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Research report

High frequency stimulation of the subthalamic nucleus improves treadmill locomotion in unilateral 6-hydroxydopamine lesioned rats

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Abstract

This study investigated the influence of electrical stimulation of the subthalamic nucleus (STN) on motor impairment induced by unilateral 6-hydroxydopamine (6-OHDA) lesions in the medial forebrain bundle. Rats were trained to walk on a treadmill and then implanted with microelectrode arrays in and near the STN. The neurotoxin 6-OHDA was injected into the medial forebrain bundle (MFB) unilaterally to produce a targeted lesion of the dopaminergic system. Successful lesions produced impaired treadmill walking behavior. High frequency stimulation (HFS) of the STN improved treadmill walking immediately and restored normal walking patterns. The same HFS failed to evoke visible side effects such as stepping, turning, raising of the head or facial muscle contraction in the absence of treadmill movement, or to change rotational behaviors elicited by the dopamine (DA) agonist apomorphine in unilateral lesioned rats. This suggests that the stimulation did not cause movement by an activation of brainstem locomotor regions or an increase attention leading to movement. Apomorphine-induced rotation may represent an imbalance of dopaminergic activation which remains during HFS. This work may provide a rodent model for deep brain stimulation (DBS) in patients with Parkinson's disease, and be suitable for further investigation of the neural mechanisms underlying the therapeutic effects of DBS.

Theme: Motor systems and sensorimotor integration

Topic: Basal ganglia

Keywords: Parkinson's disease; Deep brain stimulation; Basal ganglia; Movement disorder; Apomorphine

1. Introduction

The goal of this research was to determine whether electrical stimulation of structures within the basal ganglia can counteract at least temporarily the behavioral deficits caused by a unilateral lesion of the dopaminergic system in rats. While stimulation of neural tissue has had a long history [23,60], the mechanisms by which brain stimulation can influence movement are not well understood. Stimulation of neuronal circuits upstream of motor neurons may induce abnormal movements by direct actions on motor pathways or through general activation of 'locomotor centers' [16,20,33,53,59,63]. Conversely, stimulation of sensory systems may provide cues that can be conditioned to activate behavioral sequences [21]. Furthermore, activation of reward systems may provide substrates for reinforcement of particular movement sequences [50]. Recently it has been found that deep brain stimulation (DBS) in the basal ganglia may alter local circuit function in humans with Parkinson's disease (PD) without alterations in conscious perception of the stimuli, involuntary movement, or general arousal.

Surgical treatment of basal ganglia movement disorders by thalamotomy and pallidotomy has been performed since the 1950s [15,22,65] to alleviate tremor, rigidity and other motor impairments associated with PD [34,48,66]. Recently, long-lasting therapeutic effects of continuous DBS have been reported in Parkinsonian patients, including improvement of akinesia, rigidity and tremor [3,4,7,32,37,40,41,52]. The major advantages of DBS over surgical ablation therapy are the reversibility of possible side effects, and the flexibility to adjust the stimulation

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parameters to obtain optimal stimulation effects. Both surgery and stimulation may reestablish a mode of baseline activity which allows motor command signals to generate effective movements. In spite of becoming a common alternative to ameliorate PD, the neural mechanisms underlying the therapeutic effects of DBS have yet to be clarified [6,19,30,47,69]. Current animal models have contributed significantly to the Parkinsonian research [57,67,73]; however, there is a need for development of appropriate and reliable rodent models of DBS treatment of motor deficits resulting from dopamine (DA) depletion to compliment existing models [56].

The present study explored the effects of high frequency stimulation (HFS) of the subthalamic nucleus (STN) in a rat model of PD using a treadmill locomotion task. In this task a normal rat learns rapidly to walk on a treadmill which is cycling between on and off periods. At treadmill onset, an intact rat begins to walk voluntarily. In contrast, a rat with a unilateral 6-OHDA lesion of the medial forebrain bundle (MFB) quickly loses the ability to initiate locomotion during a session. Identification of stimulation sites in the basal ganglia that can restore the ability to move in this context may guide the development of DBS therapeutic interventions. Furthermore, it is critical to determine whether intentional movement can be facilitated by HFS without observable non-specific effects on motor output or general arousal. We report that selective stimulation in the region of the STN in rats with unilateral lesions of dopaminergic nigrostriatal projections yields an enhancement of locomotion on the treadmill, an effect that may be compared with the relief of akinesia in humans.

2. Materials and methods

2.1. Subjects

Twenty six male Sprague–Dawley rats weighing from 350 to 400 g were used in these experiments. The animals were housed individually in cages with a reversed dark–light cycle (light on from 19:00 to 07:00 h). Food and water were provided ad libitum. All animals were treated in accordance with the US Public Health Service *Guide for the Care and Use of Laboratory Animals*.

