Long-term stimulation of the subthalamic nucleus in hemiparkinsonian rats: neuroprotection of dopaminergic neurons

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Object. The goal of this study was to evaluate the neuroprotective effects conferred by long-term electrical stimulation of the subthalamic nucleus (STN) against degeneration of dopaminergic neurons by assessing motor functional and immunohistological findings in hemiparkinsonian rats.

Methods. In 13 of 25 rats, a concentric microelectrode was stereotactically implanted into the right STN under the guidance of extracellular microelectrode recording. After this had been done the animals were given an injection of 6-hydroxydopamine (6-OHDA) into the right striatum. Seven of the rats received continuous stimulation (frequency 130 Hz, intensity 80–100 μA) for 2 weeks (Group A); the other six did not receive any stimulation during this period (Group B). Twelve rats did not receive electrode implantation and underwent 6-OHDA injection only; these animals served as a control group (Group C). After 2 weeks, motor function in the rats was evaluated by conducting an amphetamine-induced rotation test. Finally, tyrosine hydroxylase–immunoreactive neurons in the pars compacta of the substantia nigra (SNc) were counted to evaluate the extent of degeneration of dopaminergic neurons. Ipsilateral rotation was significantly decreased in Group A, regardless of the effects of stimulation delivered during the test (p < 0.05). Rats in Group B demonstrated typical circling as did those in Group C, except that on stimulation Group B rats immediately stopped circling or changed direction. Tyrosine hydroxylase–immunoreactive neurons in the SNc were significantly preserved in the animals in Group A, whereas neurons in animals in Groups B and C were moderately depleted (p < 0.01).

Conclusions. Acutely, STN stimulation improved rotation symmetry in rats with moderate SNc degeneration. When STN stimulation had been applied for the preceding 2 weeks, motor function was better and SNc neural degeneration was significantly milder. Subthalamic nucleus stimulation thus appears to protect dopaminergic neurons in this hemiparkinsonian model, in addition to improving motor function in these animals.

KEY WORDS • deep brain stimulation • Parkinson disease • neuroprotection • subthalamic nucleus • 6-hydroxydopamine • rat

Deep brain stimulation directed to the STN is a promising surgical treatment for advanced PD, which is in use at many institutions worldwide. This treatment can decrease motor symptoms of the disease such as rigidity, bradykinesia, tremor, and drug-induced dyskinesia. In addition to alleviation of motor symptoms, possible neuroprotective effects of DBS of the STN are now suspected. The underlying hypothesis is that STN DBS or ablation can decrease glutamate-induced excitotoxicity, which is induced by the STN on the SNc. Any proven neuroprotective effects of DBS or ablation of the STN would assign to these modalities an important role in the arrest or delay of the progression of PD and expand the indications for these treatments to include moderate or early disease stages.

Recent preclinical research has addressed this issue. Authors of some reports have concluded that STN ablation in rats prevents further degeneration of dopaminergic neurons in the SNc. These studies involved chemical STN ablation rather than stimulation, however, and thus questions remain as to whether STN stimulation itself also can act neuroprotectively. Furthermore, controversy persists regarding whether the clinical effectiveness of DBS has the same mechanism as that underlying the benefits conferred by ablation.

One problem in resolving the matter has been the lack of a reliable long-term STN DBS model in awake rats. To overcome this obstacle, we have developed a technique of DBS of the STN in awake rats and have used it in an established rat model of PD (6-OHDA–induced nigrostriatal degeneration). Our purpose was to evaluate the neuroprotection conferred by long-term DBS of the STN as well as its effects on motor function and to consider the implications of this method’s mechanisms of therapeutic effects.

Abbreviations used in this paper: ANOVA = analysis of variance; AP = anteroposterior; DBS = deep brain stimulation; DV = dorsoventral; GABA = γ-aminobutyric acid; ML = mediolateral; 6-OHDA = 6-hydroxydopamine; PD = Parkinson disease; PLSD = protected least significant difference; SN = substantia nigra; SNc = pars compacta of the SN; SNr = pars reticulata of the SN; STN = subthalamic nucleus; TH = tyrosine hydroxylase; VPM = ventral posteriomedial (thalamic) nucleus; ZI = zona incerta.


Animal Preparation

Twenty-nine Sprague–Dawley rats were initially prepared for this study. In four of them a histological study showed an unsatisfactory location of the stimulation electrode and those animals were excluded (detailed information on the location of each electrode is described in Results). Consequently, 25 rats were included in this study. The rats, which weighed between 245 and 275 g each, were housed in a temperature-controlled environment at 24°C with a 12-hour light/dark cycle and were given free access to food and water. All animal experiments were performed in accordance with the Guidelines for Animal Experimentation of Nagoya University, which were issued in April 2000.

Overview of the Experimental Design

The rats were divided into three groups. In Group A (seven rats), a DBS electrode was stereotactically implanted into the right STN with the guidance of microelectrode recordings. On the next day, the rats were observed during a brief stimulation at various intensities to determine the optimal intensity for long-term stimulation (therapeutic intensity) for each rat. Two days after implantation, 6-OHDA was stereotactically injected into the right striatum to produce hemiparkinsonism. Subthalamic nucleus stimulation at the therapeutic intensity was begun immediately after the 6-OHDA injection and continued for 2 weeks. In Group B (six rats) the animals underwent DBS electrode implantation into the STN followed by an injection of 6-OHDA, but they did not receive long-term stimulation. This group was used to evaluate the pathological changes in the SNc as a sham surgical group and also to examine motor responses to amphetamine with and without acute stimulation; they represented rats with considerable nigrostriatal dopaminergic neuronal degeneration. In Group C (12 rats) the animals underwent only the 6-OHDA injection into the right striatum and served as a control group. Two weeks after 6-OHDA administration, the rats in all three groups were examined in an amphetamine-induced rotation test. After this test the animals were killed and their brains were examined histopathologically. The characteristics of each group are summarized in Table 1. Detailed methods for each step of the protocol are described later in this paper.

