotion used to capture exploratory behavior (Bailey & Crawley, 2009; Díaz-Morán, 2014; Walsh & Cummins, 1976). Velocity is akin to total distance traveled and can also be seen as another measure of rodent activity within the open field, though it is a less utilized measure locomotion. Total distance and velocity are variables used to evaluate the overall motor function (Walsh & Cummins, 1976).

Time spent in inner zones, or the center of the open field, compared to time spent in the outer zones, thigmotaxis near the walls, are variables used in tandem to examine arousal and anxiety-like behavior. Time spent near the wall of the open field (i.e. time spent in the outer zone) is representative of greater anxiety-like behavior (Bailey & Crawley, 2009; Seibenhener &

Wooten, 2015). In EthoVision zones were created (zones 1-16) to measure changes in locomotion from the center to the periphery. The center was 50% of the total area of the arena creating an inner zone of 30X30 cm, listed as zones six, seven, ten, and eleven within the video tracking software. The periphery comprised the rest of the outer zones (zones one through five, eight, nine, and twelve through sixteen). Appendix G shows the arena and the respective zones subdivisions in EthoVision.

Grooming and rearing were visually analyzed using ImageJ (Laboratory for Optical and Computational Instrumentation, University of Wisconsin). Self-grooming is viewed as a behavior associated with arousal and is negatively associated with freezing and thigmotaxis, indicators of anxiety-like behavior (Díaz-Morán, 2014; Estanislau et al., 2019; Spruijt, 1992). In the present study, self-grooming was operationally defined using Estanislau et al. (2009) description of rostral grooming. Estanislau and researchers defined rostral grooming as a fixed-action pattern "involving friction movements with the forepaws directed to the nose, face, head and ears" (p. 112588). Examples of rostral grooming only included the definition by Estanislau and colleagues. An example of rostral grooming from the present experiment can be seen in Appendix C. Nonexamples of self-grooming included any grooming to the body such as scratching, licking, or biting. A nonexample of self-grooming via using the back paw to scratch the body can be seen in Appendix D. Grooming was measured by duration (seconds) and began once the rat stopped in place and started the sequence of rostral grooming.

In the open field test, rearing has been described as an exploratory behavior that is negatively correlated with freezing and thigmotaxis indicating activity and arousal, opposite of anxiety-like behavior (Díaz-Morán, 2014). Rearing was defined as two paws leaving the floor of the open field test both while standing on hind legs and either supported (on the wall) or

unsupported (away from walls). Rearing was measured by count (number) of instances. An instance of rearing was counted once the rat had both front paws off the floor of the open field. Successive counts were only included if both paws came back down to the floor of the open field. An example of both supported and unsupported rearing can be seen in Appendix E. Nonexamples of rearing include only one paw coming off the floor, the rat looking upward with no paws leaving the floor, and changing walls in the open field while already rearing. A nonexample of rearing can be seen in Appendix F. Any instances of grooming and rearing that were unclear by the observer were clarified by group decision from the second and third authors. Therefore, interrater agreement was not calculated.

Results

Data were analyzed using Statistical Package for the Social Sciences Version 20® (SPSS; IBM®, New York). Data were split up into both the initial five minutes of the recording and the total ten minutes to examine behavior over time. Data for the total ten minutes are presented in Appendices H-M. Behavioral data can often be abnormally distributed; therefore, a one-sample Kolmogorov-Smirnov test to determine non-normality. All significance values were above .05 indicating data were normally distributed. Distribution data of the initial five minutes are shown in Table 1 below.

Table 1.

Descriptive Statistics and One-sample K-S Test for Measures in the First Five Minutes of the Open Field Test

Data were analyzed using Analysis of Variance (ANOVA) for normally distributed data per previous studies recommendations (Bailey & Crawley, 2009; Tatem et al., 2014). As seen in Table 2 below, a one-way ANOVA comparison for the first five minutes of the data did not yield significant results for any measure.

Table 2.

Descriptive Statistics and One-way ANOVA Results for All Measures in the First Five Minutes of the Open Field Test

Measure	% time in Outer Zone	% time in Inner Zone	Rearing Count	Grooming (seconds)	Total Distance Traveled (cm)	Average Velocity (cm/s)	16)	Sig.
							7	.77
N	19	19	19	19	19	19		
M	95.67	4.34	23.00	8.79	2475.03	12.57		
SD	2.35	2.35	12.94	6.60	860.78	3.31	7	.09
D	.62	.62	.55	.61	.33	.41		
Sig.	.843	.843	.928	.846	1.000	.997		
Grooming (seconds)	13.81	4.20	7.90	8.04	5.26	4.66	3.59	.05
Velocity (cm/s)**	13.36	3.69	11.33	4.57	12.95	1.33	.37	.70

Time Spent in Outer Zone (seconds)	286.03	6.10	282.92	6.67	289.00	7.35	1.31	.30
Time Spent in Inner Zone (seconds)	12.44	7.0	16.01	6.83	10.78	7.4	.93	.10
Percentage of Time in Outer Zone	95.84	2.32	94.63	2.27	96.41	2.46	.94	.41
Percentage of Time in Inner Zone	4.16	2.32	5.37	2.27	3.59	2.46	.94	.41

Note. **Velocity was found to be in violation of Levene's test for equality of variances, $F(2, 16)=3.85$, $p=.043$. Therefore, a Welch's test was conducted and presented above; adjusted degrees of freedom $F(2, 7.47)$.

Figure 3 below shows the time spent in the inner and outer zones for the first five minutes of the open field test. Animals that did not receive STN DBS spent most of the time in the outer zone, 96%, and STN DBS delivered acutely or chronically did not change this pattern of behavior, 95% and 96% respectively. As expected, the percentage of time spent in the inner zone matches the percentage of time spent in the outer zone. Table 2 above shows the descriptive statistics of the one-way ANOVA.

Figure 3.

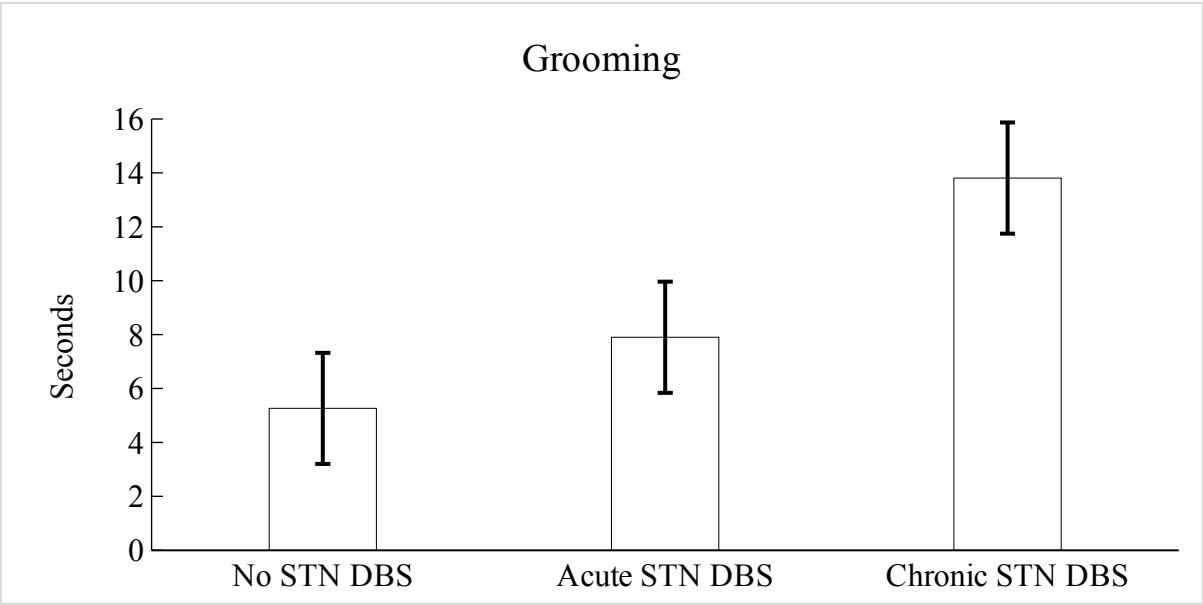
Outer and Inner Zones for First Five Minutes in the Open Field Test

A)

B)

Note. Average of percentage of time in seconds spent in the outer zone (A) and inner zone (B) of the open field arena. No STN DBS (*n*=7), Acute STN DBS (*n*=6), and Chronic STN DBS (*n*=6). Data are represented as mean ± SEM, one-way ANOVA.

Figure 4 below shows the time spent grooming in the first five minutes of the open field test. Animals that did not receive STN DBS spent less time grooming, 5s, than STN DBS delivered acutely or chronically, 8s and 14s respectively. While rats that did not receive STN DBS groomed less, Table 2 above, shows no significant difference between groups and presents descriptive statistics of the one-way ANOVA.

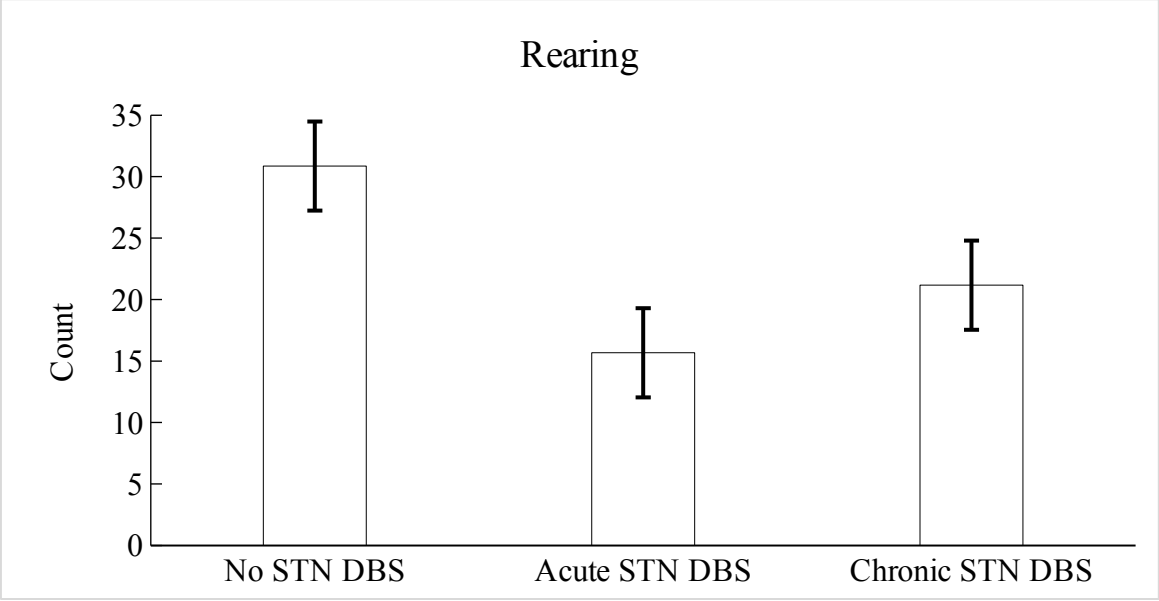


Note. Average time in seconds spent grooming in the open field arena. No STN DBS (*n*=7), Acute STN DBS (*n*=6), and Chronic STN DBS (*n*=6). Data are represented as mean ± SEM, one-way ANOVA.