2.2. Surgery procedures

Each rat was anesthetized with ketamine (100 mg/kg i.m.) and xylaxine (10 mg/kg). Aseptic surgical procedure was observed. The antibiotic, sodium ampicilin (50 mg/kg i.m.) was given prior to the surgery. Stimulation electrodes were constructed in an array of eight Teflon-insulated, stainless steel microwires (50 μm in diameter, NB Lab. Denison, TX), soldered onto a strip connector. The microwires were arranged in a 3×3×2 configuration and spaced 250 μm apart from one another. A whole array cluster was 0.75 mm in diameter. The arrays were implanted bilaterally with the goal of placing as many electrode tips as possible within the small volume of the STN (3.5 mm posterior and 2.5 mm lateral to bregma; 7.3 mm ventral to the surface of cortex). High resolution mammography X-ray imaging was employed in selected animals to confirm that electrode wires remained in close proximity after implantation. In addition, two 26-gauge cannulae were implanted 2 mm above the medial forebrain bundle (MFB) at 2.0 mm posterior and 2.0 mm lateral to the bregma to allow injection of 6-OHDA later in the experiment. Six small stainless steel screws were secured onto the skull to serve as anchors for cementing the headset in place. The microwire arrays and cannulae were embedded in dental acrylic at the end of surgery to form a 'hat' on the rat head. The behavioral experiment began 7 days after surgery.

Following baseline condition treadmill locomotion testing sessions each rat was lightly anesthetized with ketamine (80 mg/kg) and 8 μg 6-OHDA (free base in 4 μl 0.2% ascorbic acid saline solution) was injected unilaterally over a 6-min period into the MFB. The injection needle (33-gauge) was left in the cannula for at least 2 min after the completion of the injection to prevent leaking and allow diffusion of the drug into the MFB. The side of the unilateral 6-OHDA injections was selected randomly (12 in the right and 14 in the left side). Seven rats were subjected to a second 6-OHDA injection either in the same (three rats) or in the contralateral (four rats) MFB as the first injection due to a lack of motor deficits after the first injection.

2.3. Experimental procedures

Rats were trained to walk on the treadmill which was enclosed within a 38-cm long chamber at a speed of 12 cm/s. The treadmill cycled between 20-s on and 20-s off periods. Walking is triggered by the sensory cues of the treadmill onset and anticipation due to internal timing of the fixed duration time-off interval. The experimental sessions were video-taped with a synchronized timer (30 ms resolution) superimposed onto each video frame. A ruler was placed in view along with the treadmill belt to measure the body position of the rat during treadmill walking.

After establishing consistent treadmill walking patterns, rats were subjected to a unilateral 6-OHDA lesion of the dopaminergic neurons of the MFB (described above). After 6-OHDA injection, treadmill locomotor activity was assessed daily. Intra-cranial bipolar HFS (60 μs pulse width, 50–175 μA) was delivered at 130 Hz in 3-s on, 2-s off cycles during a 20-s treadmill walking phase.

To confirm unilateral DA depletion, a traditional rotation test was performed 10–15 days after 6-OHDA infusion. Each rat was placed in a spherically shaped behavioral chamber in which it could move freely and rotations were

detected by a photocell. Apomorphine (0.25 mg/kg, s.c.) was injected 20 min after the start of the behavioral test. HFS was applied via the same electrodes that induced beneficial locomotor effects in the treadmill task. The HFS was delivered 20 min after apomorphine injection (around the peak of the rotational response) for 2 min with the same stimulation parameters and same 3-s on 2-s off cycles used in successful HFS treatment during the treadmill task. 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).

2.4. Data analysis

Treadmill locomotion patterns were associated with the rat's body position in the treadmill chamber. The position of the rat's nose in the treadmill chamber was measured by a ruler placed under the treadmill belt (Fig. 1) using off-line video analysis (temporal resolution 30 ms). The distance between the rat's nose and the back wall of the treadmill chamber was measured every second over the 20-s treadmill walking period. The average distances were compared among the control (before lesion), the lesioned without HFS and the lesioned with HFS conditions. Analysis of variance (ANOVA) was used to detect significant differences between these conditions. Post hoc pair wise Tukey tests for significance differences analysis were employed to determine the sources of detected significances. Comparisons with a $P<0.05$ were considered significant in all cases. Unless otherwise indicated, mean data values were reported with standard errors.

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 10–20 μA of anodal current was passed for 10–20 s through the stimulation electrodes to deposit iron ions. The loci of microwires that produced stimulation effects were labeled in this manner. In rats with unsuccessful HFS, loci of randomly selected microwires were labeled to identify non-effective stimulation sites. The animal was then sacrificed and perfused with 4% paraformaldehyde–3% potassium ferricyanide solution. Thirty-micron thick coronal sections were cut with a cryostat through the STN and mounted on slides. Enhanced visualization of the stimulation sites was achieved by incubation of the mounted sections in a solution

