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Research report

High-frequency stimulation of the subthalamic nucleus reverses limb-use asymmetry in rats with unilateral 6-hydroxydopamine lesions

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Abstract

Deep brain stimulation (DBS) is a widely used clinical treatment for Parkinson's disease (PD). A rodent model of DBS is a necessary tool for understanding the neural mechanisms of this method. Our previous study showed that high-frequency stimulation (HFS) of the subthalamic nucleus (STN) improved treadmill locomotion in rats with unilateral 6-hydroxydopamine (6-OHDA)-induced lesions of nigrostriatal dopamine (DA) neurons. The present study tested DBS effects on limb-use asymmetry (LUA) during vertical/lateral exploration in a cylindrical chamber in rats with similar unilateral nigrostriatal DA lesions. Limb-use asymmetry assessment has been used to detect functional capacity over a wide range of dopamine depletion. Before lesioning, rats exhibited regular rearing activity and used both forelimbs equally often to support weight during exploration of the walls of the cylinder. After unilateral nigrostriatal DA lesioning, rats displayed reduced rearing activity and predominant use of the ipsilateral (good) forelimb to touch the wall. HFS of the STN, but not of other nearby regions surrounding the STN, in the lesioned rats restored normal rearing activity and reversed the limb-use asymmetry caused by the unilateral DA depletion. This study is consistent with the possibility that there can be beneficial effects of STN-DBS on behavioral impairments in unilateral DA-depleted rats and may suggest an appropriate rodent model for DBS study.

Theme: Motor systems and sensorimotor integration
Topic: Basal ganglia

Keywords: Parkinson's disease; Deep brain stimulation; Basal ganglia; Movement disorder; Limb-use asymmetry; Apomorphine; Rat

1. Introduction

Parkinson's disease (PD) is a neurological disorder characterized by a variety of motor impairments resulting from the degeneration of dopamine (DA) neurons in the substantia nigra. A reduction and slowness of movement, associated with delayed movement initiation (akinesia), is one of the major signs of motor impairment in Parkinson's disease. Researchers have reported that deep brain stimulation (DBS) can reverse the three cardinal motor symptoms found in Parkinsonian patients: akinesia, rigidity, and tremor [18,22,30]. Moreover, high-frequency stimulation (HFS) of the subthalamic nucleus (STN) in particular has been shown to be an effective therapeutic option for patients with advanced Parkinson's disease [23,24,28]. Safety (no permanent damage to brain tissue), flexibility (adjustable stimulation parameters), and reversibility (ability to stop when more advanced treatment becomes available) are three important advantages of DBS over surgical ablation treatments. Clinical improvements produced by DBS may allow reduction of DAergic drug treatments, resulting in a significant decrease in dyskinesia side effects.

The 6-hydroxydopamine (6-OHDA) lesion model of parkinsonism in the rat has provided an invaluable tool for investigating the pathophysiology of DA denervation and evaluating novel therapeutic options [37]. However, little effort has been devoted to investigating the effects of DBS

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in rodent models of PD [7,8]. In rats, unilateral 6-OHDA nigrostriatal lesions produce asymmetries of body posture and contralateral sensorimotor deficits. Lesioned animals display many impairments on the side contralateral to the lesion and are often more responsive on the ipsilateral (good) side [5,6,31,32,35,36]. 6-OHDA-lesioned rats exhibit motor deficits that share essential functional similarities with parkinsonian akinesia. Such deficits can be quantified using novel and relatively simple testing procedures [25], such as assessment of limb-use asymmetry (LUA), which can detect acute and chronic effects of a wide range of DA depletion [33,36,41,42]. This test evaluates both independent and co-use of a rat's forelimbs to support its body against the walls of a cylindrical enclosure during exploration. The test utilizes the animal's innate drive to explore a novel environment by standing on the hindlimbs and leaning towards the enclosing walls.

The current study investigated the possible effects of DBS of the STN on limb-use asymmetries in a rat model of PD. We report that selective stimulation within the STN region in rats with unilateral 6-OHDA nigrostriatal lesions had a beneficial impact on contralateral forelimb disuse—an effect that may resemble the relief of akinesia or related movement impairments in humans with PD. The preliminary study has been reported in abstract form [38].

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats weighing 350–400 g were used in this experiment. The animals were housed individually in cages with a reversed dark–light cycle (lights off from 07:00 to 19:00 h). Food and water were provided ad libitum. All animals were treated in accordance with the U.S. Public Health Service *Guide for the Care and Use of Laboratory Animals* and experiments were approved by the Institutional Animal Care and Use Committees at the Wake Forest University School of Medicine.