Figure 5 below shows the number of times reared over the first five minutes of the open field test. Animals that did not receive STN DBS reared more, 31 instances, than STN DBS delivered acutely or chronically, 16 instances and 21 instances respectively. While rats that did not receive STN DBS reared more, Table 2 above, shows no significant difference between groups and presents descriptive statistics of the one-way ANOVA.

Figure 5.

Rearing in the First Five Minutes of the Open Field Test

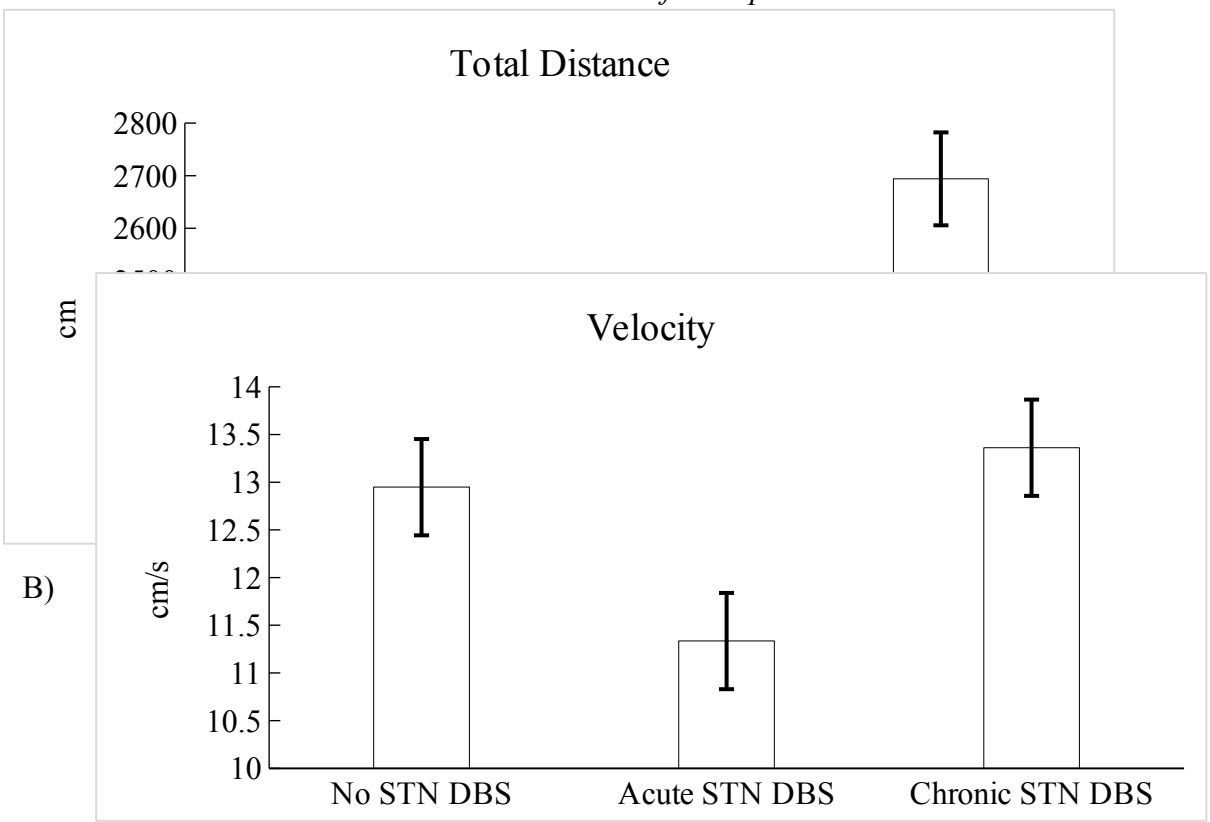


Note. Average number of rearing instances in the open field arena. No STN DBS (*n*=7), Acute STN DBS (*n*=6), and Chronic STN DBS (*n*=6). Data are represented as mean ± SEM, one-way ANOVA.

Figure 6 below shows the total distance traveled (cm) and velocity (cm/s) over the first five minutes of the open field test. Animals that did not receive STN DBS had less total distance traveled, 2338cm, than STN DBS delivered acutely or chronically, 2416cm and 2694cm respectively. Animals that did not receive STN DBS had similar velocity, 13cm/s, as STN DBS delivered acutely or chronically, 11cm/s and 13cm/s respectively. While rats that did not receive STN DBS traveled less distance and had similar velocity, Table 2 above, shows no significant differences between groups and presents descriptive statistics of the one-way ANOVA.

Figure 6.

Total Distance Traveled in the First Five Minutes of the Open Field Test



Note. Average total distance traveled in centimeters (A) and average velocity in centimeters per second (B) in the open field arena. No STN DBS (*n*=7), Acute STN DBS (*n*=6), and Chronic STN DBS (*n*=6). Data are represented as mean ± SEM, one-way ANOVA.

Discussion

In this study we demonstrate that acute and chronic STN DBS does not produce more anxiety-like behavior in a rat model of PD compared to rats that were not stimulated. Goal one was not found, as STN DBS does not increase anxiety-like behavior in a parkinsonian rat model that does not display motor deficits. Goal two was also not verified, as acute and chronic STN DBS was not found to be significantly different on any measure.

We initially hypothesized that STN DBS would induce anxiety-like behavior in the 6-OHDA PD rat model. This hypothesis was formulated based on clinical reports of transient anxiety symptoms or long-term side effects by parkinsonian patients undergoing STN DBS. Additionally, Reymann et al. (2013) found that excitotoxic lesion of the STN using ibotenic acid

induced anxiety-like behavior in rats in the elevated plus maze. However, our findings are contrary to our hypothesis and to these previous studies. A possible explanation for our diverging results is the placement of the electrode in the STN and how the electric field created by the stimulation differently affects the STN itself and surrounding fibers of passage. For example, Abulseoud et al. (2016) investigated fifteen individuals with severe PD who underwent bilateral STN DBS in the medial and lateral STN. During parameter setting, seven patients reported twelve acute episodes of anxiety (feeling apprehensive and uncertain), especially with higher voltage. In rats the electrode is quite large compared to their STN; therefore, we stimulate the entire STN leaving us unable to selectively target the dorsal or ventral areas of the STN.

Anderson et al. (2005) conducted a comparison of GPi DBS vs. STN DBS on motor deficits and non-motor symptoms. They examined 20 patients diagnosed with PD who had STN DBS (n=10) and GPi DBS (n=10). Though the focus was on postoperative outcomes, two STN DBS patients experienced acute anxiety (feeling nervous, tense, and restless) during parameter setting. No patients in the GPi DBS group reported feelings of anxiety. They suggested that anxiety during DBS parameter setting is common, indicating acute stimulation as a possible factor in patient reports of anxiety, though our results did not find a significant difference among acutely stimulated rats.

Another study by Houeto et al. (2002) retrospectively investigated twenty-four individuals with bilateral STN DBS. At 6-months post operation the researchers conducted behavioral assessments. Of the twenty-four participants, eighteen reported generalized anxiety after surgery; ten of them reporting anxiety without any specific focus, and three who's anxiety could not be described. Houeto and colleagues stated that medication (Levodopa) and STN stimulation are likely contributors to anxiety. It is unclear why our study did not find results in

the chronically stimulated STN DBS group that parallel those in Houeto and researcher's study; however, answers may be found in their stimulation parameters or in the administration of Levodopa.

Notwithstanding the evidence described above, other clinical studies reported that anxiety symptoms did not surge with STN DBS. Rothlind et al. (2007) examined neuropsychological performance following unilateral STN DBS (n=19) and unilateral compared to bilateral STN DBS (n=14) using the Speilberger State-Trait Anxiety Inventory. Rothlind and colleagues noted that all participants were on Levodopa. Neuropsychological testing was completed on average 13 days pre-op and an average of 6-months post-op of the first unilateral STN DBS surgery. No significant difference in state- or trait-anxiety scores were found for unilateral STN DBS. Patients then received another surgery an average of 7-months after the first to implant a second stimulating electrode contralaterally, and the final neuropsychological assessment was given an average of 15 months post-operation. Again, no significant differences were found before and after unilateral and bilateral STN DBS. These findings parallel our chronic STN DBS group, which was not significantly different on measures of anxiety-like behavior. A similar study by Lopiano et al. (2001) bilaterally implanted STN DBS in 16 patients. Participants received the State-Trait Anxiety Inventory and were tested before surgery and 3-months after. No significant differences were found from pre- to post-operation on the State-Trait Anxiety Inventory.

Examining the first three studies, it is possible that bilateral STN DBS, higher voltage, and medication or a combination may all play a role in eliciting anxiety. However, the results provided by Rothlind et al. (2007) and Lopiano et al. (2001) conflict with these findings; both unliteral and bilateral STN DBS, Levodopa, and high amplitude stimulation were involved.

Future studies should look to examine bilateral STN DBS, varying voltage strengths, and Levodopa to examine their independent and combined effects on anxiety.

Our results may differ from previous literature for another key reason. One of the principal limitations of the present study is the lack of histological analysis. Histological analysis is a necessary component of assessing and validating procedures conducted with animal models such as neurochemical lesions and electrode implantation (Knoblaugh et al., 2018). Without tissue examination, we cannot determine if the electrode was placed correctly into the STN nor if the 6-OHDA lesion hit the striatum and to what extent nigrostriatal degeneration occurred. Moreover, this type of analysis is important as previous reviews have noted that variation between lesions in each subject (Amalric et al., 1995; Deumens et al., 2002; Sauer & Oertel, 1994, pp. 403-404). Currently, histological analysis is pending and will help us understand if surgical errors played a role in non-significant results. Though we do not have tissue results, the second part of goal one was met. Our model did not have observable motor deficits. The total ten-minute analysis of the open field test was used to examine motor function and habituation, rats often will display less anxiety-like behavior over time and repeated testing. Thus, the first five minutes then can prove the most useful in examining differences in behavior (Badstuebner et al., 2017; Creed et al., 2013, pp. 508-509). Appendix M demonstrates similar total distance traveled and velocity for all groups over ten minutes in the open field arena.

