Non-Invasive Focused Ultrasound Treatment to Improve Depressive-Like Behavior in a Hemi-Parkinsonian Rat Model

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“Non-Invasive Focused Ultrasound Treatment to Improve Depressive-Like Behavior in a Hemi-Parkinsonian Rat Model”

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Abstract

Parkinson’s disease (PD) is a neurodegenerative disorder in which there is a depletion of dopaminergic neurons in the substantia nigra pars compacta (SNc). Symptoms include those that are motor, such as tremors and rigidity, along with symptoms that are nonmotor, such as depression. Depression in Parkinson’s disease is seen in up to 50% of PD patients, and is often treated with traditional antidepressants, with varying levels of efficacy. In this research project, focused ultrasound was used as a non-pharmacological and non-invasive treatment to improve depressive-like behavior in a hemi-Parkinsonian rat model. To induce a hemi-Parkinsonian phenotype in rats, stereotaxic surgeries were performed. The neurotoxin 6-hydroxydopamine was infused in the right medial forebrain bundle, which contains neurons extending from the SNc to the striatum. This resulted in a PD phenotype on the left side, contralateral to the lesion. The limb-use asymmetry test was then used to confirm this phenotype. Focused ultrasound treatments were given to target the celiac plexus, the downstream arm to the vagus nerve. The forced swim test was then used to see if depressive, despair-like behavior improved following focused ultrasound treatment. Brains were then collected via trans-cardiac perfusions for post-mortem tissue analysis with tyrosine hydroxylase staining. Focused ultrasound treatment was not found to alter the contralateral akinesia-like immobility seen in PD rats. Weight decreased for all groups, regardless of focused ultrasound treatment. Based on current data, the efficacy of focused ultrasound as an antidepressant treatment for despair-like behavior in hemi-Parkinsonian rats remains to be seen. Further research with alternative experimental methods is suggested to truly see if focused ultrasound can improve despair-like behavior in hemi-Parkinsonian rats.

Keywords: Parkinson’s disease, depression, despair, peripheral non-invasive focused ultrasound, forced swim test
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Disclaimer

Portions of this thesis were modified from a manuscript that was recently accepted for publication in Experimental Brain Research (Herlihy et al.).
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1. Introduction

1.1. Parkinson’s disease

Parkinson’s disease is a neurodegenerative disorder that affects up to one million people in the United States and ten million people globally (Parkinson’s Foundation, n.d.-b). Most people with PD are diagnosed in their 60s; in some rare cases, people are diagnosed before age 50. It is more common in men than women (Parkinson’s Foundation, n.d.-b).

1.1.1. Symptoms and diagnosis

PD is recognized as a whole-body disorder (Waller et al., 2021), characterized by both motor and nonmotor symptoms. Motor symptoms include tremors, bradykinesia (slowness of movement), rigidity, and issues with balance. While a typical presentation of PD is often recognized by motor symptoms, PD patients find their nonmotor symptoms to have a more pronounced negative impact on their perceived quality of life (Santos-Garcia & Fuente-Fernandez, 2013). Nonmotor symptoms include depression, anxiety, apathy, constipation, sleep disorders, and cognitive impairment (National Institute of Neurological Disorders and Stroke, n.d.). Nonmotor symptoms typically develop before motor symptoms manifest. The presentation of these nonmotor symptoms could lead to an early detection of PD but could also lead to misdiagnosis in some cases due to the lack of motor symptoms at this early stage. As PD progresses, the prevalence of psychotic symptoms and visual hallucinations increases (Waller et al., 2021).

PD is typically diagnosed by examining symptoms, patient history, and a thorough physical exam (Parkinson’s Foundation, n.d.-a). Typically, motor symptoms are examined for a diagnosis, but non-motor symptoms may also aid in diagnosis. A thorough patient history is important when diagnosing PD, as some dopamine-blocking medications may induce parkinsonism, such as antipsychotic medications (Waller et al., 2021). A thorough physical exam helps to rule out other
disorders. For example, the presence of a tremor may not necessarily indicate PD. An essential or dystonic tremor could be present, where a tremor only occurs during movement. In PD, if a tremor does happen to be present, it must be a resting tremor (Waller et al., 2021).