Twenty-eight rats were assigned to two groups. Group 1 consisted of 22 rats that were subjected to deep brain stimulation experiments after unilateral DA depletion. Group 2 consisted of 6 rats without DA depletion (sham control) to determine whether DBS could influence limb-use asymmetry in neurologically intact rats.

2.2. Surgery procedures

Rats were anesthetized with a combination of ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg). Aseptic surgical procedures were observed. Sodium ampicilin (50 mg/kg, i.m.), an antibiotic, was given prior to the surgery. Stimulation electrodes were constructed in an array of platinum–iridium microwires (50 μm in diameter, NB Lab, Denison, TX, and Biographic, Winston-Salem, NC) soldered onto a strip connector. The microwires were arranged in a $3 \times 3 \times 2$ configuration and spaced 250 μm apart from each other. The whole array cluster was 0.75 mm in diameter. For the rats in group 1, the array was randomly assigned to either side of the brain (11 in the right and 11 in the left side) and implanted within the small volume of the STN. The stereotaxic coordinates used to target this structure were: 3.5 mm posterior, 2.5 mm lateral to bregma; and 7.3 mm ventral to the surface of cortex according to the atlas of Paxinos and Watson [29]. To allow targeted intra-cranial injection of 6-OHDA later in the experiment, one 26-gauge microinjection cannula was also implanted ipsilateral to the electrode. The tip of the cannula was 2 mm above the medial forebrain bundle (MFB) at 2.0 mm posterior and 2.0 mm lateral to the bregma. Six small stainless-steel screws were secured onto the skull to serve as anchors. The implanted microwire array and cannula were embedded in dental acrylic with the anchoring screws to form a headstage on each rat's head. Comparable surgical procedures were applied in the six sham control rats with bilateral placement of electrodes in the STN.

Animals were housed individually and allowed to recover from surgery for at least 10 days before being subjected to the experiment.

2.3. Experimental procedures

Rats were placed in a transparent plastic cylinder (20 cm in diameter and 30 cm high). Experiments were performed in dim light to encourage movement. The cylinder encourages use of the forelimbs for vertical exploration and landing after a rearing movement [6,33,41,42]. No habituation to the cylinder prior to the experiment was allowed. The test was performed between 09:00 and 16:00 h. For the rats in group 1, pre-lesion sessions (10 min each) were performed as a baseline control to compare with sessions following DA depletion and DA lesion + HFS conditions. The experimental sessions were videotaped with an infrared camera located beneath the cylinder for off-line analysis of limb-use behavior; a synchronized timer (33-ms resolution) was superimposed onto each video frame. Minimizing stress during behavioral testing is crucial for acquiring reliable data; thus, rats were handled gently once per day following their arrival at the lab.

After the two baseline sessions, each rat was lightly anesthetized with ketamine (80 mg/kg, i.p.) and received an injection of desipramine HCL (15 mg/kg, i.p.), a norepinephrine uptake blocker. Thirty minutes later, 8 μg of 6-OHDA (free base dissolved in 4 μl 0.01% ascorbic acid saline solution to prevent oxidation) was injected unilaterally into the MFB over a 6-min period. The injection needle (31-gauge) extended 2 mm beyond the tip of the cannula and was left in the cannula for at least 2 min after the completion of the injection to prevent leaking and to allow diffusion of the drug into the MFB.

The rats' behavior was tested again 10–14 days after 6-OHDA injection and their limb-use asymmetry scores were

obtained. HFS of the STN (130 Hz, pulse width 60 μs for unipolar pulse, 80 μs for bipolar pulse, 75–200 μA) was delivered in the following session. Some stimulation sessions lasted longer than 10 min to enable collection of more data.

To confirm unilateral DA depletion and test the effects of DBS on rotation behavior induced by a low dose of apomorphine, a rotation test was performed 10–15 days after lesioning. Each rat was placed in a hemi-spherically shaped behavioral chamber in which it could move freely, and rotations were detected by a photocell. A low dose of apomorphine (0.05 mg/kg, s.c.) was injected at the beginning of the behavioral test. DBS was delivered 20 min after apomorphine injection (around the peak of the rotational response) for 2 min with the same electrodes and stimulation parameters used in successful DBS treatment during the test of limb-use asymmetry. The number of rotations during the 2-min stimulation period was compared with those observed during the 2-min periods immediately before and after stimulation (expressed as rotations/min).

Rats in the sham control group were tested 10–14 days after surgery, each session lasting for 10 min. DBS of each STN was applied alternately across sessions. After the DBS sessions, the sham control rats were injected with ketamine (80 mg/kg, i.p.) and the limb-use test was performed 7 days later to assess the long lasting effect of anesthesia.