Another limiting factor was the total sample size. Investigating differences between small group sizes diminishes the power of the study and allows more error to be introduced hurting the possibility of significant results unless the process under examination produces strong responses. However, as noted above, anxiety as a non-motor symptom of STN DBS has been reported as both acute and chronic, and as improving, no change, or becoming worse postoperatively. Future

studies should work to increase the number of animals included in examination of anxiety as a non-motor symptom of STN DBS.

An additional reason why we did not find differences between groups is from a possible ceiling effect of the open field. Rats have a preference to avoid open spaces and display thigmotaxis naturally as an anxiety-like behavior in response to stress (Ennaceur & Chazot, 2016; Seibenhener & Wooten, 2015). Therefore, while still an important measure, rats already display high levels of thigmotaxis, which means experimental interventions may need a strong effect to produce significant differences. Several studies have reported possible ceiling effects when using rodents in the open field test and other behavioral tests of anxiety such as the Elevated Plus Maze (Brenes et al., 2009; Shoji et al., 2021; Thompson et al., 2015).

The open field has also been criticized for being used as a test for anxiety (Harro, 2018; Walsh & Cummins, 1976). Harro (2018) and Walsh and Cummins (1976) have both reported on the unvalidated and unreliable measures used when employing the open field paradigm. Both studies argue that many factors can influence anxiety-like behavior (illumination, noise, smell, handling) to being placed in a novel environment can elicit anxiety. Additionally, Harro states that thigmotaxis is an unreliable measure because rats have a general tendency to prefer enclosed and protected spaces. These criticisms are all valid. For instance, Appendices B-E reveal another possible limitation in the present study. Depending upon the angle of the camera and brightness of lights in the room with the open field, some shadows creating darker areas, preferential areas for rats, may influence rats to spend more time in those areas. Hence, why current research aims to validate behavioral measures of anxiety-like behavior and implement standard procedures such as those followed in the current experiment.

While there are drawbacks to the open field test, Tatem et al. (2014) provide a succinct argument for its current use writing:

> a) it is a comprehensive assessment of both locomotor and behavioral activity, which is strongly, but not always correlated with locomotive function; b) it is an easy measure to perform; c) it requires no animal handling during testing; d) it is a noninvasive measure that can be performed more than once throughout the duration of a study; e) no special training is needed to perform the test; f) multiple animals can be tested at one time; and g) it is a clinically relevant outcome measure. (p. 6)

Further limitations can be found looking at the setup of the open field. First, the placement of the camera above the open field made it difficult to adequately view the rats' paws and subtle movement. As a result, verifying if both paws left the floor to count an instance of rearing was uncertain if the rat was facing with its back toward the camera. Similarly, counting how long or if a rat was engaged in grooming could be difficult for the same reason. Interobserver agreement could have helped reduce this problem by providing measure verification. Future research using open field should include interobserver agreement to increase measure reliable when completing measures by hand. Moreover, interested researchers should consider placing an additional camera closer to the open field to best capture rearing and grooming.

Another obstacle that arose during the experiment was the highly saturated color of the open field arena and reflection from the plastic (see Appendix B). Prior to video analysis in EthoVision, each video was changed to black and white to reduce difficulties during software detection. However, the contrast between the black and white open field and the silver tether

created software interference. Future analysis should consider using a matte-colored material for the open field and/or a color that is different than that of their subject and tether.

Thinking about the measures of the open field, a prior study using the open field with mice reported that unsupported rearing is similar to measures such as time spent in the center and is reduced during stress, whereas supported rearing is indicative of locomotion such as distance traveled measures (Sturman et al., 2018). While this may not translate to rats, it is worth considering counting both rearing types separately for a better understanding of anxiety-like behavior during data analysis.

Due to the present drawbacks of the open field test, researchers should contemplate additional behavioral tests of anxiety-like behavior in rodents. Bouwknecht & Paylor (2008) write about behavioral tests saying, "determining anxiety in rodents is more complicated than measuring a single parameter in a particular paradigm. It is important to use proper controls such as additional measures in the same or other procedures, as well as a conservative estimation of the chance of finding an actual effect" (p. 385). Another popular and validated test is the elevated plus maze (EPM), a cross-shaped maze elevated off the ground that has two enclosed arms and two open arms. Similar to the open field test, the EPM examines entries into the open versus enclosed spaces as measures of anxiety-like behavior, time spent in the enclosed arms indicative of more anxiety-like behavior. In the EPM, factors such as illumination and the rodent being placed into the maze did not appear to affect behavior. The EPM is also less correlated with other locomotor and exploratory measures. (Pellow et al., 1985). Rosso et al. (2021) conducted a meta-analysis on the effects of anxiolytics and the reliability of behavioral tests for mice. Reviewing 17 behavior paradigms, Rosso and colleagues concluded that the EPM's time spent in the enclosed and open arms along with the light-dark box's (LDB) time spent in the light

compartment were the two paradigms able to detect effects of anxiolytic drugs and detect the reliably with significant effect sizes. This review may explain the difficulty that the open field test has in detecting anxiety-like behavior in the presented results.

Overall, we can conclude acute and chronic STN DBS does not produce more anxiety-like behavior. Given the above limitations, future experiments investigating anxiety-like behavior in rodent models of PD using DBS should take the following factors into account: (1) Optimization of STN DBS parameters, (2) Adding a positive drug control such as caffeine to examine anxiogenic effects to demonstrate whether untreated animals are displaying exacerbated anxiety-like behavior, (3) Similarly, adding a negative drug control such as benzodiazepines to examine anxiolytic effects to demonstrate that the PD model is responsive to the behavior paradigms, and (4) Conducting an additional behavioral test such as the EPM or LDB to confirm anxiety-like behavior.

References

Abulseoud, O. A., Kasasbeh, A., Min, H.-K., Fields, J. A., Tye, S. J., Goerss, S., Knight, E. J., Sampson, S. M., Klassen, B. T., Matsumoto, J. Y., Stoppel, C., Lee, K. H., & Frye, M. A. (2016). Stimulation-induced transient nonmotor psychiatric symptoms following subthalamic deep brain stimulation in patients with Parkinson's disease: Association with

clinical outcomes and neuroanatomical correlates. *Stereotactic and Functional Neurosurgery*, *94*(2), 93–101. https://doi.org/10.1159/000445076

Amalric, M., Moukhles, H., Nieoullon, A., & Daszuta, A. (1995). Complex deficits on reaction time performance following bilateral intrastriatal 6-OHDA infusion in the rat. *The European Journal of Neuroscience*, *7*(5), 972–980. https://doi.org/10.1111/j.1460-9568.1995.tb01085.x

Anderson, V. C., Burchiel, K. J., Hogarth, P., Favre, J., & Hammerstad, J. P. (2005). Pallidal vs subthalamic nucleus deep brain stimulation in Parkinson Disease. *Archives of Neurology*, *62*(4), 554–560. https://doi.org/10.1001/archneur.62.4.554

Armstrong, M. J., & Okun, M. S. (2020). Diagnosis and treatment of Parkinson disease: A review. *JAMA*, *323*(6), 548–560. https://doi.org/10.1001/jama.2019.22360

Auten, R. L., & Davis, J. M. (2009). Oxygen toxicity and reactive oxygen species: The devil is in the details. *Pediatric Research*, *66*(2), 121–127. https://doi.org/10.1203/PDR.0b013e3181a9eafb

Aviles-Olmos, I., Kefalopoulou, Z., Tripoliti, E., Candelario, J., Akram, H., Martinez-Torres, I., Jahanshahi, M., Foltynie, T., Hariz, M., Zrinzo, L., & Limousin, P. (2014). Long-term outcome of subthalamic nucleus deep brain stimulation for Parkinson's disease using an MRI-guided and MRI-verified approach. *Journal of Neurology, Neurosurgery & Psychiatry*, *85*(12), 1419–1425. https://doi.org/10.1136/jnnp-2013-306907

Badstuebner, K., Gimsa, U., Weber, I., Tuchscherer, A., & Gimsa, J. (2017). Deep brain stimulation of hemiparkinsonian rats with unipolar and bipolar electrodes for up to 6 weeks: Behavioral testing of freely moving animals. *Parkinson's Disease*, *2017*, 5693589. https://doi.org/10.1155/2017/5693589

Bailey, K. R., & Crawley, J. N. (2009). Anxiety-Related Behaviors in Mice. In J. J. Buccafusco (Ed.), *Methods of Behavior Analysis in Neuroscience*. (2nd ed.). CRC Press/Taylor & Francis.

Ball, N., Teo, W. P., Chandra, S., & Chapman, J. (2019). Parkinson's Disease and the Environment. *Frontiers in Neurology*, *10*, 218. https://doi.org/10.3389/fneur.2019.00218

Blum, D., Torch, S., Lambeng, N., Nissou, M., Benabid, A. L., Sadoul, R., & Verna, J. M. (2001). Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: Contribution to the apoptotic theory in Parkinson's disease. *Progress in Neurobiology*, *65*(2), 135–172. https://doi.org/10.1016/s0301-0082(01)00003-x

Bologna, M., Leodori, G., Stirpe, P., Paparella, G., Colella, D., Belvisi, D., Fasano, A., Fabbrini, G., & Berardelli, A. (2016). Bradykinesia in early and advanced Parkinson's disease. *Journal of the Neurological Sciences*, *369*, 286–291. https://doi.org/10.1016/j.jns.2016.08.028

Bouwknecht, J. A., & Paylor, R. (2008). Pitfalls in the interpretation of genetic and pharmacological effects on anxiety-like behaviour in rodents. *Behavioural Pharmacology*, *19*(5-6), 385–402. https://doi.org/10.1097/FBP.0b013e32830c3658

Brenes, J. C., Padilla, M., & Fornaguera, J. (2009). A detailed analysis of open-field habituation and behavioral and neurochemical antidepressant-like effects in postweaning enriched rats. *Behavioural Brain Research*, *197*(1), 125–137. https://doi.org/10.1016/j.bbr.2008.08.014

Bryda E. C. (2013). The mighty mouse: The impact of rodents on advances in biomedical research. *Missouri Medicine*, *110*(3), 207–211.

