Neuroprotective Role of Focused Ultrasound of the Celiac Plexus in a Rat Model of Parkinson's Disease

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Many treatments for Parkinson ' s Disease (PD) are focused on managing and helping symptoms, however there is a need to further understand neuron degeneration. Additionally, PD therapies tend to be very surgically invasive, making it difficult for them to be widespread. Focused ultrasound (FUS) is a completely non-invasive technique that has the potential to cause neuroprotection. Male Sprague Dawley rats underwent a bilateral lesion of 6-hydroxydopamine (6-OHDA) PD model into the striatum. Immediately following, FUS therapy of the celiac plexus was applied for a total of twelve days. The amount of dopamine neuron (DA) degeneration in the substantia nigra pars compacta (SNc) after FUS was observed. Limb-use asymmetry tests (LAT) were used to determine if motor function improved with FUS therapy as well. Ultimately, our research goal was to show that FUS can cause neuroprotection within the brain and slow dopamine neuron degeneration. With this information, FUS has the potential to be used as a more widespread therapeutic for PD patients.

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All research involving non-human vertebrates or human subjects was conducted under the supervision of an experienced teacher or researcher and followed state and federal regulatory guidance applicable to the humane and ethical conduct of such research.

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1. Introduction:

Parkinson's Disease (PD) is a neurodegenerative disorder, caused by the death of dopamine (DA) neurons in the substantia nigra pars compacta (SNc) [1]. PD is commonly characterized by motor symptoms such as tremors, muscle stiffness, bradykinesia, and gait difficulty, along with a variety of other non-motor symptoms [2,3]. Due to increased longevity and an aging population, PD has steadily grown to be the second most common neurological disorder with numbers constantly rising [3,4].

Detection and diagnosis of PD is primarily based on the presence of motor symptoms, however, most times the disease has significantly progressed past this point. It has been estimated that there is almost a 60-80 percent loss of DA neurons when a person begins to have visible and obvious motor symptoms, such as tremors [5]. Many current treatments are focused on managing PD symptoms, protecting the remaining neural circuitry, and slowing the progression of PD. Treatments such as deep brain stimulation (DBS) and vagus nerve stimulation (VNS) have both helped PD patients, however they are both surgically invasive and ultimately do not consistently stop further neuron degeneration [6]. This has created a need for less invasive therapies that also are able to stop neuron degeneration earlier than current treatments.

Currently, researchers have been observing the gut-brain axis to better understand neuroinflammation in the early stages of PD. The gut-brain axis is a complex relationship between our brain and gastrointestinal system, allowing information from both to be exchanged through the vagus nerve [7]. PD research has found that stimulation of the vagus nerve or celiac plexus has promising anti-inflammatory effects within both the

brain and gut [8,9]. Focused ultrasound (FUS) has been found to induce neuroprotective effects like VNS and is less invasive, making it a better non-surgical approach to preventing neuron degeneration.

The aim of this study was to research the use of FUS of the celiac plexus as a neuroprotective agent against DA neuron degeneration in the SNc. Former research has observed that VNS has anti-inflammatory effects and can induce neuroprotection against DA neuron degeneration [1,3,6,10,11]. Based on these examples of protection and the hypothesis that early PD pathology can be seen in the gut, we were interested in finding if FUS can provide neuroprotection within the brain. Specifically, this study analyzed DA levels in the SNc and striatum, two weeks after the injection of 6- hydroxydopamine (6-OHDA) PD model in rats that did and did not receive FUS treatment.

2. Materials and Methods:

2.1. Animals and Cohorts

Adult male Sprague Dawley rats, ages 8 to 10 weeks, were used for all experiments of the study. Six rats were used across four cohorts for a total of 20 animals: PD with FUS, PD with mock FUS, sham with FUS, and sham with mock FUS. All Institutional Animal Care and Use Committee (IACUC) guidelines were followed for rat housing throughout the study.