2.4. Data analysis

For the rats in group 1, limb use was assessed by off-line video analysis with a temporal resolution of 33 ms in three conditions: control, DA lesion, and DA lesion plus DBS of the STN. The score was expressed in terms of (1) percent use of the ipsilateral (nonimpaired) forelimb relative to the total number of ipsilateral, contralateral, and simultaneous (both) limb-use observations; (2) percent use of the contralateral (impaired) forelimb relative to the total number; and (3) percent simultaneous or near-simultaneous stepping-type (both) limb use relative to the total number. In addition, the number of rears per minute was assessed as an indication of general vertical motor activity in all three conditions, and percent of control was expressed in lesion and DBS conditions. Analysis of variance (ANOVA) was used to detect significant differences between these conditions. Post hoc pairwise Tukey tests for significant differences were employed to determine the sources of detected significances. Comparisons that differed with a $P<0.05$ were considered significant in all cases. Unless otherwise indicated, mean values were reported with standard errors.

For the sham control rats, limb use was assessed with and without DBS of the STN. Each side of the STN was tested alternately in separate sessions. Number of rears per minute was assessed 7 days after ketamine injection without DBS.

2.5. Histological localization of stimulation sites and confirmation of lesions

At the end of the experiments, each animal was subjected to the same anesthesia as described for the surgery session, and 20 μA of anodal current was passed for 10–20 s through the stimulation electrodes to mark with local electrolytic lesions the loci of the microwires that produced stimulation effects. In rats with unsuccessful DBS, the loci of randomly selected microwires were similarly labeled to identify non-effective stimulation sites. The animals were then sacrificed and perfused intra-cardially with 4% paraformaldehyde. Thirty-micrometer-thick coronal sections were cut through the STN with a cryostat and mounted on slides. Histological staining for tyrosine hydroxylase [16] was employed to assess loss of DA cells and fibers in the substantia nigra pars compacta and striatum ipsilateral to the 6-OHDA injection. Sections with the STN were stained with Cresyl violet to reveal the stimulation lesion sites. Boundaries of the STN were assessed with reference to the rat brain atlas of Paxinos and Watson [29].

3. Results

3.1. Effects of unilateral DA depletion on LUA test

Rats' behavior during the LUA test was examined and scored by off-line video analysis (Fig. 1). The analysis

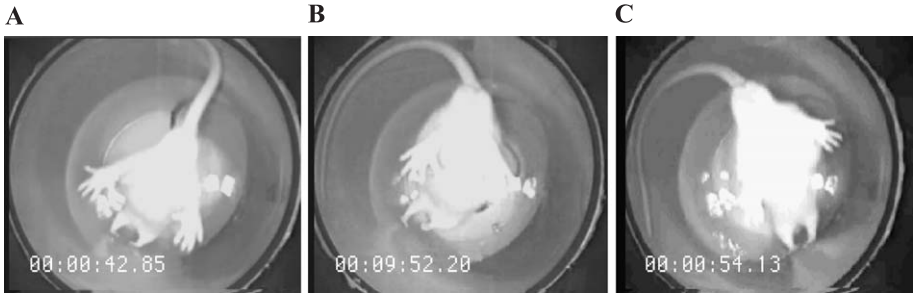

Fig. 1. Digital images showing the STN-DBS effects on the limb-use asymmetry (LUA) test in rats with unilateral DA lesions. (A) LUA test with a control rat: Normal rats use both forelimbs equally to touch the enclosure wall. (B) LUA test in a rat with a unilateral DA lesion (left side): Lesioned rats exhibit asymmetric limb use. Note that the right forelimb (contralateral) was not used to touch the wall. (C) LUA test in a lesioned rat during HFS of the STN: DBS reverses limb-use asymmetry in DA-lesioned rats. Note that the rat used both forelimbs to touch the wall.

focused on two behaviors: the average number of rears per minute and the pattern of limb use during rearing.

Normal rats were very active and reared frequently within the first 10 min of the experimental session, with approximately 3 rears per minute. They used both forelimbs equally to touch, with weight support, the cylinder wall during rearing and vertical–lateral exploration. On average, 74.9% of touches in control rats were made by both forelimbs simultaneously, and less than 20.0% of touches were made by each forelimb alone.

Rats with DA depletion were less active and reared less frequently than they did before lesioning, reaching only 30% of pre-lesion level (Fig. 2), which was significantly less than that of intact conditions ($P<0.05$, ANOVA, post hoc pairwise Tukey test). All rats showed asymmetric behavior after the 6-OHDA lesion. Unilateral DA lesions resulted in significant increases in ipsilateral (good) limb use during rearing; 80.5% of all contacts with the wall were with the ipsilateral forelimb. Correspondingly, touches with the contralateral (bad) limb and with both limbs decreased significantly to 3.8% and 15.7%, respectively ($P<0.01$).