Carlsson, A., Lindqvist, M., & Magnusson, T. (1957). 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature*, *180*(4596), 1200. https://doi.org/10.1038/1801200a0

Castrioto, A., Lhommée, E., Moro, E., & Krack, P. (2014). Mood and behavioural effects of subthalamic stimulation in Parkinson's disease. *The Lancet Neurology, 13*(3), 287-305. http://dx.doi.org/10.1016/S1474-4422(13)70294-1

Chang, C., Li, N., Wu, Y., Geng, N., Ge, S., Wang, J., Wang, X., & Wang, X. (2012). Associations between bilateral subthalamic nucleus deep brain stimulation (STN DBS) and anxiety in Parkinson's disease patients: A controlled study. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *24*(3), 316–325. https://doi.org/10.1176/appi.neuropsych.11070170

Chaudhuri, K. R., & Schapira, A. H. V. (2009). Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment. *The Lancet Neurology, 8*(5), 464-74. https://www.proquest.com/scholarly-journals/non-motor-symptoms-parkinsons-disease/docview/201490479/se-2?accountid=12259

Chen, P.-H., Wang, R.-L., Liou, D.-J., & Shaw, J.-S. (2013). Gait disorders in Parkinson's disease: Assessment and management. *International Journal of Gerontology*, *7*(4), 189–193. https://doi.org/10.1016/j.ijge.2013.03.005

Chia, S. J., Tan, E. K., & Chao, Y. X. (2020). Historical Perspective: Models of Parkinson's disease. *International Journal of Molecular Sciences*, *21*(7), 2464. https://doi.org/10.3390/ijms21072464

Costa, R., Tamascia, M. L., Nogueira, M. D., Casarini, D. E., & Marcondes, F. K. (2012). Handling of adolescent rats improves learning and memory and decreases anxiety. *Journal of the American Association for Laboratory Animal Science: JAALAS*, *51*(5), 548–553.

Couto, M. I., Monteiro, A., Oliveira, A., Lunet, N., & Massano, J. (2014). Depression and anxiety following deep brain stimulation in Parkinson's disease: Systematic review and meta-analysis. *Acta Médica Portuguesa*, *27*(3), 372–382. https://doi.org/10.20344/amp.4928

Creed, M. C., Hamani, C., & Nobrega, J. N. (2013). Effects of repeated deep brain stimulation on depressive- and anxiety-like behavior in rats: Comparing entopeduncular and subthalamic nuclei. *Brain Stimulation*, *6*(4), 506–514. https://doi.org/10.1016/j.brs.2012.09.012

Da Cunha, C., Boschen, S. L., Gómez-A, A., Ross, E. K., Gibson, W. S. J., Min, H.-K., Lee, K. H., & Blaha, C. D. (2015). Toward sophisticated basal ganglia neuromodulation: Review

on basal ganglia deep brain stimulation. *Neuroscience & Biobehavioral Reviews*, *58*, 186–210. https://doi.org/10.1016/j.neubiorev.2015.02.003

Dauer, W., & Przedborski, S. (2003). Parkinson's disease: Mechanisms and models. *Neuron*, *39*(6), 889–909. https://doi.org/10.1016/s0896-6273(03)00568-3

Denenberg V. H. (1969). Open-field behavior in the rat: What does it mean?. *Annals of the New York Academy of Sciences*, *159*(3), 852–859. https://doi.org/10.1111/j.1749-6632.1969.tb12983.x

Deumens, R., Blokland, A., & Prickaerts, J. (2002). Modeling Parkinson's disease in rats: An evaluation of 6-OHDA lesions of the nigrostriatal pathway. *Experimental Neurology*, *175*(2), 303–317. https://doi.org/10.1006/exnr.2002.7891

di Biase, L., Summa, S., Tosi, J., Taffoni, F., Marano, M., Cascio Rizzo, A., Vecchio, F., Formica, D., Di Lazzaro, V., Di Pino, G., & Tombini, M. (2018). Quantitative analysis of bradykinesia and rigidity in Parkinson's disease. *Frontiers in Neurology, 9*(121). https://doi.org/10.3389/fneur.2018.00121

Díaz-Morán, S., Estanislau, C., Cañete, T., Blázquez, G., Ráez, A., Tobeña, A., & Fernández-Teruel, A. (2014). Relationships of open-field behaviour with anxiety in the elevated zero-maze test: Focus on freezing and grooming. *World Journal of Neuroscience*, *04*(01), 1–11. https://doi.org/10.4236/wjns.2014.41001

Dorsey, E. R., Sherer, T., Okun, M. S., & Bloem, B. R. (2018). The emerging evidence of the Parkinson pandemic. *Journal of Parkinson's Disease*, *8*(s1), S3–S8. https://doi.org/10.3233/JPD-181474

Ellenbroek, B., & Youn, J. (2016). Rodent models in neuroscience research: Is it a rat race?. *Disease Models & Mechanisms*, *9*(10), 1079–1087. https://doi.org/10.1242/dmm.026120

Ennaceur, A., & Chazot, P. L. (2016). Preclinical animal anxiety research - flaws and prejudices. *Pharmacology Research & Perspectives*, *4*(2), e00223. https://doi.org/10.1002/prp2.223

Estanislau, C., Veloso, A. W. N., Filgueiras, G. B., Maio, T. P., Dal-Cól, M. L. C., Cunha, D. C., Klein, R., Carmona, L. F., & Fernández-Teruel, A. (2019). Rat self-grooming and its relationships with anxiety, dearousal and perseveration: Evidence for a self-grooming trait. *Physiology & Behavior*, *209*, 112585–112594. https://doi.org/10.1016/j.physbeh.2019.112585

Feigin, V. L., Abajobir, A. A., Abate, K. H., Abd-Allah, F., Abdulle, A. M., Abera, S. F., Abyu, G. Y., Ahmed, M. B., Aichour, A. N., Aichour, I., Aichour, M. T., Akinyemi, R. O., Alabed, S., Al-Raddadi, R., Alvis-Guzman, N., Amare, A. T., Ansari, H., Anwari, P., Ärnlöv, J., … Vos, T. (2017). Global, regional, and national burden of neurological disorders during 1990–2015: A systematic analysis for the global burden of disease study 2015. *The Lancet Neurology*, *16*(11), 877–897. https://doi.org/10.1016/s1474-4422(17)30299-5

Frank, M. J. (2011). Computational models of motivated action selection in corticostriatal circuits. *Current Opinion in Neurobiology*, *21*(3), 381–386. https://doi.org/10.1016/j.conb.2011.02.013

Frank, M. J., & Claus, E. D. (2006). Anatomy of a decision: Striato-orbitofrontal interactions in reinforcement learning, decision making, and reversal. *Psychological Review*, *113*(2), 300–326. https://doi.org/10.1037/0033-295X.113.2.300

Giovanni, A., Sieber, B. A., Heikkila, R. E., & Sonsalla, P. K. (1994). Studies on species sensitivity to the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Part 1: Systemic administration. *The Journal of pharmacology and experimental therapeutics*, *270*(3), 1000–1007.

Goetz, C. G., Poewe, W., Rascol, O., & Sampaio, C. (2005). Evidence-based medical review update: Pharmacological and surgical treatments of Parkinson's disease: 2001 to 2004. *Movement Disorders*, *20*(5), 523–539. https://doi.org/10.1002/mds.20464

Graves, S. M., Xie, Z., Stout, K. A., Zampese, E., Burbulla, L. F., Shih, J. C., Kondapalli, J., Patriarchi, T., Tian, L., Brichta, L., Greengard, P., Krainc, D., Schumacker, P. T., & Surmeier, D. J. (2020). Dopamine metabolism by a monoamine oxidase mitochondrial shuttle activates the electron transport chain. *Nature Neuroscience*, *23*(1), 15–20. https://doi.org/10.1038/s41593-019-0556-3

Hamani, C., Florence, G., Heinsen, H., Plantinga, B. R., Temel, Y., Uludag, K., Alho, E., Teixeira, M. J., Amaro, E., & Fonoff, E. T. (2017). Subthalamic nucleus deep brain stimulation: Basic concepts and novel perspectives. *Eneuro*, *4*(5), 1–14. https://doi.org/10.1523/eneuro.0140-17.2017

Harro J. (2018). Animals, anxiety, and anxiety disorders: How to measure anxiety in rodents and why. *Behavioural Brain Research*, *352*, 81–93. https://doi.org/10.1016/j.bbr.2017.10.016

Hawley, J. S., Armstrong, M. J., & Weiner, W. J. (Eds.). (2014). *Parkinson's disease: Improving patient care*. Oxford University Press, Incorporated.

Houeto, J. L., Mesnage, V., Mallet, L., Pillon, B., Gargiulo, M., du Moncel, S. T., Bonnet, A. M., Pidoux, B., Dormont, D., Cornu, P., & Agid, Y. (2002). Behavioural disorders, Parkinson's disease and subthalamic stimulation. *Journal of Neurology, Seurosurgery, and Psychiatry*, *72*(6), 701–707. https://doi.org/10.1136/jnnp.72.6.701

Huotarinen, A., Leino, S., Tuominen, R. K., & Laakso, A. (2019). Rat subthalamic stimulation: Evaluating stimulation-induced dyskinesias, choosing stimulation currents and evaluating the anti-akinetic effect in the cylinder test. *MethodsX*, *6*, 2384–2395. https://doi.org/10.1016/j.mex.2019.10.012

Isoda, M., & Hikosaka, O. (2011). Cortico-basal ganglia mechanisms for overcoming innate, habitual and motivational behaviors. *European Journal of Neuroscience*, *33*(11), 2058–2069. https://doi.org/10.1111/j.1460-9568.2011.07698.x

Jakobs, M., Fomenko, A., Lozano, A. M., & Kiening, K. L. (2019). Cellular, molecular, and clinical mechanisms of action of deep brain stimulation—a systematic review on established indications and outlook on future developments. *EMBO Molecular Medicine*, *11*(4). https://doi.org/10.15252/emmm.201809575

Jonsson, G. (1983). Methods in chemical neuroanatomy. *Handbook of Chemical Neuroanatomy*, *1*, 463-507.