1.1.2. Treatment

Treatment of PD varies, but generally targets dopaminergic systems. Pharmacologically, levodopa, dopamine agonists, and monoamine oxidase type B (MAO-B) inhibitors are often used early in treatment of motor symptoms (Armstrong and Okun, 2020). While they are relatively successful in decreasing the severity of motor symptoms, there are some side effects. Levodopa is a direct precursor to dopamine and can cross the blood-brain barrier, while dopamine itself cannot cross. Levodopa is then converted to dopamine once it reaches the brain (Camargo et al., 2014). Levodopa can result in side effects such as motor complications and dyskinesia (Porras et al., 2014), and can also become less effective over time from the desensitization of dopamine receptors and dopaminergic neuronal death (Keun et al., 2021). Thus, individuals with PD who use levodopa often must take higher, more frequent doses over time for the same effects. While it does have some side effects and may become relatively ineffective over time, levodopa is still considered the gold standard of PD therapies (Haddad et al., 2017).

Dopamine agonists, such as the non-ergoline agonists ropinirole and pramipexole, are effective compared to placebo, but less efficacious compared to levodopa in treating PD motor symptoms (Binde et al., 2020). Dopamine agonists mimic dopamine and act on dopaminergic receptors (Quinn, 1995). Dopamine agonists can be used in conjunction with levodopa. While dopamine agonists are relatively effective for treating PD motor symptoms, there are numerous side effects, such as nausea, vomiting, hallucinations, and confusion (Brooks, 2000). Use of
dopamine agonists could also result in impulse control disorders, such as gambling (Garcia-Ruiz et al., 2014).

Monoamine oxidase type B (MAO-B) inhibitors, such as selegiline and safinamide, inhibit monoamine oxidase B activity in the central nervous system, block dopamine catabolism, and ultimately enhance dopamine levels at the synapse (Chen & Swope, 2005). Like dopamine agonists, MAO-B inhibitors can be used alongside treatment with levodopa (Tan et al., 2022). Use of MAO-B inhibitors could lead to side effects such as nausea, constipation, confusion, and hallucinations (Parkinson’s Foundation, n.d.-c).

1.1.3. Mechanism

PD is caused by the degeneration of dopaminergic neurons in the nigrostriatal pathway, projecting from the substantia nigra pars compacta (SNc) to the dorsal striatum. The nigrostriatal dopamine system is involved with motor function and learning (Klein et al., 2019). This loss of dopaminergic function in the nigrostriatal pathway can influence other pathways involved with movement. The depletion of dopamine in the striatum in individuals with PD is found to increase activity in the circuits between the internal globus pallidal segment (GPi) of the ventral striatum and the pars reticulata portion of the substantia nigra (SNpr), which results in GABA dysfunction, inhibiting the thalamus and its ability to activate the frontal cortex, ultimately resulting in decreased motor activity in PD (DeMaagd & Philip, 2015). In contrast to the research for mechanisms leading to impaired motor function in PD, the neural mechanisms responsible for the non-motor symptoms in PD are less well known and researched.
1.2. Depression in Parkinson’s disease

Up to 40-50% of patients with PD experience depression (Reijnders et al., 2008). Depression in Parkinson’s disease (DPD) can be further subdivided into three types: major depressive disorder (MDD), minor depression, and dysthymia. MDD is diagnosed by the presence of at least five the following symptoms during the same 2-week period: depressed mood, loss of pleasure/interest, significant weight gain or weight loss, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue, feelings of worthlessness or guilt, decreased concentration, and thoughts of death/suicide (American Psychiatric Association, 2013).

Minor depression follows similar diagnostic criteria needed for diagnosis of major depressive disorder, but with only 2-4 symptoms for the minimum two-week period. A depressed mood and/or a loss of pleasure/interest must be present for a MDD or minor depression diagnosis (American Psychiatric Association, 1994).

Dysthymia is characterized by a depressed mood for most of the time for at least two years, and the presence of at least two of the following symptoms: significant weight loss or weight gain, insomnia, fatigue or loss of energy, feelings of hopelessness, psychomotor retardation, and low self-esteem (American Psychiatric Association, 1994).