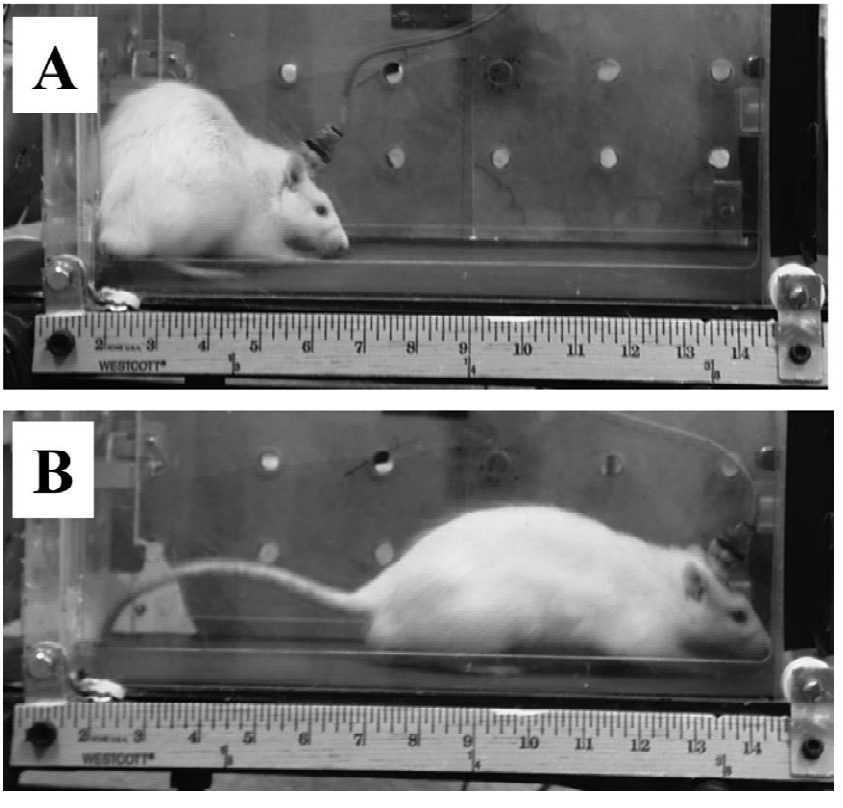

Fig. 1. Digital image showing the effects of HFS of the STN on treadmill locomotion in a rat with a unilateral lesion of the dopaminergic system. (A) The lesioned rat exhibited a passive walking pattern and was pushed back against the wall of treadmill chamber with its back arched before HFS. (B) HFS of the STN produced immediate improvement of treadmill locomotion. The rat walked with active steps and moved to the front part of treadmill chamber. The ruler underneath the treadmill belt was used to measure rat's nose position.

containing 5% potassium ferricyanide–10% HCl. Histological staining for tyrosine hydroxylase [25] was employed to assess losses of DA cells and fibers in substantial nigra pars compacta and striatum on the side ipsilateral to the 6-OHDA injection. Boundaries of the STN were assessed with reference to the rat brain atlas of Paxinos and Watson [51].

3. Results

3.1. Effects of unilateral dopamine depletion on treadmill locomotion

A treadmill-locomotion session consisted of trials with alternate periods of 20 s walking and resting. Rats typically learned to walk on the treadmill with regular, smooth steps after two to three training sessions. In these cases, rats walked forward on the treadmill belt and maintained a position at the forward part of the treadmill chamber during the entire 60-min session. Initiation of movement was apparently cued by tactile, auditory and visual sensations. This occurred without the slight aversion of a bump against the back wall of the chamber that was experienced during initial phases of training. Total walking distance for the session was 216 m.

A successful unilateral dopaminergic lesion resulted in marked locomotor deficits as revealed during the treadmill walking phase. Sixteen out of 26 rats (61%) exhibited a motor deficit after a single intra-MFB 6-OHDA injection. In these cases, the rats appeared unable or reluctant to walk and engaged only in ineffective stepping against the rear wall of the treadmill chamber with an arched back posture (Fig. 1A). The deficit developed at varying latencies after the start of the session, ranging from 1 to 25 min (652±107 s; median=520 s). Intact rats continued to initiate walking normally during the treadmill.

Twelve rats with motor deficits were tested in which treadmill locomotion was continuously monitored from day 1 after the lesion. Eleven of these exhibited treadmill locomotor deficits in the first session which was within 24 h of the 6-OHDA infusion. These deficits remained for more than 2 weeks. In the longest monitored case, the motor deficit persisted for 2 months following the lesion. Three rats exhibited functional recovery over the 5–10-day period after receiving lesions. These rats were not included as cases with motor deficits.

Seven rats were subjected to a second 6-OHDA injection either in the same (three rats) or in the contralateral MFB (four rats) of the first injection. Of the three rats that received a second intra-MFB 6-OHDA injection ipsilateral to the first, one rat manifested a motor deficit following the second injection. Of the four rats that received contralateral secondary injections, three developed motor deficits produced by DA depletion in the hemisphere which received the second injection. Table 1 summarizes the effects of unilateral lesions on treadmill locomotor behaviors.

3.2. Enhancement of treadmill locomotion by HFS of the STN in rats with unilateral nigrostriatal dopaminergic lesions

Rats that exhibited treadmill locomotor deficits after the unilateral dopaminergic lesion received HFS (130 Hz, 60 μs pulse width, 50–175 μA) of the STN ipsilateral to the lesion via a pair of microelectrodes. HFS at 50 μA did not cause any observable disturbances in animal behavior. The current was gradually increased until an enhancement effect appeared on locomotion. This presented as positive, smooth walking until the nose of the rat reached the front part of the chamber. In some cases a slight contraversive turning, raising the head and facial muscle contractions appeared as side effects of HFS. These symptoms stopped immediately upon termination of HFS.