2.2 Surgical Procedures

All surgeries were performed by members of the lab and were done within accordance to rules established by IACUC. Rats were anesthetized with isoflurane using

an inhalant system in a stereotaxic frame. Rats were given an injection intraperitoneally (IP) of desipramine (25 mg/kg) and pargyline (50 mg/kg) prior to craniotomy with body temperature maintained at 37℃ throughout the surgery. Lubrifresh was applied to the eyes to prevent dehydration and lidocaine gel was applied to the ear bars to reduce discomfort. Additionally, bupivacaine was administered subcutaneously (SC) under the shaved scalp to also minimize discomfort [12]. A burr hole was made in the cranium and 3.4 µl of 6-OHDA or a saline solution was injected bilaterally into the left and right striatum [13]. After injection of 6-OHDA or saline, rats had their incisions stapled shut and were given buprenorphine and saline SC, as well as topical bacitracin. All rats were allowed to recover for two days according to protocol established by IACUC and the Animal Resources Facility (ARF).

2.3 Focused Ultrasound

Animals in the FUS cohorts underwent two sessions of FUS therapy of the celiac plexus within four to six hours of each other for four days [6]. Each session of FUS lasted for a total of three minutes. After the four days, rats were given a two day period to recover and then they continued for four more days of FUS therapy as before (Fig. 1). Due to my schedule, I was unable to do both sessions of FUS. I did afternoon sessions and lab members helped with the morning sessions.

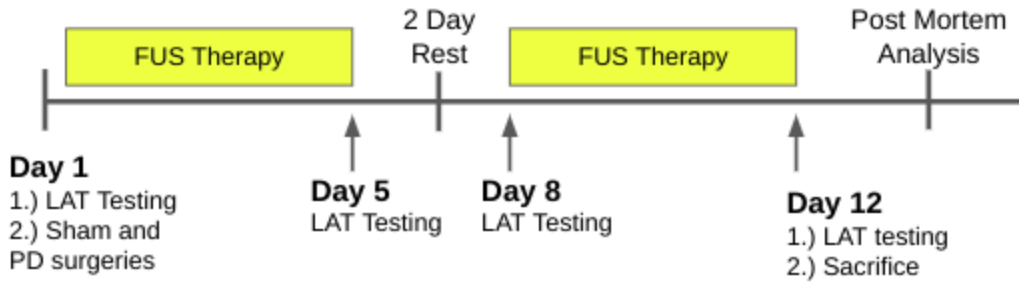

Figure 1. Experimental timeline

2.4 Behavioral Testing

A limb-use asymmetry test (LAT) on days 1, 5, 8, and 12 on all rats. Rats were placed in a transparent cylinder and the number of forepaw taps were recorded and counted for a total of five minutes [10]. LATs on day 1 were done prior to surgery in order to get a baseline score. The LAT scores are compared to the baseline and used to establish if FUS has an effect on motor symptoms within PD and to see how the disease progressed [14]. All LATs were done and analyzed by me. Body weights were also taken everyday of the study [11].

2.5 Immunohistochemistry

After all behavioral tests were concluded, animals were transcardiac perfused by lab members and subsequently their brains were extracted. Extracted brains were sectioned and underwent immunohistochemical staining in the striatum and SNc with tyrosine hydroxylase (TH). Eventually, this will be used to observe the amount of DA neurons that were in the striatum and SNc [15].

2.6 Statistical analysis

TH-immunoreactivity will be assessed in both brain regions using optical density analysis with the ImageJ software. Data collected from the striatum and SNc of all cohorts will be analyzed using GraphPad Prism software systems. ANOVAs, t-tests, and correlation analyses will be utilized to assess the effect of preoperative and postoperative FUS treatment on DA neurons in a rat model of PD. A p-value of <0.05 will be considered statistically significant.

3. Results

3.1 Body Weight

Animals in each treatment group did not show a significant change in their total body weight throughout the overall timeline (Fig. 2). Additionally there was no significance with percent weight change over time (Fig. 3).