While these three subsets of depression are also present in the general population, DPD contrasts slightly from depression in the general population, as thoughts of self-blame, suicide, and a negative self-attitude are less common in DPD (Laux, 2022). In addition, symptoms of DPD and PD often overlap, making the condition difficult to diagnose. Such symptoms include, but are not limited to, fatigue, insomnia, difficulty in concentration, and loss of energy (Laux, 2022).
1.2.1. Pharmacological treatments

Treatments for DPD include antidepressants such as dopamine agonists, selective serotonin reuptake inhibitors (SSRI), serotonin and norepinephrine reuptake inhibitors (SNRI), tricyclics, and monoamine oxidase inhibitors (MAOI). Dopamine agonists display mixed results when used to treat DPD (Laux, 2022). They may have an antidepressant effect but can lead to side effects such as confusion or hallucinations (Raskin & Durst, 2010). Furthermore, the limited research on dopamine agonists for treating DPD is mostly inconclusive (Leentjens, 2011). In comparison, the other antidepressants listed appear effective compared to placebo (Laux, 2022). However, side effects are still possible with these treatments, and some DPD patients do not see an improvement in depressive symptoms while on these medications. Some DPD patients have even experienced a worsening of their PD motor symptoms following traditional antidepressant treatment (Richard & Kurlan, 1997).

1.2.2. Nonpharmacological treatments

For cases of treatment resistant depression, nonpharmacological methods have been used, such as electroconvulsive therapy (Douyon et al., 1989) and vagus nerve stimulation (Breit et al., 2018). Electroconvulsive therapy (ECT) has also been used to treat PD and DPD, with motor symptoms and depression improving upon treatment (Borisovskaya et al., 2016), but with little research about the duration of its antidepressant effects. On the other hand, vagus nerve stimulation (VNS) has been approved for use in those with treatment resistant depression in the general population (Breit et al., 2018). With VNS, patients undergo a surgery in which an implant is placed to electrically activate the vagus nerve. VNS has been speculated to activate afferent neurons in the vagus nerve, sending information upstream to the brain. It could then activate the nucleus tractus solitarius (NTS) and affect various regions of the brain involved with depression, including
the limbic system (Andresen & Kunze, 1994). There is limited research of its effects in those with DPD.

1.3. Focused ultrasound as a novel therapeutic

Our project uses focused ultrasound (FUS) as a nonpharmacological and non-invasive treatment for DPD. We hypothesize that targeting FUS at the celiac plexus, the downstream arm to the vagus nerve, can improve depressive-like behavior in PD rats. Applying FUS to the celiac plexus has been found to indirectly innervate the vagus nerve in rats (Akhtar et al., 2021). Peripheral FUS seems to be a better alternative compared to VNS for treating DPD due to its non-invasive approach. Using FUS aimed at the celiac plexus to stimulate the vagus nerve is potentially a promising treatment for depression; if found to be effective in rats, it could potentially be used as a treatment for DPD in the clinical setting.

2. Materials and methods

2.1. Animals

Male adult Sprague Dawley rats (Taconic Farms, Rensselaer, NY) with weights between 300 g and 400 g were used. They were individually housed with a 12-hour light/dark cycle with lights on at 7 AM, temperature set at 72 degrees Fahrenheit, and humidity set at 30-70%. Rats had access to food and water ad libitum. All experiments followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Albany Medical College Institutional Animal Care and Use Committee.
2.2. Chemicals

All chemical substances were acquired from Sigma-Aldrich (St. Louis, MO), unless specified otherwise.

2.3. Experimental design

![Experimental timeline of surgery and behavioral tests.](image)

Fig. 1: Experimental timeline of surgery and behavioral tests.

Rats were randomly assigned into four groups: sham, sham+FUS, PD, and PD+FUS. Rats underwent stereotaxic surgeries and were then evaluated using the limb-use asymmetry test (LAT). They were then given focused ultrasound (FUS) treatment or the control treatment for five consecutive days, two times each day for 3 minutes each, and 6 hours apart. Rats from every group underwent another LAT before the forced swim test (FST). After all behavioral testing was completed, rats were euthanized and trans-cardiac perfusions were performed. Brains were then extracted and sliced for post-mortem tyrosine hydroxylase (TH) analysis.
2.4. Stereotaxic surgery

6-Hydroxydopamine (6-OHDA) was used to lesion the right medial forebrain bundle (MFB), creating a hemi-Parkinsonian (PD) phenotype. Rats were first anesthetized with isoflurane (Harvard Apparatus inhalant system, Holliston, MA), weighed, and given 1.0 ml of desipramine and pargyline solution via intraperitoneal (IP) injection. They were then moved to a stereotaxic frame (David Kopf Instruments, Tujunga, CA) and given continuous isoflurane through a nose cone to ensure anesthetization throughout the surgical procedure. A heating pad was placed under the rats’ bodies to prevent drops in body heat throughout the procedure. A thermometer covered in mineral oil was placed in the rectum to measure body heat (Homeothermic Monitor, Harvard Apparatus, Holliston, MA). Next, metal ear bars covered in lidocaine were placed in the rats’ ears to keep skulls in place during the procedure. Both eyes were then covered with eye lubricant (Refresh P.M., Allergan, Madison, NJ).