Different combinations of microwires were tested out of a possible set of 56 different permutations from the array of eight microelectrodes. In practice, every electrode was tested as either an anode or a cathode. No more than three microwires in any array were identified as effective

Table 1
Summary of the effects of a unilateral MFB lesion of the dopaminergic system on treadmill locomotion and dopamine receptor agonist induced turning behaviors

Rat	First lesion			Second lesion		
	Turn	Deficit	Onset of deficit	Side	Turn	Deficit
1	−	−		contra	−	−
2	−	−		contra	+	+
3	+	+	8 min			
4	+	+	7 min			
5	+	+	6 min			
6	+, ipsi	+	14 min			
7	+	+	5 min			
8	+	+	3 min			
9	+	+	17 min			
10	−	−		contra	+	+
11	+	+	25 min			
12	+	−				
13	+	+	2 min			
14	+, ipsi	−				
15	+	−				
16	+	+	15 min			
17	+	+	8 min			
18	+	+	25 min			
19	+	+	14 min			
20	−	−		contra	NA	+ at 8 min
21	−	−		ipsi	−	−
22	−	+	10 min			
23	−	−		ipsi	−	−
24	+	+	9 min			
25	+	+	1 min			
26	−	−		ipsi	+	+ at 10 min

ipsi, ipsilateral; contra, contralateral.

stimulation electrodes that caused improvement of treadmill walking.

HFS of the STN induced immediate improvement in treadmill locomotion in MFB lesioned rats. The pattern of treadmill walking became more active during HFS, as characterized by positive, smooth steps that carried the rat toward the front part of treadmill chamber (Fig. 1B). Due mainly to the constant speed of treadmill, the walking speed and number of steps were similar between the conditions with and without HFS. Body position in the treadmill chamber was the most reliable indicator of normal versus passive walking patterns. When lesioned rats exhibited a passive walking pattern without HFS, their bodies were pushed back towards the rear part of the chamber by the treadmill belt movement. While executing normal walking patterns during HFS, rats walked actively towards the front part of the chamber. The effects of HFS lasted for five to 15 trials and then diminished, and effects often reappeared in the session 24 h later.

Fig. 2A,B depicts the effects of HFS on treadmill locomotion measured by body position in the treadmill chamber. A unilateral lesion resulted in a significantly shortened distance between the rat's nose and the back wall of the chamber (rats were carried away from the forward wall by the moving treadmill belt). HFS of the STN ipsilateral to the DA depleted side significantly increased this distance as normal walking patterns were resumed and reached the same level as that seen during the control condition before the MFB lesion.

3.3. Side effects and the free field condition

The side effects of HFS were assessed by turning off the treadmill during HFS so that any body movement associated with the onset of HFS could be detected. Side effects induced by HFS included stepping in the chamber, contraversive turning, raising the head and facial muscle contractions. All data included in Fig. 2 were obtained

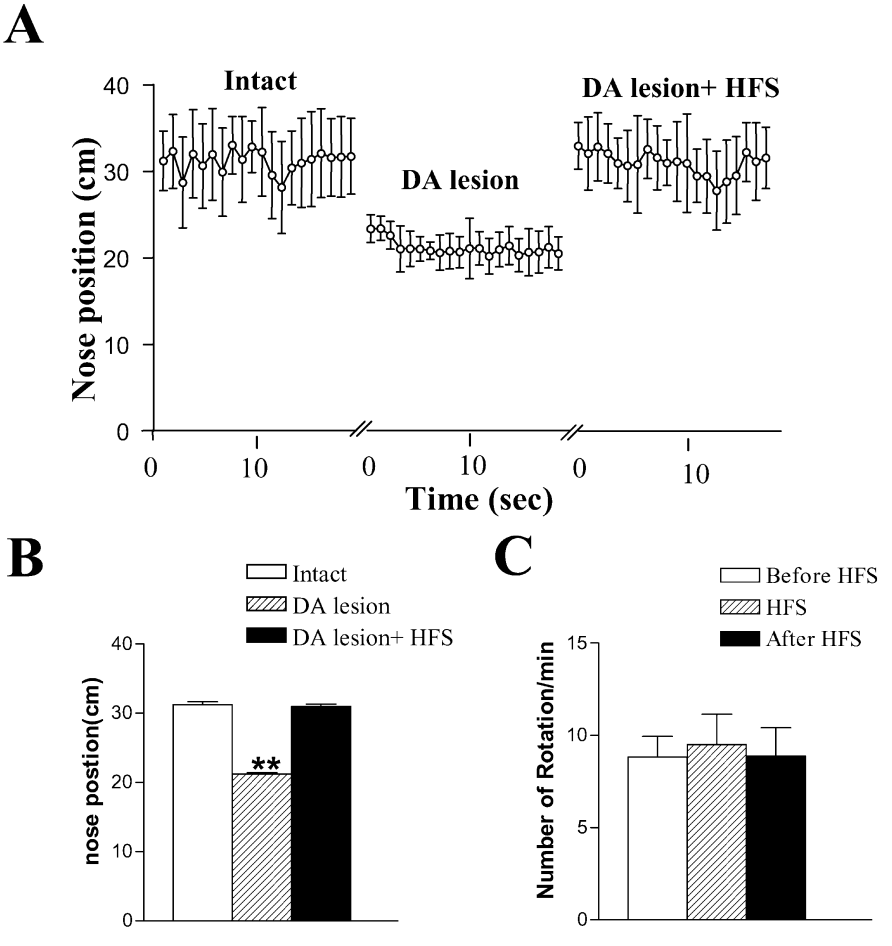

Fig. 2. Effects of HFS of the STN in treadmill locomotion and apomorphine induced rotational behaviors. (A) Distance between the rat's nose position and the rear wall of treadmill chamber in the intact, the lesioned and the lesioned plus STN-HFS conditions during 20-s treadmill walking periods. The mean distances (±S.E.M.) per 1-s interval over the 20-s treadmill walking period are shown. Data were obtained from six rats with one trial per condition. Note the decrease in distance in the lesioned condition and the complete return to the intact level during successful HFS. (B) The average distance between the rats' noses and the back wall of the chamber was significantly reduced during the lesioned condition relative to the intact and the lesioned plus HFS conditions. (**$P<0.01$). (C) HFS of the STN delivered through the same electrodes that generated beneficial effects on treadmill walking with the same stimulation parameters did not affect rotational behavior (P values >0.05).