3.2. Reversal of limb-use asymmetry and increase in rearing activity after HFS of the STN

Rats that exhibited clear asymmetry of limb use after the unilateral nigrostriatal DA lesions were subjected to HFS (130 Hz, pulse width 80 μs for biphasic pulse, 75–200 μA) of the STN ipsilateral to the lesion via bipolar platinum–irridium microelectrodes. HFS generally started at 50 μA and was gradually increased until an anti-asymmetric effect appeared, manifested as a restoration of normal rearing activity and simultaneous use of both forelimbs. Different pairs of microwires were tested for an optimal combination out of 56 permutations from an array of 8 microelectrodes. No more than three microwires in any array were identified as effective stimulation sites.

High-frequency stimulation of the STN attenuated limb-use asymmetry in seven out of eight rats in which correct placement of electrodes within the STN had been identified by histological staining (see histology segment for detail). The effective DBS of the STN in these seven rats was manifested by an increase in simultaneous use of both forelimbs, reaching 69.7% of total touches, which did not

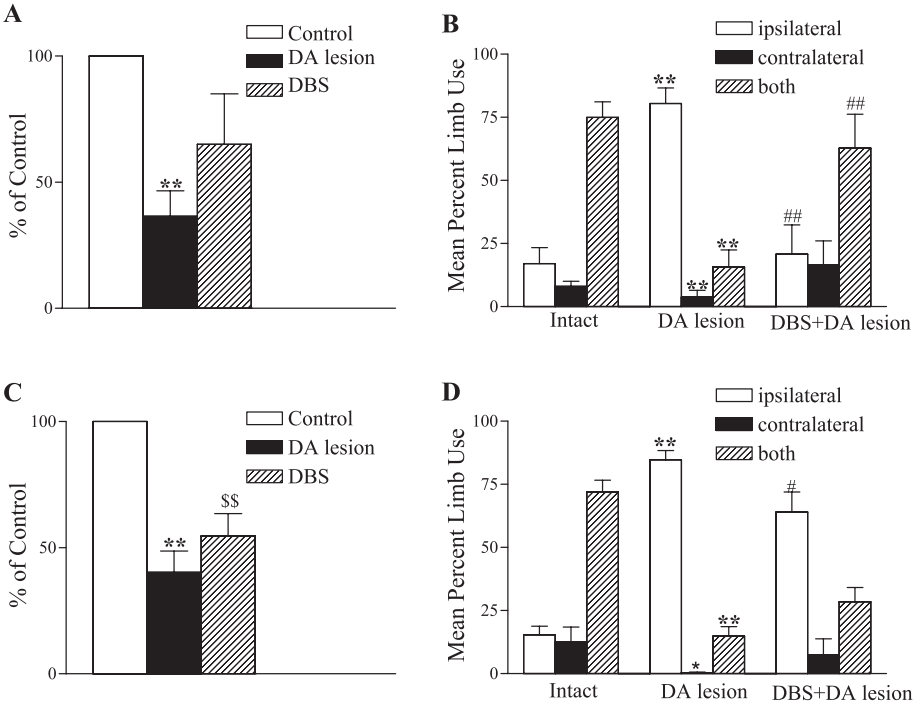

Fig. 2. STN-DBS on a limb-use asymmetry test in rats with unilateral DA lesions. Data from rats with stimulation electrodes located in the STN are shown in panels A and B; data from rats with stimulation electrodes located outside of the STN are shown in panels C and D. (A) Rearing activity in control, DA lesion, and DA lesion + DBS conditions measured as rears/min and expressed as percent changes. Intact rats were very active with approximately 3 rears/min. 6-OHDA lesioning decreased rearing activity. DBS in the STN of lesioned rats increased rearing activity towards the control level (control vs. DBS conditions, $P>0.05$). (B) Limb-use asymmetry in intact, unilateral DA lesion and lesion + DBS conditions. Intact rats predominately used both forelimbs simultaneously or alternating in a wall-stepping movement to touch the wall during rearing. DA-lesioned rats predominately used the ipsilateral (good) limb independent of the contralateral limb. DBS of the STN reversed the limb-use asymmetry by significantly increasing bilateral wall touches and decreasing use of the ipsilateral limb only. (C) DBS outside the STN did not affect rearing activity. (D) DBS outside the STN did not reverse limb-use asymmetry in DA-lesioned rats. (lesion vs. control, $^{*}P<0.05$ and $^{**}P<0.01$; lesion vs. lesion + DBS, $^{\#}P<0.05$ and $^{\#\#}P<0.01$; $^{\$\$}$lesion + DBS vs. control, $P<0.01$, ANOVA).

