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Current Amplitude-Dependent Modulation of Rotational Behavior with GPi Stimulation in the Rodent Model of Parkinson's Disease

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***Abstract*—The globus pallidus interna (GPi) is the main output nucleus of the basal ganglia, the neural circuit involved in motor and cognitive performance which is impacted by Parkinson's Disease (PD). Although deep brain stimulation (DBS) of the GPi is an effective treatment for the motor symptoms of PD in humans, the link between the stimulation signal space and the therapeutic benefits of DBS is not well understood. The rodent model of PD is useful for characterization of ameliorative DBS, though prior work focuses on the rodent model for DBS of the subthalamic nucleus (STN). This work investigates GPi-DBS in the rat model of PD under the framework of an amphetamine-induced rotational behavior. This work elucidates the relationship between stimulation current intensity and the motor effects of the dopaminergic lesion. Our results show that rotational behavior is modulated by the current intensity and validates GPi-DBS as a beneficial treatment of PD.**

I. Introduction

There are two typical targets for DBS used to treat the motor symptoms of humans with PD: the STN and GPi [1]–[3]. While both targets are effective in treating symptoms such as tremor, bradykinesia and rigidity [1]–[3], the mechanism of action for either target is not well understood. The results in human studies are equivocal in terms of which target achieves better treatment of the motor symptoms [2], [4], [5]. However, there are fewer investigations of the therapeutic benefits and cognitive side effects of GPi-DBS relative to the literature on STN-DBS. There is some indication, though, that there are more cognitive side effects of STN due to its relationship with the limbic system [6], [7] and that GPi-DBS may have more benefits [4].

In order to address this argument, there must be further study regarding GPi-DBS in order to make comparisons with STN-DBS. Previously, there has been no studies of GPi-DBS in the rodent model. We have studied the motor improvement associated with GPi-DBS in the rodent model using a reaction time lever-pressing task, as well as the potential cognitive side effects of DBS, such as depression and impulsivity, using several behaviors including an open field test and a sucrose preference test. However, in the interest of space, this paper will focus on the therapeutic capacity of GPi-DBS evaluated using a rotation test.

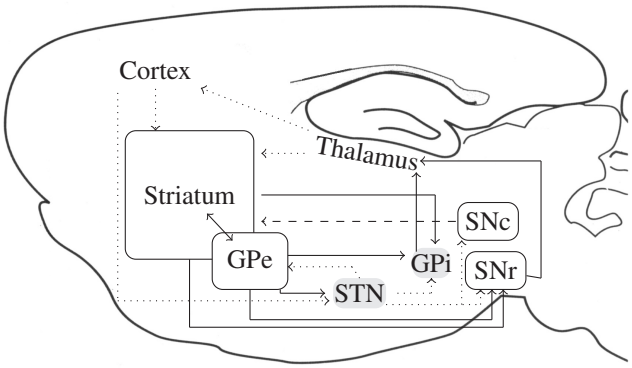

Fig. 1. Major pathways within the basal ganglia, and the pathways for the main input and output nuclei for the basal ganglia. The GABAergic pathways (inhibitory) are shown with solid lines and the glutamatergic pathways (excitatory) are with dotted lines. The dopaminergic projection from the substantia nigra pars compacta (SNc) to the striatum is depicted with a dashed line. Marked with gray shading are the two main target nuclei, GPi (a.k.a. the entopeduncular nucleus in the rat brain) and STN, for DBS to treat the motor symptoms of PD.

It is well known that apomorphine and amphetamine induce a rotational behavior in a unilaterally lesioned rodent at rest, with rotations in the direction contralateral and ipsalateral to the lesion, respectively [8]–[13]. A rotation test quantifies this circling activity, serving as a measure of severity of the motor deficits associated with the lesion. Attenuation of the rotation rate under a given treatment indicates that the treatment, at least partially, corrects the asymmetrical motor ability attributed to the lesion.

We chose to use amphetamine to investigate the motor behavior in the unilateral PD rat model with and without stimulation, with the goal of determining to what extent GPi-DBS was able to attenuate the rotation rate. We consider the amphetamine-induced rotations of the hemiparkinsonian rats in a cylindrical environment for two different stimulating signals. This behavior is compared with the rat's behavior without stimulation, which acts as the control.