from HFS trials that did not induce any of the visible side effects listed above. Effective stimulation electrodes were located mainly in the STN and surrounding regions (see Section 2.5 and Fig. 3). Most of the ineffective stimulation electrodes were located outside the STN regions except in two cases in which stimulation of electrodes located in the zona incerta dorsal to the STN resulted in the improvement of treadmill locomotion, one produced effects on treadmill locomotion that lasted for more than an hour. However, because HFS in this case induced stepping responses when the treadmill was turned off, the case was considered to produce non-specific side effects and not included as an effective HFS case. In another rat, HFS via electrodes located within the STN induced transient improvement of treadmill locomotion which lasted only 3 s during the walking phase of the first stimulation cycle. Due to the brevity of this effect, this rat was not included as an effective stimulation case (marked by ★ in Fig. 3).

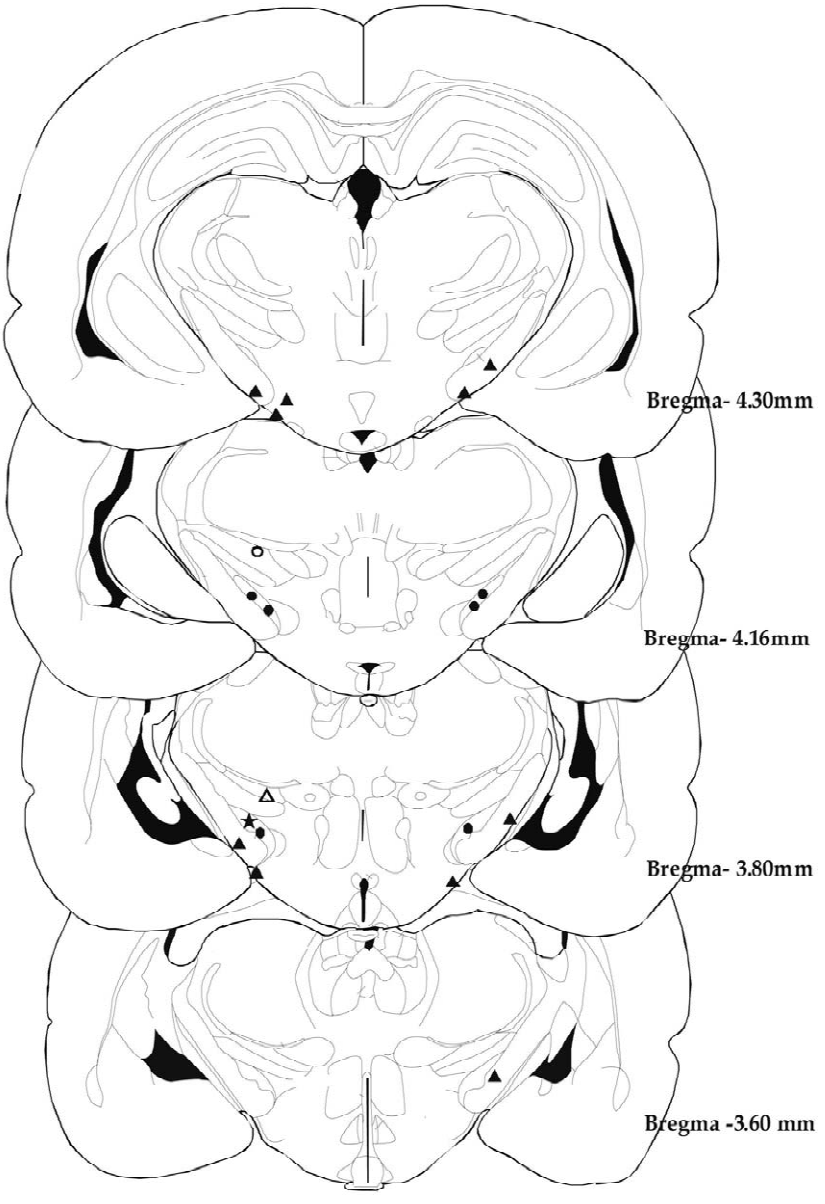

Fig. 3. Histological localization of the tips of stimulation electrodes in the STN. One label is shown for each rat. Other electrodes in the array were located within 500 μm of the labeled site. The stimulation electrodes in two rats were misplaced outside these maps. Stimulation electrode loci in the STN that enhanced locomotor behavior in the final results demonstrated in Fig. 2 are indicated by ●. The electrode location within the STN that produced only marginal effects is indicated by a ★. The stimulation electrode loci that missed the STN and had no effects on treadmill locomotion are indicated by ▲. The electrode location in the dorsal zona incerta that mediated enhanced treadmill locomotion is indicated by a △. The electrode location in the dorsal zona incerta that produced involuntary stepping during stimulation without treadmill movement is indicated with an ○.