differ from that of pre-lesion control sessions (74.9%, $P>0.05$). Contralateral limb use was increased to 16.4%, which was significantly higher than that of the nigrostriatal DA lesion condition without DBS (3.8%, one-way ANOVA, post hoc pairwise Tukey test, $P<0.01$). The rats became more active during effective HFS of the STN. Their rearing behavior scores increased to 62% of pre-lesion control level. Though still lower than those of the control condition, rearing scores during HFS did not differ significantly from those of the control condition. (Fig. 2A and B). In rats with effective DBS of the STN, the effect appeared at the first rearing occurrence. Rats returned to pre-stimulation condition behavior with fewer rears and predominant ipsilateral limb use immediately following the termination of DBS. Effects on rearing and asymmetry of limb use were not observed when the electrodes were not correctly placed in the STN (Fig. 2C and D).

In one rat, one of the two stimulation electrodes was located within the STN and the other was located in cerebral peduncle. Initial response to the DBS (200 μA) in this rat was contraversive turning. The current was subsequently reduced to a level at which the contraversive turning disappeared, and then was gradually increased to 200 μA. At this point, the rat started using both limbs to touch the wall without contraversive turning. This case was excluded from the final result since it could not be clearly classified as either inside or outside of the STN.

3.3. Effects of HFS of the STN in sham control rats

In order to determine whether DBS of the STN could increase contralateral limb use and enhance rearing activity in intact animals, limb-use asymmetry tests were performed in six sham control rats with and without DBS of the STN. DBS was delivered unilaterally in five STN sites from three rats (two bilaterally located and one unilaterally located sites). Stimulation intensity was set just below the level that induced visible side effects (sniffing, facial muscle contraction, contralateral turning). In comparison with the session without DBS, no significant difference in the percentage of contralateral limb use was detected during DBS of the STN in intact animals (Fig. 3A).

To evaluate whether DBS itself could increase rearing activity, rearing rate was measured in intact rats during DBS of the STN and compared with that in the session without DBS. As indicated in Fig. 3B, no significant difference in rearing rate could be detected between the sessions with and without DBS in intact animals.

Possible reasons for the decrease in rearing activity after unilateral DA lesion are the long-lasting effect of anesthesia during DA lesion procedure and habituation caused by multiple exposures to the same experimental environment. To test these possibilities, six intact, sham control rats received the same dose of ketamine as used in DA lesion procedure after the completion of DBS study. The rats were tested 7 days later to assess rearing activity, and the result was compared with that before ketamine injection. Fig. 3B depicts the percentage changes of rearing activity before and after ketamine administration. No significant difference in rearing activity could be found 7 days after ketamine administration ($P>0.05$, ANOVA post hoc pairwise Tukey test).

3.4. Effects of DBS of the STN on apomorphine-induced rotation

Possible effects of DBS of the STN on rotational behavior in unilateral DA lesioned rats were examined. Successfully lesioned rats started turning contraversively within 6–10 min following apomorphine injection (0.05 mg/kg, s.c.). One rat exhibited clear limb-use asymmetry without contraversive turning at the dose of 0.05 mg/kg, but displayed contraversive turning when given 0.25 mg/kg. Application of DBS that attenuated LUA did not affect rotation behavior ($P>0.05$). Fig. 4 summarizes the apomorphine-induced rotational behavior before, during, and after DBS.

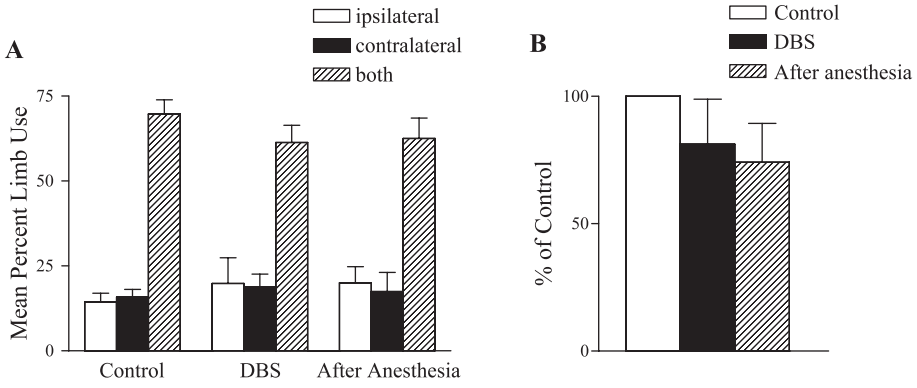

Fig. 3. The effect of DBS and anesthesia on limb use and rearing activity in sham control, intact rats. (A) The effects of anesthesia and DBS of the STN on limb-use asymmetry. DBS of the STN did not significantly change percent of different limb uses in comparison with control group without DBS. There was no significant change in percent of limb use 7 days after ketamine injection. (B) The effects of anesthesia and DBS of the STN on rearing activity. DBS performed following the control session did not significantly change rearing activity. Rearing activity did not change 7 days after ketamine injection (80 mg/kg, i.p.) without DBS.