Kaindlstorfer, C., Granata, R., & Wenning, G. K. (2013). Tremor in multiple system atrophy - A review. *Tremor and Other Hyperkinetic Movements (New York, N.Y.)*, *3*, tre-03-165-4252-1. https://doi.org/10.7916/D8NV9GZ9

Kaiser, I., Kryspin-Exner, I., Brücke, T., Volc, D., & Alesch, F. (2008). Long-term effects of STN DBS on mood: Psychosocial profiles remain stable in a 3-year follow-up. *BMC Neurology*, *8*, 43. https://doi.org/10.1186/1471-2377-8-43

Kalteis, K., Standhardt, H., Kryspin-Exner, I., Brücke, T., Volc, D., & Alesch, F. (2006). Influence of bilateral STN-stimulation on psychiatric symptoms and psychosocial functioning in patients with Parkinson's disease. *Journal of Neural Transmission*, *113*(9), 1191–1206. https://doi.org/10.1007/s00702-005-0399-9

Knoblaugh, S. E., Hohl, T. M., & La Perle, K. (2018). Pathology principles and practices for analysis of animal models. *ILAR Journal*, *59*(1), 40–50. https://doi.org/10.1093/ilar/ilz001

Kowal, S. L., Dall, T. M., Chakrabarti, R., Storm, M. V., & Jain, A. (2013). The current and projected economic burden of Parkinson's disease in the United States. *Movement Disorders*, *28*(3), 311–318. https://doi.org/10.1002/mds.25292

Kraeuter, A. K., Guest, P. C., & Sarnyai, Z. (2019). The open field test for measuring locomotor activity and anxiety-like behavior. *Methods in Molecular Biology*, *1916*, 99–103. https://doi.org/10.1007/978-1-4939-8994-2_9

Kuncel, A. M., & Grill, W. M. (2004). Selection of stimulus parameters for deep brain stimulation. *Clinical Neurophysiology*, *115*(11), 2431–2441. https://doi.org/10.1016/j.clinph.2004.05.031

Lill, C. M., & Klein, C. (2017). Epidemiologie und ursachen der parkinson-erkrankung [epidemiology and causes of Parkinson's disease]. *Der Nervenarzt*, *88*(4), 345–355. https://doi.org/10.1007/s00115-017-0288-0

Lin, H.-Y., Yeh, W.-L., Huang, B.-R., Lin, C., Lai, C.-H., Lin, H., & Lu, D.-Y. (2012). Desipramine protects neuronal cell death and induces heme oxygenase-1 expression in MES23.5 dopaminergic neurons. *PloS ONE*, *7*(11). https://doi.org/10.1371/journal.pone.0050138

Linnoila, M., Karoum, F., Calil, H. M., Kopin, I. J., & Potter, W. Z. (1982). Alteration of norepinephrine metabolism with desipramine and zimelidine in depressed patients. *Archives of General Psychiatry*, *39*(9), 1025–1028. https://doi.org/10.1001/archpsyc.1982.04290090027006

Lopiano, L., Rizzone, M., Bergamasco, B., Tavella, A., Torre, E., Perozzo, P., Valentini, M. C., & Lanotte, M. (2001). Deep brain stimulation of the subthalamic nucleus: clinical effectiveness and safety. *Neurology*, *56*(4), 552–554. https://doi.org/10.1212/wnl.56.4.552

Maserejian, N. M., & Vinikoor-Imler, D. A. (2020). Movement Disorder Society Virtual Congress 2020. In *International Parkinson and Movement Disorder Society – Meeting Abstracts*. Wiley. https://www.mdsabstracts.org/abstract/estimation-of-the-2020-global-population-of-parkinsons-disease-pd/

Mink, J. W. (1996). The basal ganglia: Focused selection and inhibition of competing motor programs. *Progress in Neurobiology*, *50*(4), 381–425. https://doi.org/10.1016/s0301-0082(96)00042-1

Mirelman, A., Bonato, P., Camicioli, R., Ellis, T. D., Giladi, N., Hamilton, J. L., Hass, C. J., Hausdorff, J. M., Pelosin, E., & Almeida, Q. J. (2019). Gait impairments in Parkinson's disease. *The Lancet Neurology, 18*(7), 697-708. http://dx.doi.org/10.1016/S1474-4422(19)30044-4

Mogenson, G. J., Jones, D. L., & Yim, C. Y. (1980). From motivation to action: Functional interface between the limbic system and the motor system. *Progress in Neurobiology*, *14*(2-3), 69–97. https://doi.org/10.1016/0301-0082(80)90018-0

Mottaghi, S., Buchholz, O., & Hofmann, U. G. (2020). Systematic evaluation of DBS parameters in the hemi-parkinsonian rat model. *Frontiers in Neuroscience*, *14*, 1–13. https://doi.org/10.3389/fnins.2020.561008

National Center for Biotechnology Information (2022). *Compound summary: Oxidopamine.* PubChem. https://pubchem.ncbi.nlm.nih.gov/compound/Oxidopamine.

National Research Council. (2011). *Guide for the care and use of laboratory animals: Eighth edition*. The National Academies Press. https://doi.org/10.17226/12910

Nonnekes, J., Giladi, N., Guha, A., Fietzek, U. M., Bloem, B. R., & Růžička, E. (2019). Gait festination in parkinsonism: Introduction of two phenotypes. *Journal of Neurology*, *266*(2), 426–430. https://doi.org/10.1007/s00415-018-9146-7

Nonnekes, J., Post, B., Tetrud, J. W., Langston, J. W., & Bloem, B. R. (2018). MPTP-induced parkinsonism: An historical case series. *The Lancet Neurology*, *17*(4), 300–301. https://doi.org/10.1016/s1474-4422(18)30072-3

Obeso, J. A., Olanow, C. W., Rodriguez-Oroz, M. C., Krack, P., Kumar, R., & Lang, A. E. (2001). Deep-brain stimulation of the subthalamic nucleus or the pars interna of the

globus pallidus in Parkinson's disease. *New England Journal of Medicine*, *345*(13), 956–963. https://doi.org/10.1056/nejmoa000827

Okun M. S. (2012). Deep-brain stimulation for Parkinson's disease. *The New England Journal of Medicine*, *367*(16), 1529–1538. https://doi.org/10.1056/NEJMct1208070

Okun, M. S., Fernandez, H. H., Wu, S. S., Kirsch-Darrow, L., Bowers, D., Bova, F., Suelter, M., Jacobson, C. E., 4th, Wang, X., Gordon, C. W., Jr, Zeilman, P., Romrell, J., Martin, P., Ward, H., Rodriguez, R. L., & Foote, K. D. (2009). Cognition and mood in Parkinson's disease in subthalamic nucleus versus globus pallidus interna deep brain stimulation: The COMPARE trial. *Annals of Neurology*, *65*(5), 586–595. https://doi.org/10.1002/ana.21596

Palakurthi, B., & Burugupally, S. P. (2019). Postural instability in Parkinson's disease: A review. *Brain Sciences*, *9*(9), 239. https://doi.org/10.3390/brainsci9090239

Pahwa, R., & Lyons, K. E. (2012). *Diagnostic and treatment guidelines in Parkinson's disease*. Oxford University Press, Incorporated.

Paxinos, G., & Watson, C. (2004). *The rat brain in stereotaxic coordinates* (5th ed.). Elsevier.

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open : closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience methods*, *14*(3), 149–167. https://doi.org/10.1016/0165-0270(85)90031-7

Peng, L., Fu, J., Ming, Y., Zeng, S., He, H., & Chen, L. (2018). The long-term efficacy of STN vs GPi deep brain stimulation for Parkinson disease: A meta-analysis. *Medicine*, *97*(35), e12153. https://doi.org/10.1097/MD.0000000000012153

Pereira, E. A., & Aziz, T. Z. (2006). Parkinson's disease and primate research: Past, present, and future. *Postgraduate Medical Journal*, *82*(967), 293–299. https://doi.org/10.1136/pgmj.2005.041194

Péron, J., Frühholz, S., Vérin, M., & Grandjean, D. (2013). Subthalamic nucleus: A key structure for emotional component synchronization in humans. *Neuroscience & Biobehavioral Reviews*, *37*(3), 358–373. https://doi.org/10.1016/j.neubiorev.2013.01.001

Porter, C. C., Totaro, J. A., & Burcin, A. (1965). The relationship between radioactivity and norepinephrine concentrations in the brains and hearts of mice following administration of labeled methyldopa or 6-hydroxydopamine. *The Journal of Pharmacology and Experimental Therapeutics*, *150*(1), 17–22.

Porter, C. C., Totaro, J. A., & Stone, C. A. (1963). Effect of 6-hydroxydopamine and some other compounds on the concentration of norepinephrine in the hearts of mice. *The Journal of Pharmacology and Experimental Therapeutics*, *140*, 308–316.

Przedborski, S., Levivier, M., Jiang, H., Ferreira, M., Jackson-Lewis, V., Donaldson, D., & Togasaki, D. M. (1995). Dose-dependent lesions of the dopaminergic nigrostriatal pathway induced by intrastriatal injection of 6-hydroxydopamine. *Neuroscience*, *67*(3), 631–647. https://doi.org/10.1016/0306-4522(95)00066-r

Ramirez-Zamora, A., & Ostrem, J. L. (2018). Globus pallidus interna or subthalamic nucleus deep brain stimulation for Parkinson disease: A review. *JAMA Neurology*, *75*(3), 367–372. https://doi.org/10.1001/jamaneurol.2017.4321

Rand, M. S. (2008). Selection of biomedical animal models. In M. P. Conn (Eds.), *Sourcebook of models for biomedical research* (pp. 9-15). Humana Press Inc. https://doi.org/10.1007/978-1-59745-285-4_2

Reymann, J.-M., Naudet, F., Pihan, M., Saïkali, S., Laviolle, B., & Bentué-Ferrer, D. (2013). Subthalamic nucleus modulates social and anxogenic-like behaviors. *Behavioural Brain Research*, *252*, 356–362. https://doi.org/10.1016/j.bbr.2013.05.059

Robinson, T. E., Mocsary, Z., Camp, D. M., & Whishaw, I. Q. (1994). Time course of recovery of extracellular dopamine following partial damage to the nigrostriatal dopamine system. *The Journal of Neuroscience*, *14*(5), 2687–2696. https://doi.org/10.1523/jneurosci.14-05-02687.1994

Rosso, M., Wirz, R., Loretan, A. V., Sutter, N. A., Pereira da Cunha, C. T., Jaric, I., Würbel, H., & Voelkl, B. (2021). Reliability of mouse behavioural tests of anxiety: A systematic review and meta-analysis on the effects of anxiolytics. *BioRxiv*. https://doi.org/10.1101/2021.07.28.454267

Rothlind, J., Cockshott, R., Starr, P., & Marks, W. (2007). Neuropsychological performance following staged bilateral pallidal or subthalamic nucleus deep brain stimulation for Parkinson's disease. *Journal of the International Neuropsychological Society, 13*(1), 68-79. https://doi.org/10.1017/S1355617707070105

Ruge, D., Cif, L., Limousin, P., Gonzalez, V., Vasques, X., Hariz, M. I., Coubes, P., & Rothwell, J. C. (2011a). Shaping reversibility? Long-term deep brain stimulation in dystonia: The relationship between effects on electrophysiology and clinical symptoms. *Brain: A Journal of Neurology*, *134*(Pt 7), 2106–2115. https://doi.org/10.1093/brain/awr122

Ruge, D., Tisch, S., Hariz, M. I., Zrinzo, L., Bhatia, K. P., Quinn, N. P., Jahanshahi, M., Limousin, P., & Rothwell, J. C. (2011b). Deep brain stimulation effects in dystonia: Time course of electrophysiological changes in early treatment. *Movement Disorders*, *26*(10), 1913–1921. https://doi.org/10.1002/mds.23731

Sauer, H., & Oertel, W. H. (1994). Progressive degeneration of nigrostriatal dopamine neurons following intrastriatal terminal lesions with 6-hydroxydopamine: A combined retrograde tracing and immunocytochemical study in the rat. *Neuroscience*, *59*(2), 401–415. https://doi.org/10.1016/0306-4522(94)90605-x

Seibenhener, M. L., & Wooten, M. C. (2015). Use of the open field maze to measure locomotor and anxiety-like behavior in mice. *Journal of Visualized Experiments: JoVE*, (96), e52434. https://doi.org/10.3791/52434

Shoji, H., & Miyakawa, T. (2021). Effects of test experience, closed-arm wall color, and illumination level on behavior and plasma corticosterone response in an elevated plus maze in male C57BL/6J mice: A challenge against conventional interpretation of the test. *Molecular Brain*, *14*(1), 34. https://doi.org/10.1186/s13041-020-00721-2

Sonne, J., Reddy, V., & Beato, M. R. (2021). Neuroanatomy, substantia nigra. In *StatPearls*. StatPearls Publishing.