3.4. Effects of HFS of the STN on apomorphine-induced rotation in unilateral MFB lesioned rats

The possibility of an effect of HFS of the STN on rotational behavior of unilateral MFB lesioned rats was examined during apomorphine treatment [54]. In successfully lesioned cases, rats started turning to the side contralateral to the lesion within 2–3 min of the apomorphine injection (0.25 mg/kg s.c.) and made more than 200 rotations/h. After the first lesion, 18 of 26 (69%) rats exhibited rotational responses following the apomorphine injection. Among these 18 rats, two turned toward the lesioned side. Two contraversive-turning rats and one ipsiversive-turning rat did not develop treadmill locomotion deficits. One rat exhibited a treadmill locomotion deficit without contraversive turning. Immunohistochemical staining for tyrosine hydroxylase, however, confirmed considerable depletion of dopaminergic terminals in the striatum and cell bodies in the substantial nigra pars compacta in this rat. HFS was applied to the same electrodes that induced beneficial locomotor effects in the treadmill task. The HFS was delivered 20 min after apomorphine injection (around the peak of rotational response) for 2 min with the same stimulation parameters and same 3-s on 2-s off cycle used in successful HFS during treadmill task. No significant difference in the number of rotations could be detected between the periods with and without HFS of the STN ($P>0.05$). Fig. 2C summarizes the apomorphine-induced rotational behavior before, during and after HFS in rats following 6-OHDA lesions of the MFB.

3.5. Histological localization of stimulation electrodes and confirmation of lesions

Insertion of an array of eight to 10 microwires increased the chance of reaching the target area with at least one precisely placed electrode. Nevertheless, accurate targeting of a small nucleus in a deep region such as the STN remains especially difficult. As a result, the present success rate for targeting the STN and at the same time induce an effective 6-OHAD lesion was seven of 26 rats. Cases in which the microelectrode arrays were not implanted within the STN in this study provided control cases for assessment of the location specificity of the STN-HFS effects on locomotion. Moreover, an additional potential site for therapeutic stimulation may be identified from such a 'miss'.

Localization of the tip of stimulation microwires was visualized by potassium ferricyanide staining for deposited iron ions. As demonstrated in Fig. 3, all but one of the effective stimulation electrodes was located in the STN. Conversely, all but one of the ineffective stimulation electrodes was located outside of STN region. Thus the benefits of localized STN-HFS were highly effective and selective. Two stimulation electrodes located in the zona incerta dorsal to the STN improved treadmill locomotion in two lesioned rats but one of these electrodes generated a stepping response when stimulated in the resting condition, and the result was thus classified as a non-specific side effect. Stimulation of one electrode located within the STN produced less robust benefits on locomotion.

Histological staining for tyrosine hydroxylase [25] revealed extensive losses of dopaminergic cells and fibers in ventral tegmental area and striatum on the side ipsilateral to 6-OHDA injections. It is worth noting that tyrosine hydroxylase immunohistochemistry revealed considerable depletion of dopaminergic terminals in the striatum and cell bodies in the substantial nigra pars compacta in the rat that exhibited a treadmill locomotion deficit in the absence of contraversive turning behavior with apomorphine treatment. A representative example of depleted tyrosine hydroxylase immunoreactivity in the striatum ipsilateral to 6-OHDA infusion for a rat with locomotor deficits is shown in Fig. 4.

4. Discussion

This study demonstrated that HFS in the region of the STN improves treadmill locomotion in rats with unilateral MFB lesions of the dopaminergic system. Equivalent stimulation in the absence of treadmill movement did not evoke locomotor or other movements. While similarities are clear, the precise degree to which this effect is similar to the enhanced movement induced by DBS in human is not known. DBS experiments in rats have investigated its effects on preventing seizures [46,68]. However its effects in the 6-OHDA lesion behavioral model of PD have not been investigated previously. Establishing a rodent DBS model will provide insight into the mechanisms underlying the clinical benefits of DBS in humans.

4.1. DBS in rat locomotion as a model for Parkinson's disease

In the current study, treadmill locomotion was tested at a moderate speed (12 cm/s) on a level surface similar to a normal walking environment in a natural setting. Thus, the treadmill locomotor deficit manifested after a unilateral 6-OHDA lesion is relevant to akinesia observed in Parkinsonian patients. Several experiments have investigated 6-OHDA induced akinesia in other models. Schallert et al. [54] used a 'brace test' to identify the akinesia associated with monoamine lesioned rats. When pushed forward and backward by the investigator, the DA depleted rats failed to initiate steps to balance their posture. They instead utilized a bracing approach to prevent fall, revealing difficulties in initiating the required steps for normal movement. Chang et al. [13] investigated the effects of a