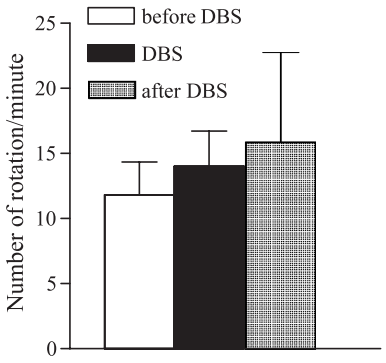

Fig. 4. Effects of DBS of the STN on apomorphine-induced rotational behaviors in unilateral nigrostriatal DA-lesioned rats. Contraversive rotation was induced by administration of a low dose of apomorphine (0.05 mg/kg, s.c.). The number of rotations during the 2 min of DBS did not differ from that during the 2 min before or after DBS delivery with the same DBS protocol that generated beneficial effects during the LUA test.

3.5. Histological localization of stimulation electrodes and staining for tyrosine hydroxylase

The successful rate for targeting the STN and concurrently inducing an effective 6-OHDA lesion was 7 out of 22 rats. Cases in which the microelectrode arrays missed the STN target provided controls for assessment of the location specificity of the DBS effects on limb-use asymmetry behavior. Fig. 2C,D depicts behavioral data from rats with microelectrodes misplaced outside of the STN ($n = 13$). Note that DBS in these cases did not affect limb use or rearing behaviors during the test for limb-use asymmetry.

The loci of stimulation microwire tips were marked by a small hole caused by the lesion-level current used at the end of the experiment. As shown in Fig. 5, all of the effective stimulation electrodes were located in the STN. Conversely, all but one of the ineffective stimulation electrodes was located outside of STN region.

Histological staining for tyrosine hydroxylase [16] revealed extensive losses of DA cells and fibers in the substantia nigra pars compacta and striatum on the side ipsilateral to the 6-OHDA injections.

4. Discussion

Our previous studies established the first DBS model in parkinsonian rats using a treadmill locomotion task [7,8]. Here, we tested the effects of DBS on limb-use asymmetry in rats with unilateral nigrostriatal DA lesions to extend further the test repertoire that can be affected by DBS. Our results demonstrated that: (1) DBS of the STN attenuated lesion-induced reductions in rearing activity; (2) DBS of the STN reversed lesion-induced asymmetry of limb use; (3) the STN-DBS protocol, though beneficial during limb-use asymmetry testing, did not affect low dose apomorphine-induced rotation behavior in lesioned rats; and (4) effective stimulation electrodes were located specifically within the STN region.

The limb-use asymmetry test has been shown to be sensitive to a range of dopamine depletion levels and correlated with the extent of dopamine depletion in other labs. For example, increases in physical activity in the forelimb contralateral to the DA lesion can reduce or prevent limb-use asymmetry [9,41,42,43]. Lundblad et al. [25] demonstrated that both L-DOPA and bromocriptine improved rats' ability to use their parkinsonian forelimb in this test.

Using a limb-use asymmetry test has several advantages in the assessment of the effectiveness of DBS. On a conceptual level, the nature of the motor manifestation being tested is unequivocal. The postural support and weight-shifting movements observed are identical to those typically performed by a rat in its home cage, and are examined without experimenter handling. Practically, the test is objective, simple to carry out, fast in its execution, and does not require animal training, aversive motivation, or food deprivation, which are known to influence function in parkinson models. The behavior is stable chronically and easy to quantify. The test is very sensitive even to partial DA depletion [33], and, importantly, the absence of a drug