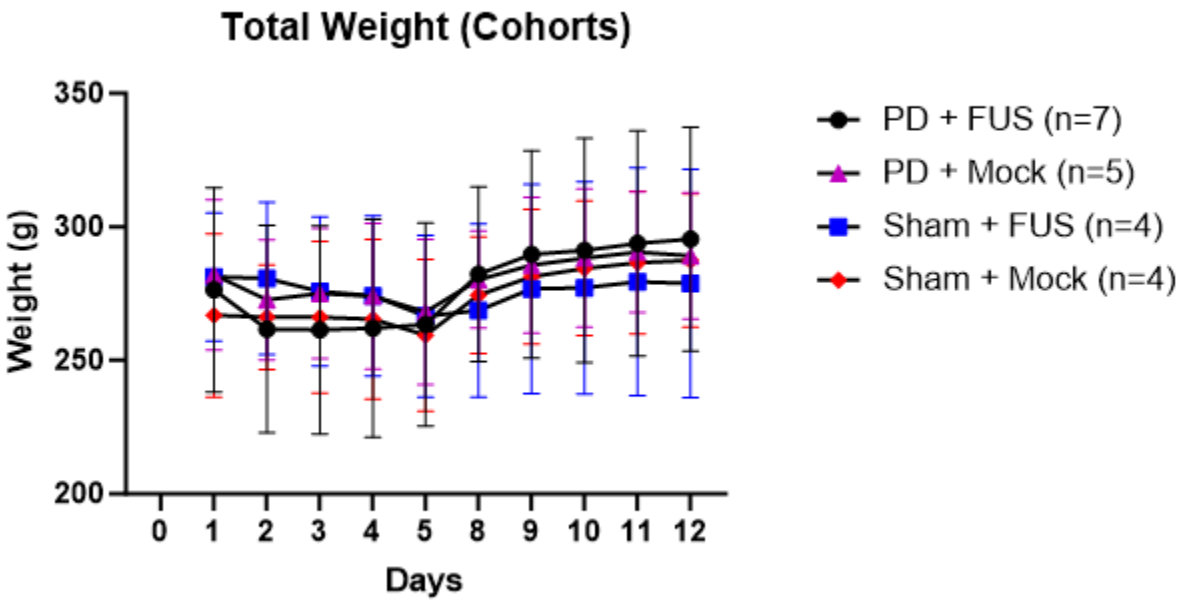

Figure 2. Total Body Weight Results

All animals showed a slight decrease in body weights without a significant difference from baseline weight.