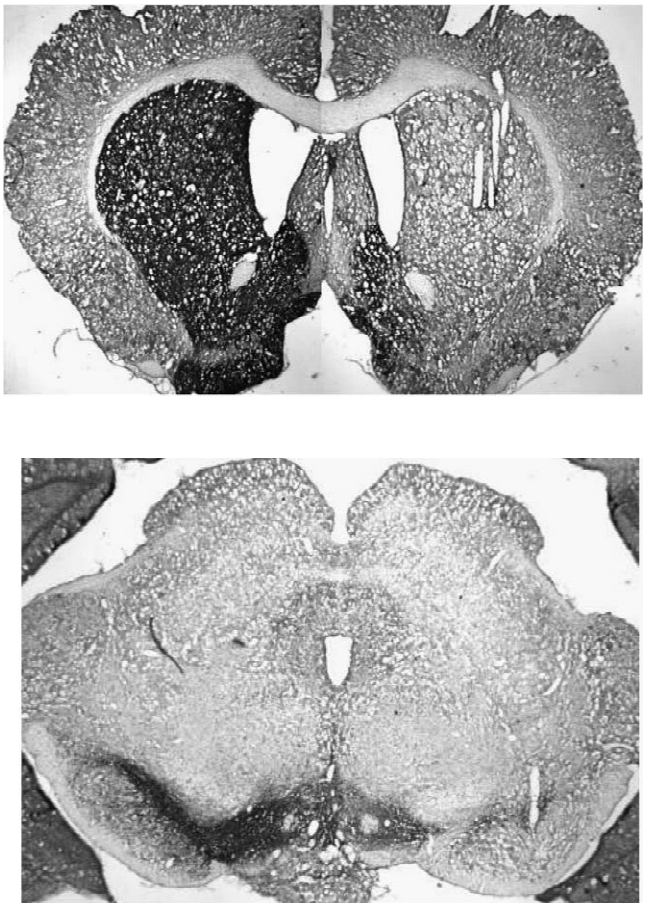

Fig. 4. Immunohistochemical staining for tyrosine hydroxylase (TH) in a unilateral dopaminergic cell lesioned rat with locomotor deficits during treadmill walking. Note the extensive depletion of TH immunoreactive fibers in the striatum ipsilateral to the 6-OHDA injection (top figure). Substantially decreased TH staining was also observed in the substantia nigra pars compacta of the lesioned side in the same rat (bottom figure).

unilateral dopaminergic cell lesion on forward adjusting steps. When a rat walked on the treadmill with forelimbs only, the adjusting steps on the side contralateral to the lesion were significantly reduced. This deficit may be attributed to the inability of an animal to respond when shifting weight or to initiate adjusting steps during treadmill walking. All these akinesia symptoms could be reversed by administration of DA receptor agonists, supporting the view of a dopaminergic origin of these deficits [13,54–56]. The ability of the rat to respond rapidly to avoid shock (reactive capacity) has also been reported to be impaired by 6-OHDA lesions of the dopaminergic system [62].

In the present treadmill task, normal rats could walk smoothly and actively in this cue-activated mode of locomotion for 216 m during a 60-min session. Subsequent unilateral dopaminergic lesions induced locomotion deficits at varying latencies after the commencement of treadmill sessions, with an average delay latency of approximately 10 min. Locomotion deficits were characterized by hesitant and irregular walking patterns. These characteristics resemble the difficulties in step initiation and weight shifting observed in other studies [13,54]. Delayed onset of treadmill movement impairment resembles the rapid fatigue phenomenon observed with Parkinsonian akinesia [18]. Similarly, animals with moderate DA depletion are known to behave normally in basal conditions, but to reveal Parkinsonian symptoms when challenged [61,64].

The treadmill locomotion task has previously been shown to be a suitable means of assessing locomotion impairment following nigrostriatal DA lesions. For example, Hattori et al. [29] reported a treadmill locomotor deficit after unilateral dopaminergic nigrostriatal lesions and used the method to investigate the effects of a dopaminergic tissue graft on motor activity. In their study, rats were trained to run rapidly (30 cm/s) on an uphill positioned treadmill. To facilitate treadmill running, adverse electric shock was delivered to the tail when the rat was unable to follow the treadmill speed. Many shock deliveries were required for the lesioned rats to maintain a constant speed, indicating impairment of treadmill locomotor capacity [29]. The current study did not use such adverse stimulation to activate movement.

Partial bilateral depletion of nigrostriatal DA by multiple intra-striatal 6-OHDA injection sites [1,10,39] is a possible alternate method to induce a form of akinesia more relevant to Parkinsonism in the rat [11,35]. However, the magnitude of DA depletion seen with such lesions varies greatly. Moreover, accurate bilateral placement of stimulating microwires in the STN is extremely difficult (we encounter a 30% success rate for correct unilateral targeting). Based on these concerns, we consider the bilateral DA lesion model to be less practical for routine study of DBS in rat.

4.2. Motor improvement following high frequency stimulation

A significant improvement of treadmill locomotion was found during STN-HFS. After initial trials the stimulation intensity could be selected at which HFS of the STN was able to restore normal treadmill walking patterns without causing abnormal motor side effects. Significantly, no locomotion or other movements were evoked when stimulation was applied while the treadmill was stationary. Thus it appears that the HFS targeted to the STN can effectively and selectively alleviate motor deficits. These benefits cannot be attributed to effects on general arousal or direct activation of motor pathways.

As yet, the precise action is not known on the neuronal structures located within the ‘indirect’ pathway including the striatum, globus pallidus external (GPe), globus pallidus internal (GPi), STN and thalamus. It is likely that HFS blocks either the outflow of disruptive signals from these regions, or normalizes circuit function so that

cortical-striatal signals can again assert normal control. These issues are presently under investigation in this laboratory using large scale recording of neuronal populations within this system.