Mitigation of the motor symptoms associated with PD depends strongly on the stimulating signal and typically the signal used in the rodent model is a sequence of brief

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constant-current square pulses [13]–[16]. We chose to fix the stimulating frequency to a value found to be efficacious for STN-DBS (130 Hz) [14], [15] and also to fix the pulse width (60 μs), in order to focus on the current amplitude as the parameter of interest. Two current amplitudes were studied, 65μA and 100μA. This design allows us to directly relate current amplitude of the simulation signal to the change in motor behavior. We found that there is a significant reduction in the average number of ipsalateral rotations of the rats under amphetamine when stimulation was given compared to the control. This highlights the potential of GPi-DBS and encourages further work in this domain.

II. Rodent Model

A. Subjects

All subjects were adult male Long Evans rats ($n = 9$, bred by Charles River Laboratories and housed at the animal facility at Rice University, Houston, TX), weighing between 450g and 550g. The rats were housed individually and were kept under a 12/12-hr light/dark cycle (lights on from 7:00 h to 19:00 h). All animals had food and water ad libitum at least 3 days prior to the behavior, although in the weeks preceding this they were on a restricted diet during which time they received 2 - 5 pellets of standard laboratory chow, reducing their weight to approximately 85-90$\%$ of their free feeding weight. Additionally, they were given Reese's Pieces and/or peanuts as treats after the behavior. All experiments were approved by the Institutional Animal Care and Use Committee of Rice University.

B. Surgical procedure

All rats ($n = 9$) received a unilateral injection of 6-hydroxydopamine (6-OHDA) in the right hemisphere and were implanted with a stereotrode in the entopeduncular nucleus (EP), the rat equivalent of the GPi, ipsalateral to the 6-OHDA injection. The rats were anesthetized throughout the procedure using 0.5 - 5$\%$ isoflurane in oxygen at a flow rate of 1-2 liters/min. Prior to the procedure, buprenorphine (0.01-0.05 mg/kg) was administered subcutaneoulsy (SQ) for analgesia and desmethylimipramine (DMI, 10-20mg/kg) was administered intraperitoneally (IP) to protect noradrenergic neurons from the neurotoxin. Rats were placed in a stereotactic apparatus (Kopf Instruments, California, USA) throughout the procedure.

Two burr holes were made: one for the 6-OHDA injection and one for the stimulating electrode. A hemiparkinsonian model was created by unilaterally lesioning dopamine neurons via an injection of the neurotoxin into the medial forebrain bundle (MFB) for retrograde transport to the substantia nigra pars compacta (SNc). The 6-OHDA was injected into the MFB (coordinates from Bregma: AP -4, ML 1.2, V -8.1) at an injection speed of 0.2ul/min. Each rat received an injection of 2ul 6-OHDA (4 μg/μl dissolved in 0.9$\%$ saline; Sigma, Zwijndrecht, The Netherlands) and the needle was left in place for an additional 7-10 minutes following the injection. The subjects then underwent an implantation of a tungsten bipolar stereotrode (MicroProbes, Maryland, USA) in the EP (coordinates from Bregma: AP -2.5, ML 3, V -7.9). Additionally, 6-12 holes were drilled through the skull and bone achor screws were implanted. The electrode was affixed to the skull in such a manner that a plug connected to the electrode remained accessible for connection to the stimulator. The electrode and plug were fixed in position using dental acrylic and the skull screws assisted in stability of the acrylic.

The rats were given 2 days of post-operative care and were allowed two weeks of ad libitum food and water before starting a restricted diet. All rats were tested 4-6 weeks following the injection of 6-OHDA, which is sufficient time for a lesion to develop [17]. The loss of dopaminergic neurons is supported by the behavioral results of the subjects when amphetamine was administered.

C. Cylinder test

A cylinder test is used to measure the asymmetric forelimb use in the hemiparkinsonian rat model, with the extent of the asymmetry indicating the extent of the unilateral lesion induced by the 6-OHDA injection [18]–[20]. Typically rats rear on their hind limbs and lean against the wall of the environment using their forelimbs. A unilateral 6-OHDA lesioned rat uses the forelimb ipsalateral to the lesion with higher frequency than a normal rat which uses each forelimb with equal likelihood [13], [21]. This test was used to verify prior to the rotation test that the rats displayed a quantifiable unilateral motor deficit. In this test, the rat is placed in a cylindrical environment (inner diameter 20 cm, height 46 cm) and permitted to behave spontaneously. This test was performed at two time points: one week post-op and two weeks post-op. The duration of each test was dictated by the amount of time it took for the rat to complete 25 wall touches.