Once secured in the stereotactic frame, the fur atop the rats’ heads was shaved. The shaved area was then cleaned with betadine and ethanol in a circular motion moving outward, three times
each. 0.1 ml of 0.5% bupivacaine (Hospira, Lake Forest, IL) was injected subcutaneously at the incision site. To ensure that the bupivacaine was properly absorbed, a minimum waiting time of five minutes was necessary before moving to the next step. After five minutes, an incision was made on the scalp using a scalpel. A burr hole was then made (from bregma: 4.4 mm posterior and 1.5 mm lateral), and 4.5 µl of 6-OHDA neurotoxin were infused in the right MFB (from dura: 7.5 mm ventral) using a 10 µl injection syringe (Hamilton Company, Reno, NV) (Fig. 2). The neurotoxin was delivered for 9 minutes total (0.5 µl per minute). Upon completion of the infusion, the syringe was left in place for five minutes to ensure that the 6-OHDA did not reflux along the incision tract. Following removal of the syringe, wound clips were used to close the incision and a triple antibiotic ointment (Cardinal Health, Dublin, OH) was applied atop the incision site to prevent infection. For post-operative pain management, rats were given a subcutaneous injection of 1 ml of buprenorphine immediately after surgery and a 2 mg carprofen tablet (Bio-Serv, Flemington, NJ) each day for two consecutive days.

Sham rats were given 0.9% NaCl instead of 6-OHDA for lesioning the right MFB. All other conditions during surgery were kept constant between the two groups.

2.5. Focused ultrasound treatment
Fig. 3: The FUS transducer probe was placed between the lower left rib cage and xiphoid process to target the celiac plexus. Adapted from Herlihy et al.

FUS treatment was administered to PD and sham rats. Rats were anesthetized with isoflurane and had their abdomens shaved prior to treatment. The transducer probe was placed between the lower left rib cage and xiphoid process to target the celiac plexus, with ultrasound gel previously applied atop the targeted area on the skin (Fig. 3). FUS was given with bursts repeated at 5 Hz, a carrier frequency of 2.5 MHz, and a peak-to-peak output amplitude of 300 mV. Treatments were given twice each day, for three minutes each, six hours apart, over five consecutive days. Rats not given FUS underwent a similar procedure. Rats were anesthetized with isoflurane, their abdominal areas were shaved, and ultrasound gel was placed on the abdomen. The probe was positioned between the lower left rib cage and xiphoid process to target the celiac plexus, but the power amplifier (ENI 350L, Electronic Navigation Industries, Rochester, NY) was turned off. No FUS was delivered through the probe for sham rats.

2.6. Behavioral tests

![Image A](image1.png) ![Image B](image2.png)
**Fig. 4:** Schemata of behavioral tests. **A,** The limb-use asymmetry test (LAT). Rats were individually placed in a plexiglass cylinder to observe for asymmetrical forepaw use following the 6-OHDA or sham lesion. **B,** The forced swim test (FST). Rats were individually placed in a plexiglass cylinder filled with water and observed for climbing, swimming, and immobile behavior. Images were constructed with BioRender (Toronto, Ontario, Canada). Adapted from Herlihy et al.

### 2.6.1. Limb-use asymmetry test

The LAT is a behavioral test used to confirm locomotor asymmetry in various movement disorders. Here, it was used to confirm on-target lesioning and the hemi-Parkinsonian phenotype following stereotaxic surgery. A rat was placed in a transparent plexiglass cylinder (Fig. 4A) and recorded to observe the number of times its forepaws tapped the cylinder, with a minimum of 20 total taps. If the 20-tap minimum was met in five minutes, the test was completed; if not, the test was run for five more minutes. Rats that tapped with their right forepaw for at least 80% of the total taps were designated as hemi-Parkinsonian due to the contralateral akinesia-like immobility seen. If a rat underwent 6-OHDA lesioning, but did not display meet the 80% minimum, the lesion was likely off-target. Second surgeries and limb-use asymmetry tests were then performed to ensure on-target lesioning before proceeding with further behavioral tests and focused ultrasound treatment.