Spruijt, B. M., van Hooff, J. A., & Gispen, W. H. (1992). Ethology and neurobiology of grooming behavior. *Physiological Reviews*, *72*(3), 825–852. https://doi.org/10.1152/physrev.1992.72.3.825

Sturman, O., Germain, P. L., & Bohacek, J. (2018). Exploratory rearing: A context- and stress-sensitive behavior recorded in the open-field test. *Stress, 21*(5), 443–452. https://doi.org/10.1080/10253890.2018.1438405

Tatem, K. S., Quinn, J. L., Phadke, A., Yu, Q., Gordish-Dressman, H., & Nagaraju, K. (2014). Behavioral and locomotor measurements using an open field activity monitoring system for skeletal muscle diseases. *Journal of Visualized Experiments: JoVE*, (91), e51785-e51792. https://doi.org/10.3791/51785

Temel, Y., Blokland, A., Steinbusch, H. W. M., & Visser-Vandewalle, V. (2005). The functional role of the subthalamic nucleus in cognitive and limbic circuits. *Progress in Neurobiology*, *76*(6), 393–413. https://doi.org/10.1016/j.pneurobio.2005.09.005

Thompson, T., Grabowski-Boase, L., & Tarantino, L. M. (2015). Prototypical anxiolytics do not reduce anxiety-like behavior in the open field in C57BL/6J mice. *Pharmacology, Biochemistry, and Behavior*, *133*, 7–17. https://doi.org/10.1016/j.pbb.2015.03.011

Tieu K. (2011). A guide to neurotoxic animal models of Parkinson's disease. *Cold Spring Harbor Perspectives in Medicine*, *1*(1), a009316. https://doi.org/10.1101/cshperspect.a009316

Tranzer, J. P., & Thoenen, H. (1968). An electron microscopic study of selective, acute degeneration of sympathetic nerve terminals after administration of 6-hydroxydopamine. *Experientia*, *24*(2), 155–156. https://doi.org/10.1007/BF02146956

Tranzer, J. P., & Thoenen, H. (1973). Selective destruction of adrenergic nerve terminals by chemical analogues of 6-hydroxydopamine. *Experientia*, *29*(3), 314–315. https://doi.org/10.1007/BF01926498

Turner, P. V., Pang, D. S. J., & Lofgren, J. L. S. (2019). A review of pain assessment methods in laboratory rodents. *Comparative Medicine*, *69*(6), 451–467. https://doi.org/10.30802/aalas-cm-19-000042

Ungerstedt U. (1968). 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. *European Journal of Pharmacology*, *5*(1), 107–110. https://doi.org/10.1016/0014-2999(68)90164-7

Volkmann, J., Moro, E., & Pahwa, R. (2006). Basic algorithms for the programming of deep brain stimulation in Parkinson’s disease. *Movement Disorders*, *21*(S14), S284–S289. https://doi.org/10.1002/mds.20961

Voon, V., Kubu, C., Krack, P., Houeto, J.-L., & Tröster, A. I. (2006). Deep brain stimulation: Neuropsychological and neuropsychiatric issues. *Movement Disorders*, *21*(S14), 305–327. https://doi.org/10.1002/mds.20963

Voon, V., Krack, P., Lang, A. E., Lozano, A. M., Dujardin, K., Schüpbach, M., D'Ambrosia, J., Thobois, S., Tamma, F., Herzog, J., Speelman, J. D., Samanta, J., Kubu, C., Rossignol, H., Poon, Y. Y., Saint-Cyr, J. A., Ardouin, C., & Moro, E. (2008). A multicentre study on suicide outcomes following subthalamic stimulation for Parkinson's disease. *Brain: A Journal of Neurology*, *131*(Pt 10), 2720–2728. https://doi.org/10.1093/brain/awn214

Walsh, R. N., & Cummins, R. A. (1976). The open-field test: A critical review. *Psychological Bulletin*, *83*(3), 482–504.

Wang, J.-W., Zhang, Y.-Q., Zhang, X.-H., Wang, Y.-P., Li, J.-P., & Li, Y.-J. (2016). Cognitive and psychiatric effects of STN versus GPI Deep brain stimulation in Parkinson's disease: A meta-analysis of randomized controlled trials. *PLOS ONE*, *11*(6). https://doi.org/10.1371/journal.pone.0156721

Wong, J. K., Viswanathan, V. T., Nozile-Firth, K. S., Eisinger, R. S., Leone, E. L., Desai, A. M., Foote, K. D., Ramirez-Zamora, A., Okun, M. S., & Wagle Shukla, A. (2020). STN versus GPi deep brain Stimulation for action and rest tremor in Parkinson's disease. *Frontiers in Human Neuroscience*, *14*, 578615. https://doi.org/10.3389/fnhum.2020.578615

Xu, W., Miocinovic, S., Zhang, J., Baker, K. B., McIntyre, C. C., & Vitek, J. L. (2011). Dissociation of motor symptoms during deep brain stimulation of the subthalamic nucleus in the region of the internal capsule. *Experimental Neurology*, *228*(2), 294–297. https://doi.org/10.1016/j.expneurol.2010.08.007

Yang, W., Hamilton, J. L., Kopil, C., Beck, J. C., Tanner, C. M., Albin, R. L., Ray Dorsey, E., Dahodwala, N., Cintina, I., Hogan, P., & Thompson, T. (2020). Current and projected future economic burden of Parkinson's disease in the U.S. *Npj Parkinson's Disease*, *6*(1), 1–15. https://doi.org/10.1038/s41531-020-0117-1

York, M. K., Dulay, M., Macias, A., Levin, H. S., Grossman, R., Simpson, R., & Jankovic, J. (2008). Cognitive declines following bilateral subthalamic nucleus deep brain stimulation for the treatment of Parkinson's disease. *Journal of Neurology, Neurosurgery and Psychiatry, 79*(7), 789. http://dx.doi.org/10.1136/jnnp.2007.118786

Appendix A

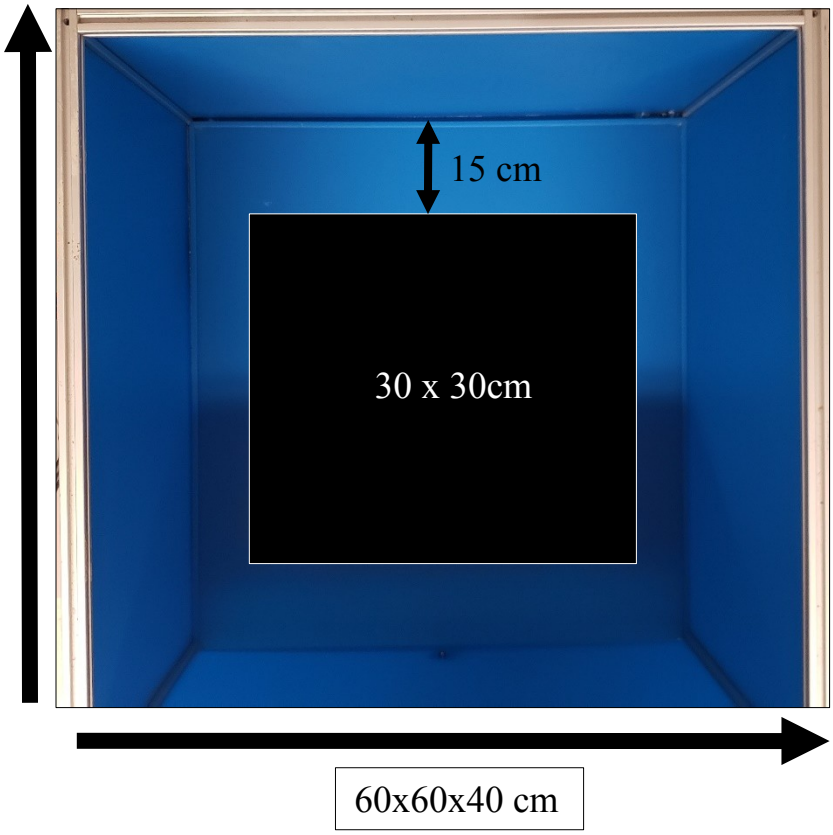
Rat Information Including Number, Sex, Group, Exclusion, and Attrition

Rat Number	Sex	Group/Reason Not Included	Attrition
R4	m	Chronic	NR= 8
R5	m	Acute	SE= 1
R6	m	Chronic	CDI= 2
R7	m	NR	HD= 4
R8	m	Acute	Total Attrition= 15
R9	m	SE	Total %= 44
R10	m	No Stim	
R11	m	No Stim	
R12	m	NR	
R13	m	Chronic	
R14	f	NR	
R15	f	Chronic	
R16	m	Acute	
R17	m	Acute	
R18	f	Chronic	
R19	f	No Stim	
R20	m	HD	
R21	m	CDI	
R22	m	NR	
R23	m	NR	
R24	f	No Stim	
R25	f	HD	
R26	f	NR	
R27	f	CDI	
R28	f	No Stim	
R29	m	Acute	
R30	m	Chronic	
R31	m	No Stim	
R32	f	NR	
R33	f	Acute	
R34	f	No stim	
R35	m	NR	
R36	m	HD	
R37	m	HD	

Note. NR= Non-responder, did not respond to stimulation; HD= Headcap detached and was euthanized; SE= Surgical error, died during surgery; CDI= Cord detection issues, could not be stimulated.