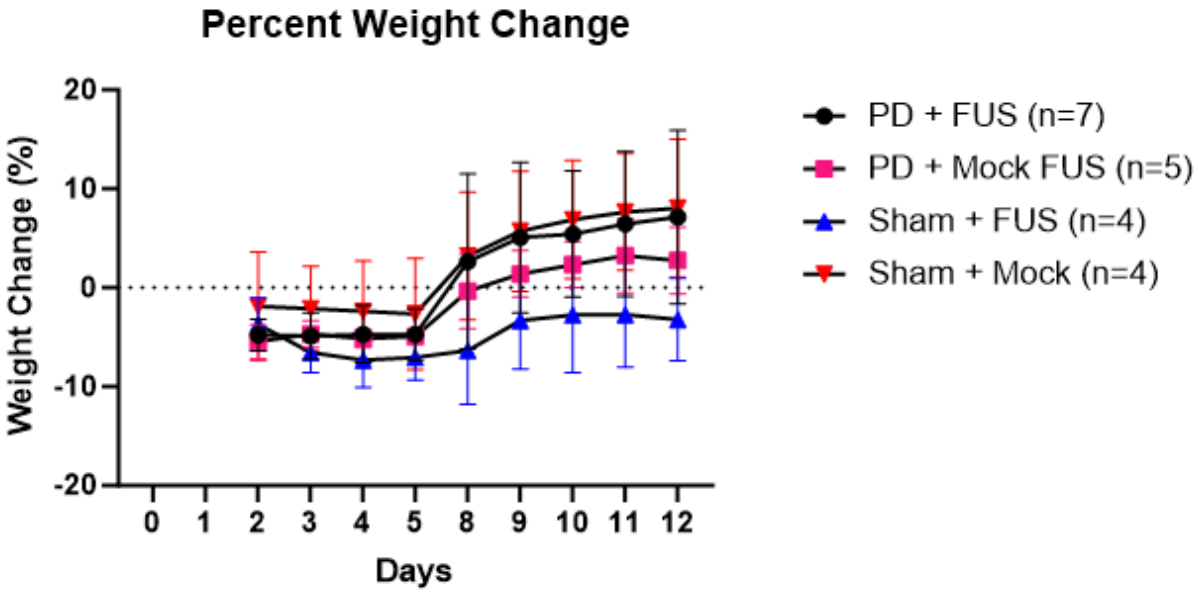

Figure 3. Percent Weight Change Results

During week two, there is a trend toward a significant change between Sham + FUS and all other groups. Additionally, all animals had an increase in percent change, but it was not significant.

3.2 Limb-use Asymmetry Test

Overall, there was no significance in the preliminary data shown in percent of left paw taps from baseline (Fig. 4). There was significance between some treatment groups and time at the point of day 8 and day 12 ($p<0.0055$). Additionally, there was no significance in the total number of left paw taps from baseline (Fig. 5). However, there was significance within the PD+FUS cohort from baseline to day 8 and day 12 ($p<0.0209$).

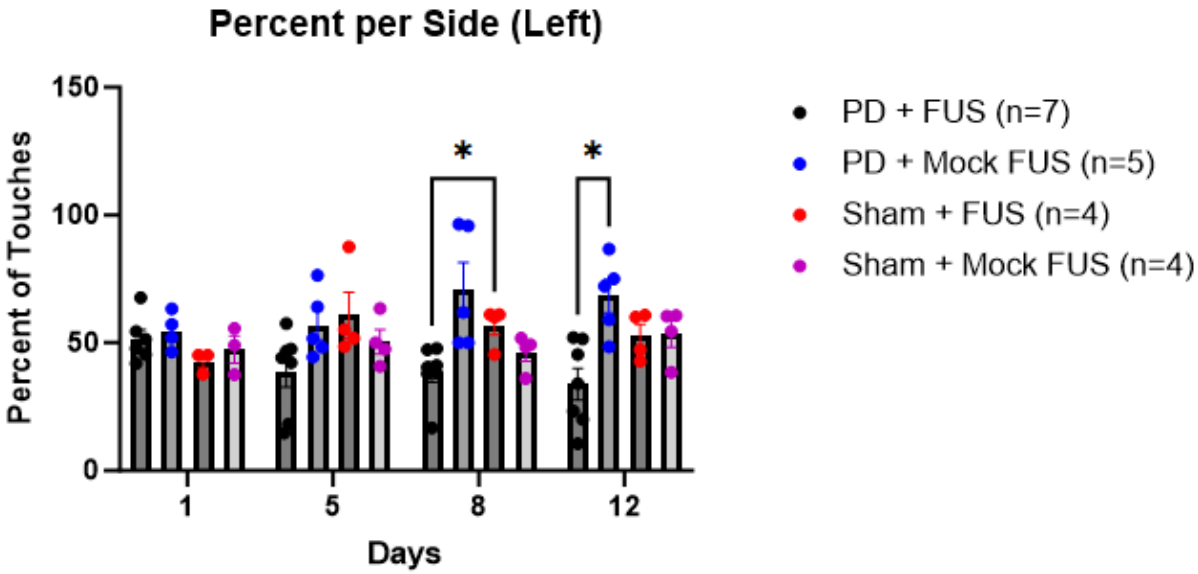

Figure 4. Percent of Left Paw Taps from Baseline Score Results

No significance between treatment groups over time. There was significance within the PD+FUS treatment groups ($p<0.0209$).