LATs were conducted at two different time points. The first was one week after stereotaxic surgery and the second was immediately before the forced swim test.

### 2.6.2. Forced swim test

The FST was used to measure behavioral despair, which is one subset of depressive-like behavior seen in rats (Yankelevitch-Yahav et al., 2015). This test was divided into two sessions, 24 hours apart: the training phase and the test phase. During both phases, rats were individually placed in a transparent plexiglass cylinder filled with water at a height of 30 centimeters and a temperature of 25 ± 1 °C. If there was more than one rat undergoing the FST on the same day, the
cylinder was cleaned between rats, and the water was replaced. The training phase and the test phase had the same exact conditions, with the only difference being the length of time spent in water. For the training phase, rats were kept in water for 15 minutes, whereas in the test phase, rats were kept in water for 5 minutes (Fig. 4B). The five-minute test phase was recorded and analyzed to determine the rat’s behavior for each second spent in the cylinder. After both sessions, rats were taken out of the water and placed under a heating lamp to help re-adjust after swimming.

A rat’s behavior in the cylinder could be described as either immobility (lack of movement of the entire body, except for small movements responsible for keeping the head above water), swimming (large forepaw movements in the water, more than necessary to keep the head above the water), or climbing (vigorous forepaw movement towards the walls of the cylinder, both in and out of the water) (Santiago et al., 2010). A longer time spent immobile in the FST was associated with despair-like behavior.

2.7. Post-mortem analysis

2.7.1. Trans-cardiac perfusion

After all behavioral testing was completed, trans-cardiac perfusions were used to extract brains for post-mortem analysis. First, rats were anesthetized with isoflurane gas and then euthanized with a urethane IP injection. Following injection, the loss of response to reflex stimulation was confirmed with a toe-pinches. If the toes on a rat’s hindlimbs were pinched and there was no reaction seen, the rat was thought to be fully anesthetized, and the procedure could then proceed.

A bilateral thoracotomy was performed. A small incision was then made in the right atrium of the heart to allow blood to flow out. 60 milliliters of heparin (a blood thinner) and 60 milliliters
of paraformaldehyde (a fixative) were injected in the left ventricle of the heart, followed by decapitation and brain extraction. Brains were stored in paraformaldehyde for two days. They were then transferred to 30% sucrose solution for cryoprotection. Frontal sections of the striatum, SNC, and ventral tegmental area (VTA) were sliced using a cryostat (Leica Biosystems, Danvers, Massachusetts).

2.7.2. Tyrosine hydroxylase immunohistochemistry

Fig. 5: Frontal sections of the striatum following TH staining from a sham rat (A) and a PD rat (B). A, In sham rats, bilateral staining was seen. B, In PD rats, unilateral staining was seen in the left (unlesioned) striatum. The right (lesioned) striatum was not stained.

TH immunohistochemical staining was used to quantify dopaminergic depletion in the SNC, VTA, and striatum. Slices were first stained using anti-TH primary antibody (rabbit IgG CAT# NB300-109, Novus Biologicals, Centennial, CO), followed by peroxidase conjugated goat anti-rabbit IgG secondary antibody (CAT# 45-A0545, Sigma-Aldrich, St. Louis, MO). 3,3-diaminobenzidine (DAB) solution (Vector Labs, Newark, CA) was used to visualize the staining. Slices were then mounted on positively charged slides (Diamond Frosted, Globe Scientific, Mahwah, NJ) and scanned (PathScan Enabler IV, Electron Microscopy Sciences, Hatfield, PA) (Fig. 5).
2.7.3. Tyrosine hydroxylase analysis

Slices of the SNc, VTA, and striatum were analyzed for TH pixelation density using ImageJ software. A small circle (width of 300 pixels and a height of 300 pixels) was used as the region of interest for the striatum, on the left and right side. For SNc, the region of interest was the entire SNc. For the VTA, the region of interest was a small circle (width of 80 pixels and a height of 80 pixels) within the VTA. Each region of interest for the three brain areas was analyzed for raw integrated density, measured in arbitrary units (AUs). Analyzers were blinded to experimental groups.