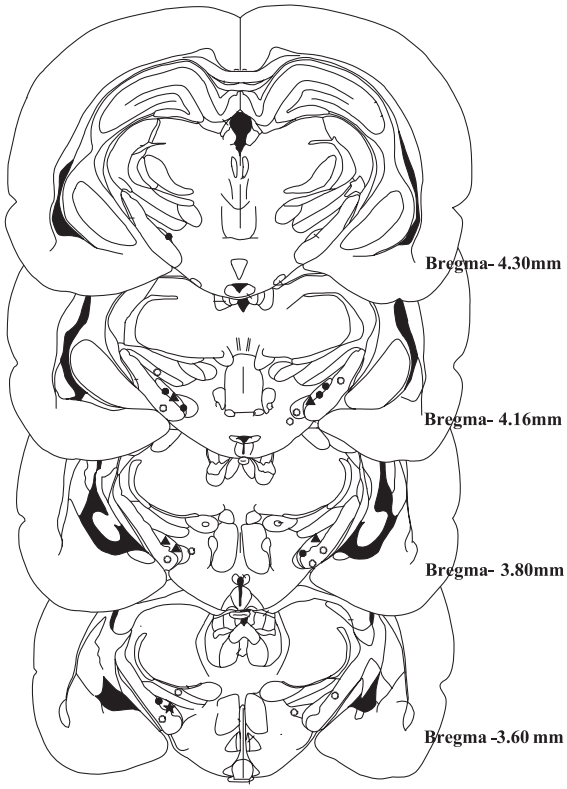

Fig. 5. Histological verification of stimulating electrode loci relative to the STN. Stimulation electrode loci in the STN that enabled reversal of LUA (included in data analysis of Fig. 2A,B) are indicated by ●. The electrode located in the STN that did not produce beneficial effect is indicated by ★. Electrodes located outside of the STN and without stimulation effects (included in data analysis of Fig. 2C,D) are indicated by ○. Electrodes located in the STN in sham control rats are indicated by ▲.

challenge eliminates confounding factors for interpreting the experimental results.

Limb-use asymmetry may also have clinical relevance. The forelimbs are used to initiate movements that require weight shifting, much like legs are used by humans when they walk. Difficulty in initiating steps from a standing posture, or regaining center of gravity, is one of the primary signs of extensive degeneration of DAergic neurons in the substantia nigra [14,20]. Decreased rearing activity observed after unilateral DA depletion is more likely attributed to a movement-related impairment rather than habituation to the experimental environments or long-lasting anesthetic effect since sham control group did not exhibit significant changes in the rearing activity. The STN-DBS in the present study had benefits similar to the therapeutic effects of DBS observed in parkinsonian patients. Namely, during DBS, rearing activity was no longer significantly impaired relative to the control condition and limb-use asymmetry was reversed. Beneficial DBS could be maintained for 8–15 min without causing any abnormal side effects. The beneficial effects of DBS on limb use seem to be specific to DA depletion since DBS of the STN in the intact animal neither influenced the limb-use asymmetry nor increased the rearing activity.

In a previous study, we found that the effect of DBS attenuated in 5–15 trials in a treadmill task using uniphasic DBS through stainless-steel microwire electrodes. One possible cause for this rapid "tolerance" acquisition is the redox reaction of water and metal with biological substrates, the byproducts of which develop around the tips of the small 50-μm stainless-steel electrodes with unbalanced current pulses [8]. We made a critical improvement by delivering biphasic stimulation pulses through platinum–iridium microelectrodes to minimize accumulation of local redox products and tissue damage. As a result, the effectiveness of DBS of the STN in this study lasted longer than that in our previous treadmill locomotion study (8–15 min in this study vs. 2–5 min in the treadmill study). In the clinic, DBS is applied continuously and the therapeutic effects have been reported to last several years [19]. The stimulation parameters used in our experiment are the same as those used in parkinsonian patients. The major difference is the size of stimulation electrodes, electrodes used in human patients are 2 mm in diameter which ensures low current density and less tissue damage, our electrodes are 50 μm in diameter and may induce tissue damage due to the high current density [17]. Since the limb-use test can only last around 15 min (rats will stop rearing after 10–15 min), we cannot determine how long the effective DBS lasts. This issue needs to be addressed by other behavioral tasks in the future.