D. Rotation test

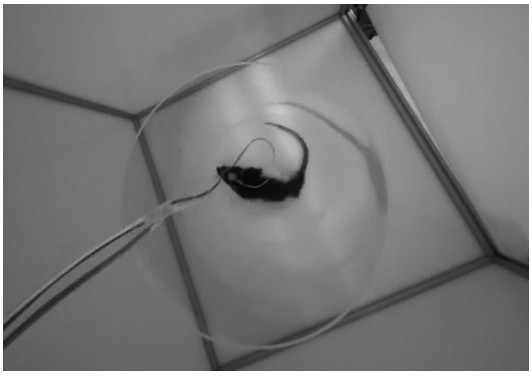

Fig. 2. Photo of the cylindrical chamber used in the Rotation Test. The cylinder was kept in an isolation chamber (built at the Kemere Lab, Rice University) to limit distractions to the rat throughout the test. The cable from the stimulator to the connector fixed to the rats' skulls was hung from the ceiling of the isolation chamber so as not to impair the rats' movements.

Under methamphetamine, the hemiparkinsonian rats experience drug-induced rotations (circling behavior) in the direction ipsalateral to the SNc lesion. The number of rotations per minute is used as an indicator of extent of the lesion, i.e. the loss of dopamine function in 6-OHDA lesioned rats [11], [12], [16], [21], as this circling behavior is not present if striatal dopamine is not depleted. With effective DBS stimulation, the number of rotations is attenuated [13], [15].

The rats were placed in an induction chamber with 5% isoflurane in oxygen until they became unconscious and then 0.5 ml of 2 mg/ml methamphetamine solution was administered IP. This dose is consistent with previous work [15]. The rats regained consciousness 1-2 minutes following the injection and were allowed to recover for an additional 15 minutes prior to beginning the test. This resting period allowed the methamphetamine to take effect in the rats.

The rats were observed in a cylindrical chamber (diameter 30 cm, height 45 cm) made of clear acrylic and were allowed to behave spontaneously; an overhead view of a subject inside the cylinder is shown in Fig. 2. The test consisted of four epochs, each lasting 5 minutes: no stimulation, low amplitude stimulation, no stimulation, and high amplitude stimulation. The stimulating current consisted of a series of rectangular pulses with a pulse width of 60 μs and frequency of 130 Hz. The low amplitude signal had current intensity of 65 μA while the high amplitude signal had current intensity of 100 μA. These values for pulse width, frequency and current amplitude were selected based on their efficacy in STN-DBS for the rodent model [15], [16] and results from human studies [1], [2]. The two current amplitudes were found experimentally to be the the lowest value which induced a reduction in rotations and the highest value which did so without negative effects, e.g., freezing and tremor, across the population.

We calculated the number of ipsalateral rotations experienced by the rat for each epoch and computed the mean number of rotations as our main performance metric. At the start of the test, the stimulator was connected to the implanted stereotrode via the plug mounted on the rats' heads. Throughout all epochs the stimulator was physically connected, even when the stimulation was off, in order to account for any change in behavior in the rats due to presence of the connecting wire. The stimulator (Model 2100, A-M Systems, Washington, USA) was switched off and on at the beginning of each epoch, as dictated by the protocol design. Video from an overhead camera (USB 2.0 Digital Camera, Point Grey Research, British Columbia, Canada) recorded each 20 minute session and the rotation data for each rat was manually extracted from the video recording.

E. Histological Processing

Following the experiments, the rats were anesthetized and the stimulating sites were marked by electrolytic lesions. The rats were perfused intracardially with a 30% isotonic sucrose solution followed by 4% paraformaldehyde in phosphate buffered saline (PBS). Fixed brains were sliced along the coronal plane and 50μm sections were immunostained for tyrosine hydroxylase (TH; primary goat anti-TH antibody, 1:200 dilution; and biotinylated rabbit anti-goat secondary antibody, 1:400 dilution). A red fluorescent Nissl stain was used (1:200 dilution) and slices were mounted using ProLong Gold Antifade Reagent with DAPI. The slices were imaged using a Nikon A1-rsi Confocal Microscope. Analysis using NIS Elements software revealed a significant 6-OHDA-induced depletion of 77.74% THir cells in the SNc (results of one-way ANOVA: F(1,16) = 25.57, $p < 0.001$). A sample image, shown in Fig. 3, demonstrates the considerable difference in TH positive cells in the left lateral and right lateral SNc.