2.8. Statistical analysis

GraphPad Prism software (Ver 10.0.2, GraphPad Software, Boston, MA) was used for all statistical analyses, and all data were represented as mean ± standard error of the mean (SEM). Two-way ANOVA was used for all statistical analyses, with repeated measures as needed, followed by the Bonferroni post hoc test.

3. Results

3.1. FUS did not affect behavior in the LAT
**Fig. 6:** LATs were performed twice on PD and sham rats ± FUS. Data represent the mean ± SEM. Two-way ANOVA with repeated measures followed by the Bonferroni post hoc test. (ns) for treatment effect. (***) denotes p ≤ 0.001 comparing PD to sham and (****) denotes p ≤ 0.001 comparing PD+FUS to sham+FUS. N=10-11/group. Adapted from Herlihy et al.

PD rats displayed akinesia-like immobility in the contralateral forepaw to the 6-OHDA lesion (≥80% right taps/total), regardless of FUS treatment (Fig. 6). PD ± FUS rats scored above 80% for the right forepaw touches over the total taps both before and after treatment. Sham ± FUS LAT scores did not pass the 80% threshold, regardless of treatment (Fig. 6).

### 3.2. Effects of FUS on behavior during the FST

![Graphs A, B, C](image)

**Fig. 7:** Time spent immobile (A), swimming (B), and climbing (C) during the FST for sham and PD rats ± FUS. Less time spent immobile indicates improved despair-like behavior. Data represent the mean ± SEM. Two-way ANOVA followed by the Bonferroni post hoc test. (*) represents p ≤ 0.05. Otherwise (ns) p > 0.05 for group & treatment effects. n=10-11/group. Adapted from Herlihy et al.

Sham and PD rats ± FUS underwent the FST. As seen in Figure 7, time spent immobile was similar between all groups. Time spent swimming was also similar between all groups, with no significant group or treatment effects. PD rats given FUS treatment spent significantly less time (p ≤ 0.05; Fig. 7C) climbing compared to sham rats given control treatment. Otherwise, there were no group or treatment effects.
3.3. FUS did not affect weight

The weight of PD and sham animals decreased during the five consecutive days of ± FUS treatment. PD+FUS rats weighed 381.6 ± 11.4 g before treatment (day 20) and 367.3 ± 10.5 g after treatment (day 24) \((p \leq 0.001; \text{Fig 8})\). Similarly, PD rats weighed 373.5 ± 17.3 g before treatment and 357.7 ± 15.7 g after treatment \((p \leq 0.001; \text{Fig. 8})\). Sham+FUS rats weighed 419.9 ± 13.0 g before treatment and 405.1 ± 12.3 g after treatment \((p \leq 0.001; \text{Fig. 8})\). Lastly, sham rats weighed 427.3 ± 9.3 g before treatment and 410.2 ± 9.4 g after treatment \((p \leq 0.001; \text{Fig. 8})\).

3.4. FUS did not affect TH expression
Fig. 9: TH staining confirms dopaminergic degeneration in the right striatum (A), VTA (B), and SNc (C) of PD rats compared to sham rats. Data represent the mean ± SEM. Two-way ANOVA followed by the Bonferroni post hoc test. (ns) for treatment effect. (**) denotes p ≤ 0.01 and (****) denotes p ≤ 0.0001 for 6-OHDA lesion effect. n=6-11/group.

PD rats had significantly less TH expression in the right (lesioned) striatum (p ≤ 0.0001; Fig. 9A), VTA (p ≤ 0.01, Fig 9B), and SNc (p ≤ 0.01; Fig. 9C) compared to sham rats, regardless of FUS treatment. The greatest numerical difference in TH expression between sham rats and PD rats was seen in the striatum (p ≤ 0.0001; Fig. 9A). As expected, for the three brain regions, two-way ANOVA revealed that there were significant effects of the 6-OHDA lesion, but not FUS treatment.

4. Discussion

FUS treatment had no observable effects on the contralateral akinesia-like immobility seen in PD rats. If there did happen to be a difference in LAT scores between PD rats before and after FUS treatment, starting with ≥80% right taps over the total before treatment to less than 80% after treatment, it would posit that FUS targeted at the celiac plexus reversed the dopaminergic depletion or regenerated the dopaminergic neurons in the lesioned SNc, VTA, and striatum. However, this was not the case and FUS delivered in our timeframe of five consecutive days did not improve motor symptoms in PD rats. Our aim was to instead see if FUS improved depressive-like behavior (specifically behavioral despair), a nonmotor symptom of PD, in PD rats.