Comparable results have been observed in Parkinsonian patients with DBS of the STN [3,4,32,37,40,41,52]. A recent study in PD patients showed that areas surrounding the STN, including the adjacent pallido-subthalamic tract and zona incerta, were effective regions to generate beneficial stimulation effects [70]. This finding suggests that in addition to direct stimulation of cell bodies in the STN, stimulation of passing nerve fibers may partially account for the therapeutic effects of DBS observed in Parkinsonian patients. Computer modeling studies indicated that anode stimulation preferentially activated cell bodies while cathode stimulation was more likely to activate nerve fibers [43,45]. Clinical investigation has revealed that cathode stimulation more effectively suppresses Parkinsonian tremor [5], probably by stimulating fibers [2,31]. However, the bipolar configuration used in the present study, as well as in many clinical settings, makes it difficult to distinguish between soma and fiber components of DBS.

HFS produced clear and immediate improvement in rat locomotor function. However, enhancement effects during STN-HFS typically attenuated in five to 15 trials, indicating rapid development of tolerance. Our present view is that this effect is due mostly to probable changes in stimulation electrode characteristics; however, a mode of biological tolerance may also contribute. Results from electric field modeling indicated that current density is concentrated at the tip of the microelectrode and at the electrode–insulation interface. Nearly uniform current-density distribution along the surface of an electrode can be achieved only with electrodes larger than 500 μm [44]. The most likely causes for rapid tolerance in the present study are the redox reactions of water, metal and biological substrates that become apparent around the tip of the small 50-μm stainless steel electrodes with unbalanced current pulses. For example, a recent study revealed that 4-h sub-chronic HFS caused considerable tissue damage around stimulation electrodes in the rat [26]. The large (2 mm diameter), platinum/iridium electrodes used in humans are optimized with stimulation circuitry to carry charge-balanced current over time which minimizes accumulation of local redox products. The effective point source of the small stainless steel electrodes could also be expected to disrupt brain tissue locally due to high local voltage gradients, and more damage should occur in the rat, considering the small size of the STN, 1 vs. 12 mm in humans.

A critical future step, therefore, will be to optimize the surface of stimulation electrodes and associated electronics specifically for the needs of brain stimulation research in the rat. Nevertheless, the immediate efficacy seen in the present model allows effective anatomical sites to be identified and further enables neurophysiological mechanisms of the beneficial effects of DBS to be investigated [14].

4.3. Actions of stimulation on basal ganglia

The organization of the basal ganglia–thalamocortical system suggests that degeneration of midbrain dopaminergic cells in PD may disrupt the normal neuronal processing within basal ganglia circuits [17,49]. One simple hypothesis for production of PD symptoms is that depletion of striatal DA disinhibits medium spiny neurons that send GABAergic projections to the GPe [58,74]. This elevated inhibitory input to the GPe in turn may reduce the inhibitory output of GPe to the STN, resulting in enhanced activity of glutamatergic projections of the substantia nigra pars reticulata (SNr) and the GPi [28,38,71]. The enhanced inhibitory output from SNr/GPi to thalamic motor nuclei may thus cause impairment of movement [17,24,36,38,71].

DBS of the STN is postulated to suppress the hyperactivated STN and to disinhibit the thalamocortical pathways by decreasing excitatory input to the SNr/GPi, the output station of the basal ganglia. A number of reports have in fact reported a decrease in firing rate in both STN and GPi during DBS in both anesthetized and behaving animals [8,9,12]. Contrary indications have come from studies reporting an increase in firing rate and glutamate release in the GPi during HFS of the STN [27,72]. In addition to simple changes in firing rate, modification of firing patterns or ‘unjamming of coded signals’ could also contribute to the therapeutic effects of DBS [6,19,69]. The current report indicates that neural coding in the rat of the ‘intention to locomote’ is effectively unblocked by HFS.

Uncertainty exists regarding the nature of the rotation movements activated by apomorphine after a unilateral lesion of the dopaminergic system. One possibility is that rotation is an abnormal dyskinetic movement stimulated by the action of the drug upon the supersensitive DA receptors of the denervated striatum. One suggestion [55] is that apomorphine may activate the unimpaired ipsilateral forelimb and thus shift the weight of animals to a new location while the contralateral forelimb simply makes catch-up steps to accommodate a newly formed center of gravity. Although there is no direct evidence, a reversal of sensory neglect, and attending to the previously neglected side, may also contribute to the drug-induced turning. Marshall et al. [42] described sensory neglect following lesions of the dopaminergic system in rats which was observed as a failure to orient to stimuli applied to the body opposite to the lesion and which was partially reversed by apomorphine treatment. Though the rotational test is a reliable measurement for unilateral DA depletion [67], the excessive rotational movement is a drug-induced abnormality rather than a primary motor deficit such as akinesia. Our view is that behavioral tests in the absence of any drug action such as self initiated treadmill locomotion

provide a better behavioral model to test the effects of DBS.

In summary, these results establish the enhancing effects of HFS of the STN on facilitation of treadmill locomotion in rats with unilateral lesions of the dopaminergic system. Establishing a rodent research model of DBS treatment for PD will facilitate efforts in future neurophysiological research to clarify underlying therapeutic effects of DBS.

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