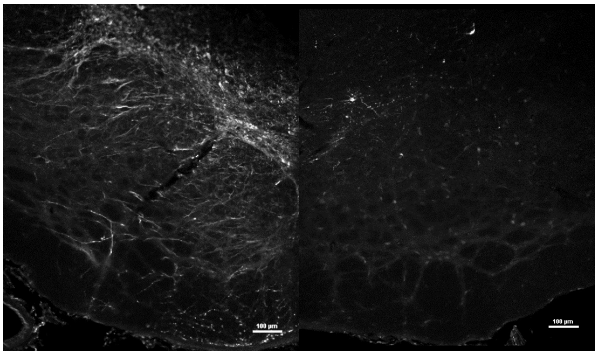

Fig. 3. Representative grayscale images of TH positive cells in the SNc on the left lateral and right lateral sides of a 50μm slice. Scale bars in each image are 100μm.

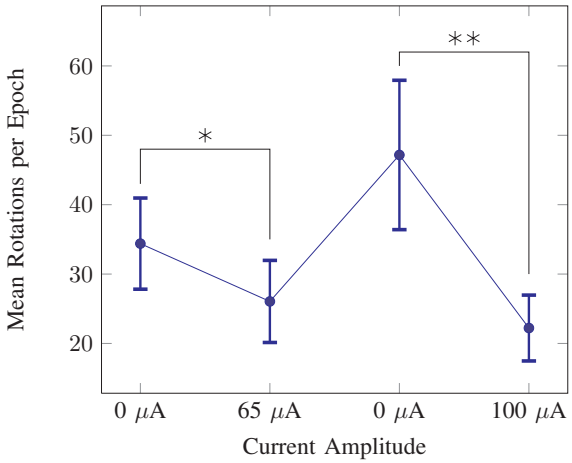

Fig. 4. Average number of amphetamine-induced rotations ipsalateral to the lesion. The total number of rotations per rat were computed during each 5 min epoch. During the OFF epoch, no stimulation was applied. Bars represent mean $\pm$ SEM ($n = 9$). Repeated measures ANOVA revealed that there was a significant difference in the mean number of rotations ($p < 0.001$). The p values for the pairwise post hoc LSD tests are provided as $^*p < 0.005$ and $^{**}p < 0.01$.

III. Results and Discussion

To capture the development of the 6-OHDA lesion, we performed the cylinder test on the rats in the two weeks

following surgery. One week after the 6-OHDA injections, the mean ratio of contralateral wall touches to total wall touches was 0.1989 ± 0.0412. After two weeks the ratio was 0.1467 ± 0.0231, which is a value consistent with previously reported proportions of contralateral wall presses in hemiparkinsonian rats [16].

After the subjects were confirmed to be unilaterally lesioned, the rotation test was performed. In Fig. 4, the mean number of rotations per 5 minute epoch ($n = 9$) is shown with error bars indicating the standard error of the mean (SEM). Results of repeated-measures ANOVA indicate a significant change in the rotational behavior of the rat induced by the stimulation ($F(3, 24) = 12.67, p < 0.001$).

The mean number of rotations per epoch was attenuated following 65 μA stimulation ($p < 0.005$), as well as following 100 μA stimulation ($p < 0.01$) when pairwise comparisons were made with their preceding no stimulation state; post hoc comparisons were made use Least Significant Difference (LSD) test. There was a significant difference between the mean rotations induced by the two different amplitudes of stimulation ($p < 0.0675$) and anecdotally there was a larger number of contralateral rotations associated with the lower lever of stimulation. During both stimulation epochs, contralateral rotations were observed as well as a large number of immobile periods in which the rats appeared to have greater control over their motor movements. Contralateral rotations were rarely observed in either epoch without stimulation. This indicates to the authors that further study is needed for low amplitude (65μA) stimulation.

IV. Conclusion

In our experiments we found that GPi-DBS did influence the number of rotations that the rats exhibited. The reduction in rotations caused by stimulation relative to the parkinsonian state was statistically significant for both low and high amplitude current. Additionally, the mean number of rotations was different for the two current intensities, which indicates that circling behavior is differentially modulated by the current amplitude. We conclude that GPi-DBS is shown to be effective in the hemiparkinsonian rodent model and modulates the amphetamine-induced circling behavior. This supports the validity of this rodent model for the study of GPi-DBS and the translational value to human studies.

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