In the FST, an improvement in despair-like behavior was denoted by a decrease in time spent immobile. In previous studies, non-PD rats given antidepressants were found to have significantly less time spent immobile, as well as an increase in swimming and/or climbing time (Detke et al., 1995). Our data from the FST shows that PD and sham rats ± FUS were immobile for roughly the same amount of time, and that no differences were seen in the time spent immobile.
between PD-FUS rats and PD+FUS rats. Thus, the antidepressant effect of FUS was not seen in PD rats in the FST.

In the FST, swimming and climbing behavior could possibly be connected to different monoaminergic systems in the brain. Detke et al. (1995) found that rats given SSRIs showed an increase in swimming behavior, while rats given SNRIs displayed an increase in climbing behavior, suggesting that swimming behavior could potentially be stimulated by the serotonergic system, whereas climbing behavior could be controlled by the noradrenergic system (Detke et al., 1995). Excluding how PD rats given FUS treatment spent significantly less time climbing compared to sham rats given control treatment, a possible explanation for the lack of significance for group and treatment effects in the FST could be that FUS possibly did not affect noradrenergic or serotonergic systems involved in despair-like behavior.

While FUS to the celiac plexus has not yet been found to improve despair-like behavior in PD rats in the FST following treatment, a previous part of this study found that a different subset of depressive-like behavior measured in rats did improve. This part of the study utilized a different behavioral test called the sucrose-preference test (SPT), which measures anhedonia (loss of pleasure) (data not shown; Herlihy et al.). The improvement in anhedonic-like behavior following FUS treatment, but not despair-like behavior, could potentially imply that FUS targeted at the celiac plexus modulated brain regions involved with pleasure, but not those involved with behavioral despair.

To further investigate if there are effects of FUS on despair-like behavior in PD rats, different models of PD could be used, or a different experimental timeline. Instead of using a unilateral PD model with lesioning in the right MFB, bilateral lesioning of the MFB could be
utilized. Interestingly, many studies that used the FST to measure behavioral despair in PD rats used a bilateral lesioning model as opposed to a unilateral lesioning model (Mou et al., 2022).

As a future consideration, we can also consider using a slightly different experimental timeline, where a behavioral test is used to measure depressive-like behavior before treatment in addition to the FST after treatment. We did not use a behavioral test to measure despair-like behavior before FUS because of the stress involved with the FST—undergoing the FST more than once for the acclimation and test phase could be considered too stressful for the rats and unethical. One potential avenue for future research could be to use a less stressful behavioral test, such as the SPT, to observe which rats display depressive-like behavior (anhedonia) prior to treatment, and if this behavior changes in the FST, conducted after ± FUS treatment. However, the FST and the SPT do not measure the same phenotypes of depressive-like behavior seen in rats, so it may not be ideal to use the SPT prior to treatment.

If further research reveals that despair-like behavior in PD rats does not improve following FUS, but anhedonic-like behavior still does, the results of the overarching study are still promising, as peripheral FUS aimed at the celiac plexus could potentially be used as a treatment for individuals with DPD who experience anhedonia, but not despair.

There are some important considerations to examine. The first is that only male rats were used in this project. This was because PD in humans is about two times more common in males compared to females (Baldereschi et al., 2000), possibly because of estradiol’s neuroprotective effects on dopaminergic neurons (Siani et al., 2017). Repeating these experiments with both male and female rats could possibly be a future area of study. Another consideration is the use of isoflurane anesthesia during treatment; isoflurane administration during treatment is likely an explanation for the decrease in weights following the five days of treatment for all groups,
regardless of ± FUS. This is supported by previous studies, such as one by Baden and colleagues, in which mice given isoflurane for 4 hours per day over 5 days weighed less than mice who were only exposed to air (Baden et al., 1988).

5. Conclusions

This study is the first of its kind to consider FUS as a therapeutic for DPD. It revealed that FUS does not impact the contralateral akinesia-like immobility seen in PD rats, ± FUS treatment resulted in a decrease in weight for all groups, and that FUS treatment does not influence TH expression. While we have not yet found an antidepressant effect of FUS in PD rats in this project, specifically when measuring despair-like behavior, further research with alternative experimental methods may reveal if FUS can improve despair in PD.
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