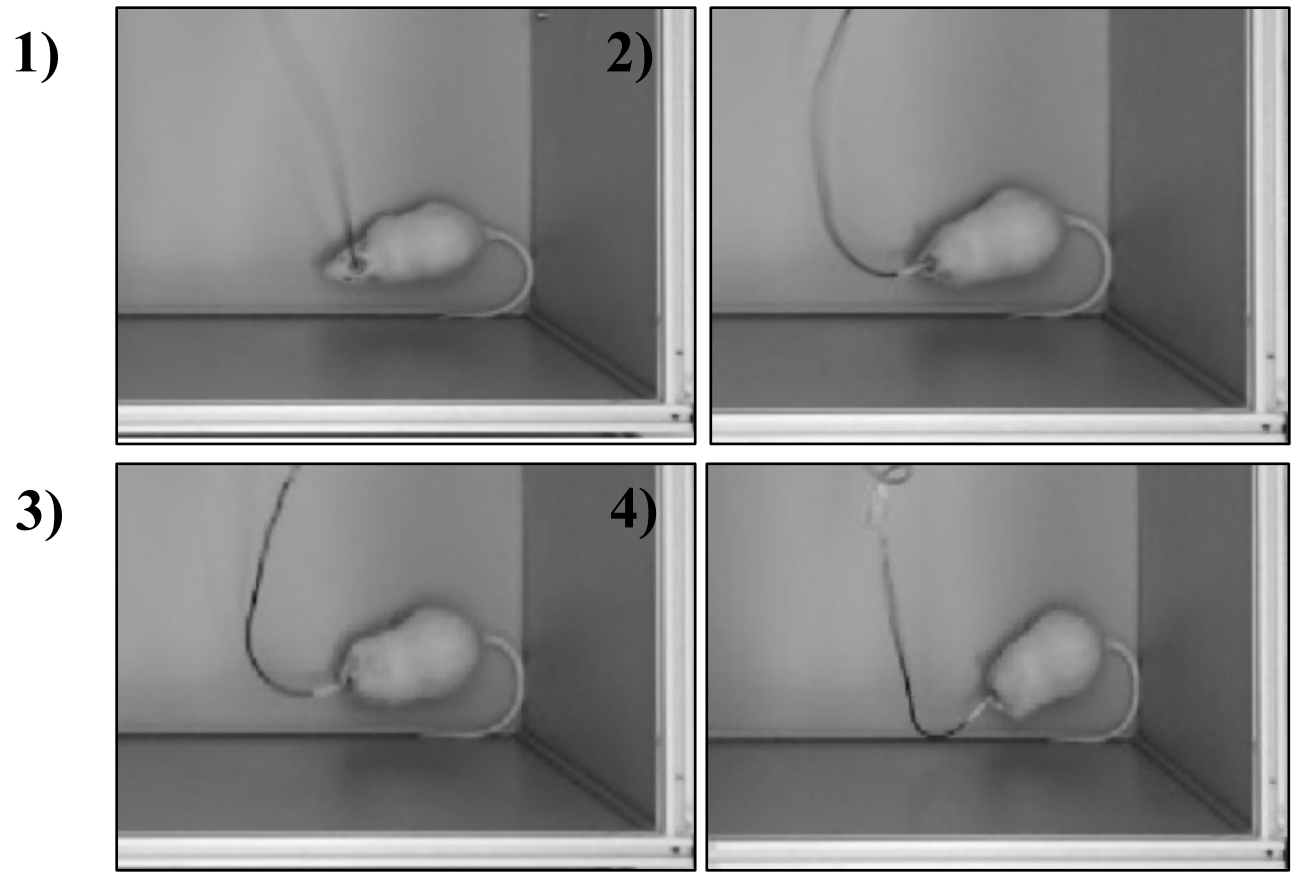
Appendix B

Open Field Arena Utilized in the Present Experiment



Appendix C

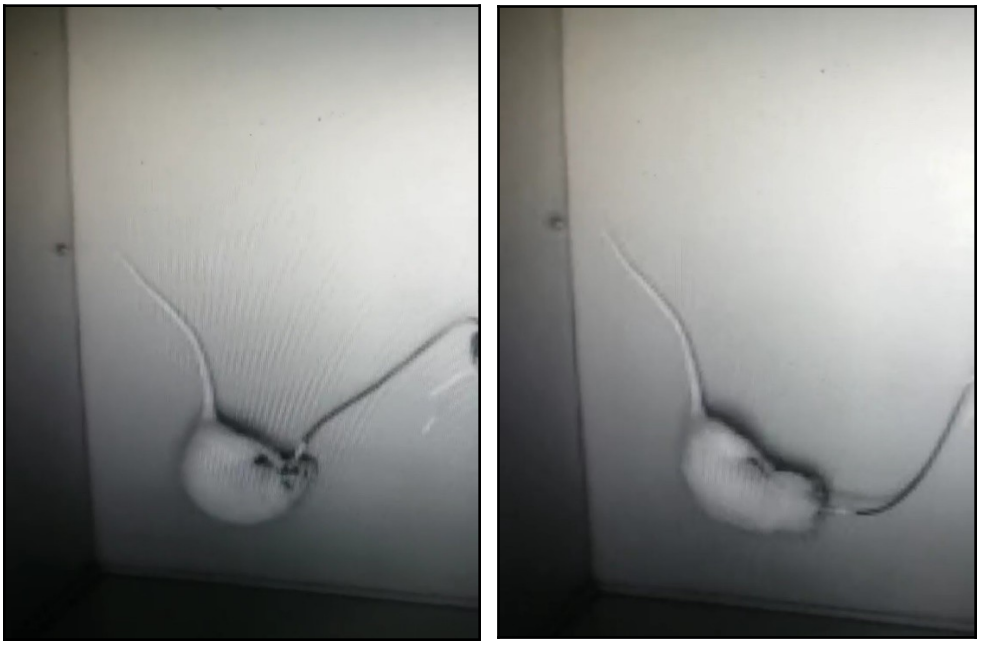
An Example of the Grooming Sequence



Note. Seen in image 1 the rat has stopped, moving image 2 the rat has begun to lick and clean its paws. In images three and four, the rat has raised its left and right paw over its head and across the ears back to the mouth.

Appendix D

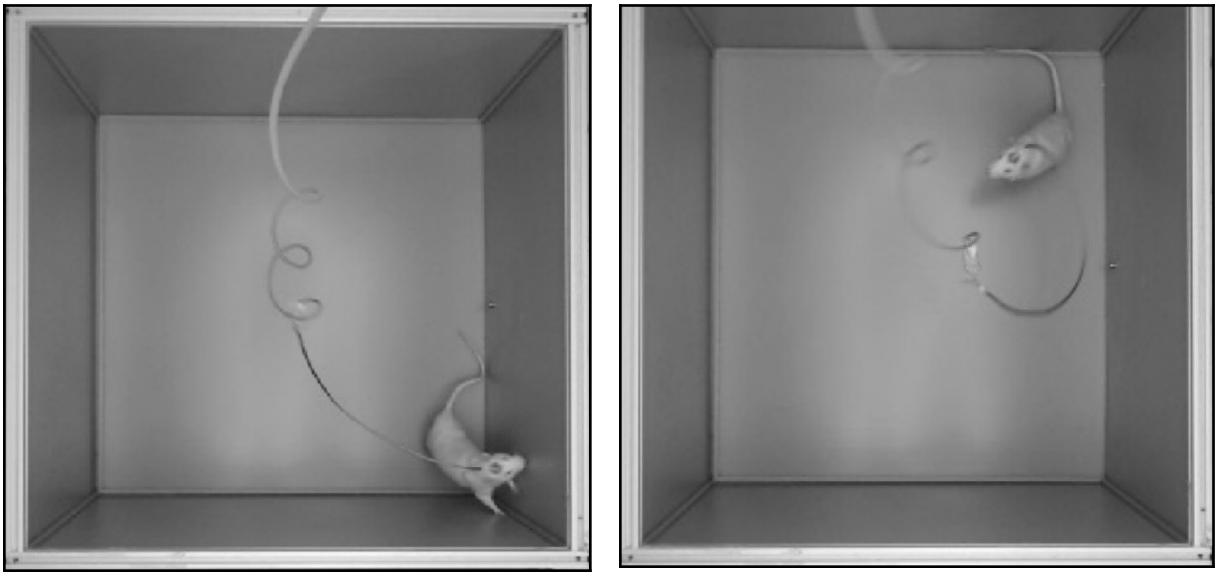
Nonexamples of Grooming Behavior



Note. Nonexample of the rat using its hind paw to scratch its body.

Appendix E

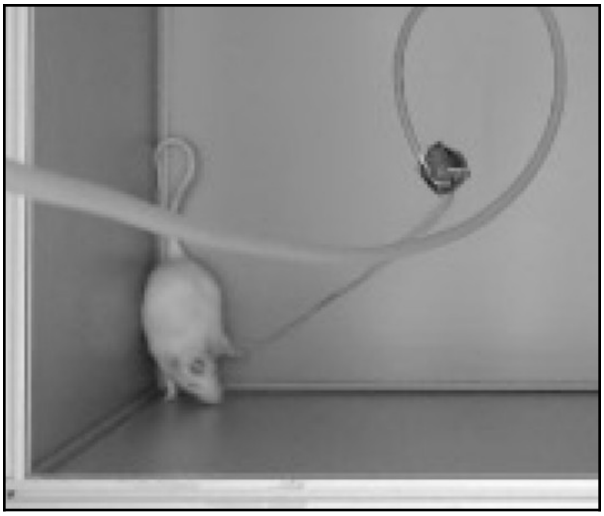
Examples of Supported and Unsupported Rearing



Note. The photo on the left illustrates supported rearing with both paws on the wall. The photo on the right shows unsupported rearing.

Appendix F

Nonexample of Rearing Behavior



Note. The rat has one paw on the floor and one on the wall, which is considered a nonexample of rearing.