Similar to our findings in the treadmill study, effective stimulation was localized to the STN. This result provides additional evidence that the benefits of DBS are anatomically specific, and that the STN is a key target for these positive effects. The precise mechanisms mediating the benefits of DBS remain unknown. The STN has been regarded as a pivotal structure in the indirect pathway of basal ganglia thalamocortical circuitry. It receives GABAergic input from the external segment of globus pallidus (GPe) and sends glutamatergic projections to the basal ganglia output nuclei, the internal segment of globus pallidus and substantial nigra pars reticulata. In addition, the STN receives excitatory input from cortical regions and projects to the pedunculopontine nucleus and other brain stem regions. As a result of dopamine neuron degeneration in midbrain, GABAergic medium spiny neuron in the striatum may be activated, and this in turn increases inhibitory input to the GPe. Inhibition of the GPe would give rise to the disinhibition of STN neurons. A consequence of this circuitry is that hyperactivity of the STN may play a role in the pathogenesis of parkinsonian symptoms [1,11,21,39]. One hypothesis is that chronic HFS may achieve a functional inhibition of the STN neurons, mimicking the effect of a lesion [2,3,13]. A number of in vitro studies have investigated the channel and membrane property changes induced by DBS. Beurrier et al. reported that STN-HFS transiently prevents the activation of STN neurons by blocking Ca^{2+}-dependent channels [4]. Magarinos-Ascone et al. found time-dependent responses of STN neurons during DBS in an in vitro slice preparation. The neurons in the STN initially followed HFS beyond 100 spikes/s. After 10 s of HFS, the cells switched to a burst firing mode and then totally shut down after another 10 s, which may be due to the inactivation of Na^+ mediated action potentials [26]. Do and Bean examined dynamic firing patterns in dissociated rat STN neurons. They found that a resurgent Na^+ current, together with a persistent Na^+ current, may be responsible for the generation of high tonic and burst firing patterns in STN neurons [12]. A recent study by Garcia et al. [15] suggested that DBS could inhibit spontaneous spike activity and simultaneously evoke a burst spike mode. Notwithstanding its insightful investigatory power, in vitro study performed in the isolated slice may not be able to reveal the precise neural processing during behaviorally effective DBS in parkinsonian animals and human patients with PD. To this end, a rodent model of DBS as described in this study can be used in combination with other investigatory tools to explore the mechanisms underlying DBS effects [7,38].

In our previous study, we reported that effective DBS in the treadmill task did not change rotation behaviors elicited by a high dose of apomorphine (0.25 mg/kg, s.c.) [8]. Here, we included a test of DBS effects on rotation elicited by a low dose of apomorphine (0.05 mg/kg, s.c.). We tested both low (0.05 mg/kg) and high (0.25 mg/kg) doses of apomorphine because they may cause different behavioral phenomenology and activate different receptors. For example, low and high doses of apomorphine may act differently in presynaptic D_2 vs. postsynaptic D_1 receptors due to differences in receptor subtype ligand affinities [27,40]. Additionally, a high dose may act on DA receptors in both the intact and lesioned hemispheres, whereas a low dose may act only on supersensitive receptors in the lesioned side [44].

In unilateral nigrostriatal DA lesioned rats challenged by apomorphine, the good hind limb (ipsilateral) serves as a pivot while the bad hind limb (contralateral) steps. The high number of steps with the bad limb in response to the apomorphine challenge may be a sign of enhanced reactivity to a shift of weight. Ziegler and Szechtman [45] reported that step asymmetry between the two hind limbs was higher in the low dose group even though the number of rotations was lower. Failure of DBS to block turning induced by both high and low doses of apomorphine suggests that the stimulation may not be sufficient to counteract the activation of supersensitive DA receptors. A recent study by Darbaky et al. [10] reported that unilateral STN-HFS applied in unilaterally DA-depleted rats did decrease apomorphine-induced turning behavior. Different experimental procedures may partially account for this discrepancy with our results. Two doses of apomorphine were used in Darbaky's study. The initial apomorphine administration was 0.1 mg/kg in the control experiment. A higher dose of apomorphine (0.3 mg/kg) was used in the DBS experiment two weeks later in consideration of possible acquired tolerance. Although the doses differed, it is more likely that the important experimental difference was in the duration of DBS. In Darbaky's study, HFS was applied immediately after the apomorphine injection and continued for an hour during the test, while in our study the stimulation lasted for only 2 min during the peak of rotational behavior. It could be that the effects of DBS on rotation require an extended period of time to appear. However, in both our treadmill and limb-use asymmetry tests, the stimulation effects appeared very quickly. The rotation test is a sensitive method to detect a severe level of DA depletion; however, pharmacological activation of supersensitive DA receptors may confound the interpretation of results. Behavioral tests in the absence of a drug challenge, such as bracing/stepping reactions to forced weight shifts [34] and spontaneous limb-use tests, have been suggested as additional ways to examine behavioral effects of treatments in rat models of PD [25].

In conclusion, the present study investigated the effects of DBS of the STN on limb-use asymmetry and apomorphine-induced rotation behavior in unilateral DA-lesioned rats. The results demonstrate that targeted DBS can attenuate nigrostriatal DA lesion-induced reduction of rearing activity and substantially increase use of the forelimb impaired by the lesion. The same DBS protocol that produced beneficial effects in the limb-use asymmetry test, however, did not alter rotational behavior induced by a low dose of apomorphine.

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