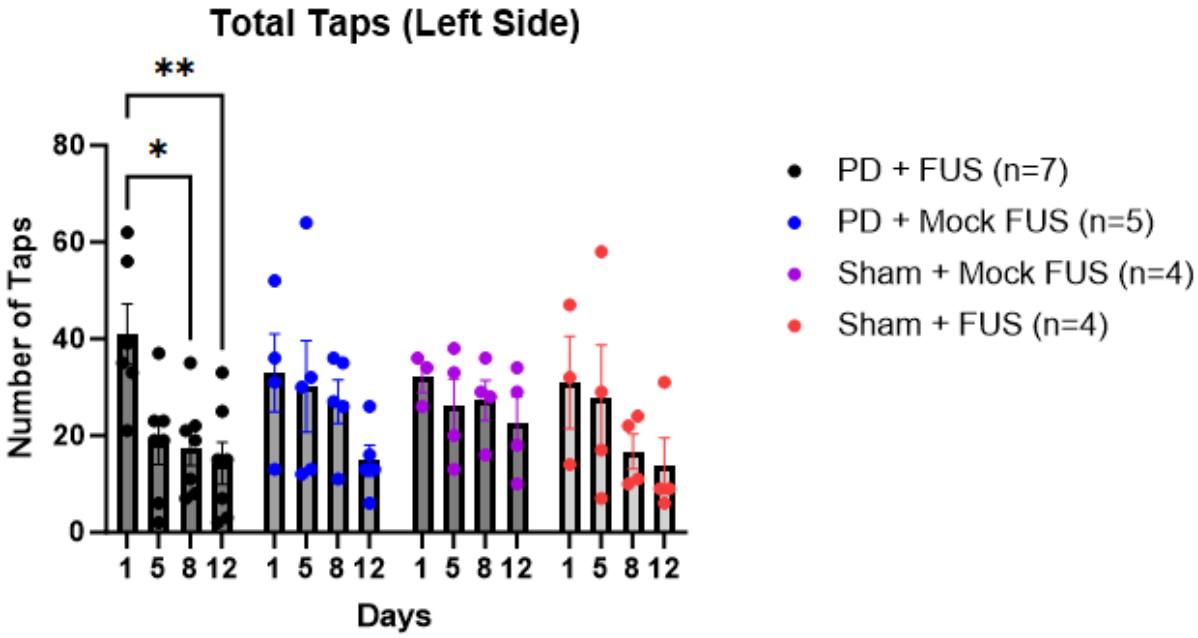

Figure 5. Total Taps (Left Side) Results

No significance in the percent per left side over the course of the study. Significance between cohorts and dates was found ($p<0.0055$).

3.3 TH Staining

FUS treatment should have a higher number of TH-positive neurons in the SNc and the striatum [1, 17, 18]. PD rats without FUS should have a smaller amount of TH positive neurons compared to PD rats with FUS therapy. This will show that FUS demonstrates neuroprotective therapeutics, preventing DA neuron degeneration. Additionally, the neuron loss should appear to be more gradual and not as extreme as it usually is.

4. Discussion

In this study, our goal was to demonstrate the therapeutic effects of FUS on a rat model of early stages of PD. Most of our data is preliminary, however we are able to still notice many trends. Eventually, when we complete our TH staining, we will have a better idea of the specific effects that FUS has on the DA neurons.

All of the results in the total body weight were expected. Similar to other literature, all of the animals in each treatment group decreased in total weight [10,11]. The animals not having significant weight loss helps show that the FUS is not causing excessive reduction in fat and weight from the ultrasound. This data is important to note, because for potential human patients, the FUS therapies should not cause any issues with increased fat and weight loss that are harmful. Although none of the LAT scores were significant across the 12 day timeline, this is still important. The lack of significance helps to show that there was no extreme change in the progression of the PD.

Additionally, it shows that there were not any present motor symptoms. The significance that we did see with our LAT tests could mainly be attributed to the animals being more familiar with their environment and not feeling like they needed to explore it.

With the addition of the TH staining, we will be able to both visualize and quantify data from the actual tissue. The data from this will allow us to analyze how effective the FUS was with providing neuroprotection. Also, by examining the DA neurons, we will have a better understanding of the possible process that prevents the progression of PD.

5. Conclusion

With the use of FUS, future PD clinical therapies have the potential to be less invasive [19]. FUS also has the potential to slow progression of PD. We have shown the safety of FUS and how it does not excessively cause weight or fat loss. Since FUS is a therapy that is meant to target the exact pathology of PD, it also may provide protection and a reduction of inflammation in earlier stages of PD before the disease has progressed significantly. We hope to better show this when we complete our TH stains.

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