Appendix G

Zone Setup in EthoVision for Center and Border Measures

Measure	% time in Outer Zone	% time in Inner Zone	Rearing Count	Grooming (seconds)	Total Distance Traveled (cm)	Velocity (cm/s)
N	19	19	19	19	19	19
M	96.71	3.29	41.80	24.50	4456.91	12.09
SD	1.99	1.99	22.01	15.66	1614.21	3.01
D	.92	.92	.69	.44	.45	.41
Sig.	.361	.361	.723	.991	.987	.996

Appendix H

Descriptive Statistics and One-sample K-S Test for Measures for Total Ten Minutes of the Open Field Test

Appendix I

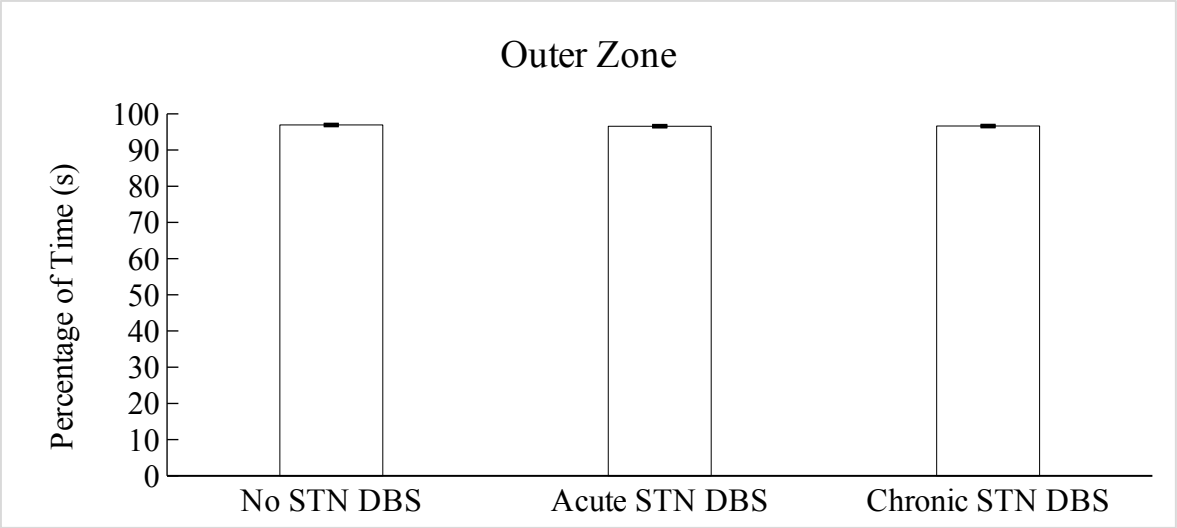
Descriptive Statistics and One-way ANOVA Results for All Measures in the Total Ten Minutes of the Open Field Test

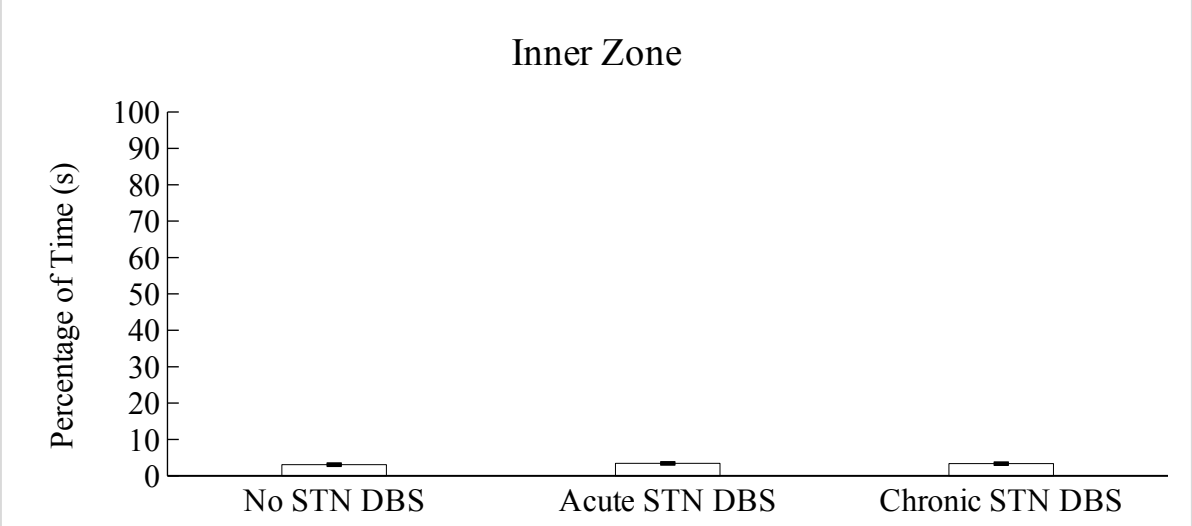
Measure	Chronic Stimulation		Acute Stimulation		No Stimulation		*F*(2, 16)	Sig.
	M	*SD*	*M*	*SD*	*M*	*SD*		
Total Distance Traveled (cm)	4914.71	1567.04	4483.80	2067.28	4041.46	1340.75	.45	.65
Rearing Count	44.50	17.20	27.50	18.49	51.71	24.40	.13	.23
Grooming (seconds)	31.03	15.65	23.35	18.61	19.88	13.21	.83	.46
Velocity (cm/s)	12.49	3.55	11.50	4.12	12.26	1.39	.16	.85
Time Spent in Outer Zone (seconds)	573.27	10.90	571.04	19.31	579.21	14.63	.50	.62

Time Spent in Inner Zone (seconds)	20.09	11.17	20.42	12.87	18.40	13.62	.05	.95
Percentage of Time in Outer Zone	96.62	1.85	96.56	2.13	96.92	2.27	.06	.95
Percentage of Time in Inner Zone	3.38	1.85	3.44	2.13	3.08	2.27	.06	.95

Appendix J

Outer and Inner Zones for Total Ten Minutes in the Open Field Test

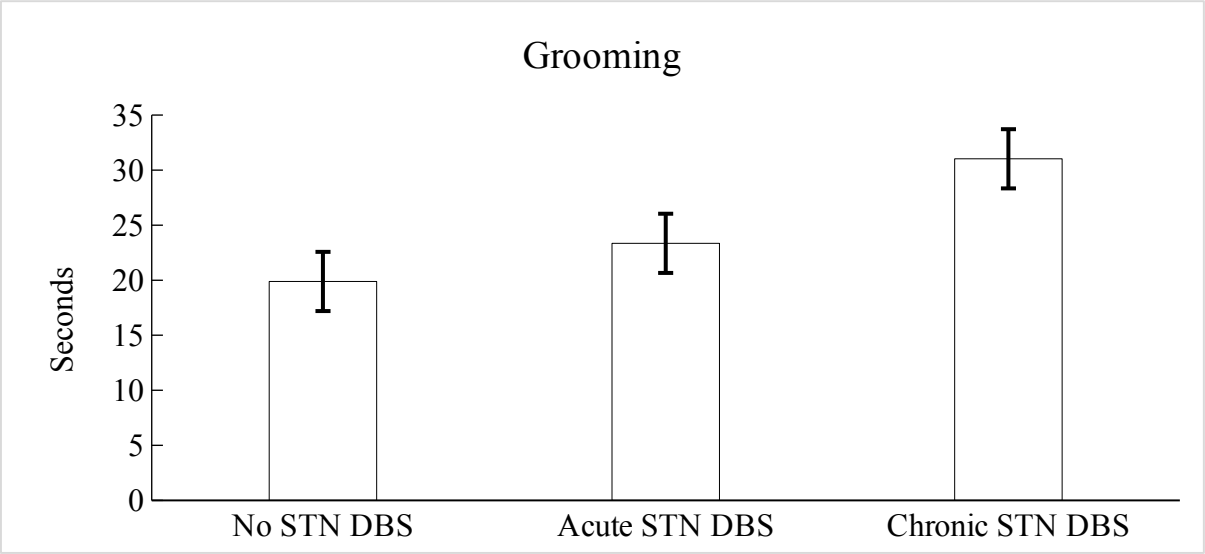

Outer Zone
Percentage of Time (s)
100
90
80
70
60
50
40
30
20
10
0
No STN DBS
Acute STN DBS
Chronic STN DBS


Inner Zone
Percentage of Time (s)
100
90
80
70
60
50
40
30
20
10
0
No STN DBS
Acute STN DBS
Chronic STN DBS

Note. Average of percentage of time in seconds spent in the outer zone (A) and inner zone (B) of the open field arena. No STN DBS (*n*=7), Acute STN DBS (*n*=6), and Chronic STN DBS (*n*=6). Data are represented as mean ± SEM, One-way ANOVA.

Appendix K

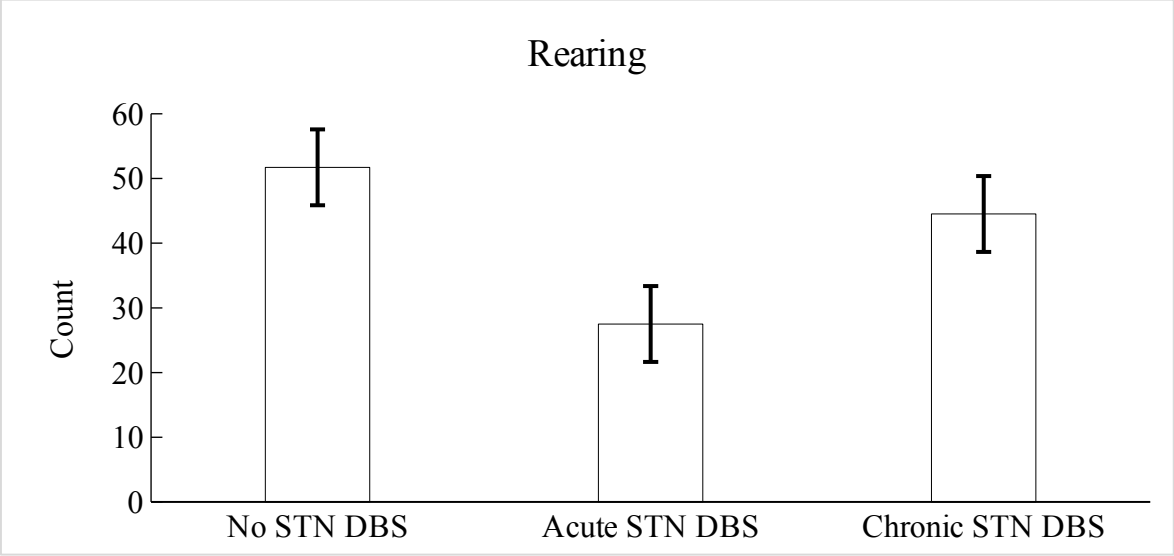
Grooming in the Total Ten Minutes of the Open Field Test



Note. Average time in seconds spent grooming in the open field arena. No STN DBS (*n*=7), Acute STN DBS (*n*=6), and Chronic STN DBS (*n*=6). Data are represented as mean ± SEM, one-way ANOVA.

Appendix L

Rearing in the Total Ten Minutes of the Open Field Test



Note. Average number of rearing instances in the open field arena. No STN DBS (n=7), Acute STN DBS (n=6), and Chronic STN DBS (n=6). Data are represented as mean ± SEM, one-way ANOVA.

Appendix M

Total Distance Traveled in the First Five Minutes of the Open Field Test

A)

B)

Note. Average total distance traveled in centimeters (A) and average velocity in centimeters per second (B) in the open field arena. No STN DBS (*n*=7), Acute STN DBS (*n*=6), and Chronic STN DBS (*n*=6). Data are represented as mean ± SEM, one-way ANOVA.