Microelectrode Recording and DBS Electrode Implantation

Each rat was anesthetized by an intraperitoneal injection of chloral hydrate (400 mg/kg). Afterward, the head was secured in a stereotactic frame (David Kopf Instruments, Tujunga, CA) and the body temperature of each animal was maintained at 37°C. The incisor bar was set at −3.5 mm to orient the horizontal calvarial surface. An incision was made in the skin at the midline and a small burr hole was drilled in the right posterior skull. The initial target representing the STN was set at a point 2.5 mm lateral to the midline, 3.8 mm posterior to the bregma, and 7.6 mm deep from the surface of the dura mater, according to the atlas of Paxino and Watson.24 For a precise identification of STN location, we performed extracellular microelectrode recording. A tungsten microelectrode (tip diameter 40 μm) was inserted through the burr hole and aimed at the initial target. Then, it was advanced slowly by using a micromanipulator (Narishige, Tokyo, Japan). Potentials at the electrode were amplified 10,000 times and conducted through a bandpass filter (10–10,000 Hz; Dam 80i; World Precision Instruments, Aston, UK) and monitored with the aid of an oscilloscope display and an audio output. Activities of neurons in various anatomical structures, such as the VPM, ZI, STN, and SNr, during microelectrode recording. In the VPM, typical neuron activity was characterized by discrete high-amplitude potentials and burst activity. After the electrode was passed through the silent area (ZI) and into the STN, an irregular firing pattern and a high firing rate were observed (40.9 ± 12.9 Hz). If the electrode was placed too far posteriorly, we encountered SNr neuron activity, which demonstrated a frequent, high-amplitude, and irregular pattern, resembling that in the STN, although the frequency of neuronal firing in the SNr is usually higher than that observed in the STN (68.3 ± 8.6 Hz).

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Treatment protocol for the experimental groups</th>
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<tbody>
<tr>
<td>Factor</td>
<td>Group A</td>
</tr>
<tr>
<td>no. of rats</td>
<td>7</td>
</tr>
<tr>
<td>DBS electrode implanted in STN</td>
<td>yes</td>
</tr>
<tr>
<td>continuous stimulation of STN</td>
<td>yes</td>
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<tr>
<td>6-OHDA injection</td>
<td>yes</td>
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</table>
Neuroprotective effects of chronic STN stimulation

DBS electrode at the same position. This electrode was a bipolar concentric microelectrode with a tip diameter of 100 μm, a shaft diameter of 200 μm, and had a distance between its two contacts of 100 μm (Unique Medical, Osaka, Japan). The electrode configuration is shown in Fig. 2A. After implantation, the electrode was affixed to the skull with dental cement. Stimuli were delivered by a biphasic stimulus isolator (BAK Electronics, Mount Airy, MD), connected to the stimulating electrode. To allow the rats to move freely while under continuous stimulation and to prevent mechanical interference with the lead connection, each rat was housed in a cage designed to permit movement.16

Evaluation of the Behavioral Response to DBS of the STN

One day after the rats underwent implantation of the DBS electrode, each rat in Group A was stimulated at various intensities for a short period (30 seconds–5 minutes) and the behavioral response was evaluated. Only the stimulus intensity was varied from 25 to 600 μA; all other parameters remained fixed at a frequency of 130 Hz and a pulse duration of 60 μsec. The purpose of this evaluation was to determine the optimal intensity for long-term stimulation (therapeutic intensity) in each rat. In general, when the intensity increased, each rat responded with involuntary movements that ranged from minor to major. The maximum intensity that did not cause such involuntary movements was chosen as the therapeutic intensity.

Administration of 6-OHDA

Two days after implantation of the DBS electrodes, 6-OHDA was administered stereotactically into the right striatum in the two groups of rats in which electrodes had been implanted and in the control group of rats without electrodes. The injection was given in the manner described by Cadet and Zhu.6 Each rat was anesthetized by an intraperitoneal injection of chloral hydrate (400 mg/kg) and was placed in a stereotactic frame. Each animal received two infusions of 6-OHDA (20 μg in 2 μl of normal saline containing 0.02% ascorbic acid) separated into two sites in the right striatum that corresponded to the following coordinates: AP 1.6 mm, ML 2.4, and DV 4.2; and AP 0.2, ML 2.6, and DV 7. The coordinates were determined according to information contained in the atlas of Paxinos and Watson.24 The drug was infused slowly at a rate of 1 μl/minute by using a Hamilton syringe. After infusion the needle was kept in place for 5 minutes before its removal.

Amphetamine-Induced Rotation Test

Two weeks after administration of 6-OHDA, amphetamine-induced rotation was tested in all groups. Each rat was placed in an individual cage and given 30 minutes to adapt to this environment. After acclimation, amphetamine was intraperitoneally administered (2.5 mg/kg) to each animal and the rats were observed for the following 60 minutes. The numbers of full clockwise and counterclockwise turns per minute in response to amphetamine injection were recorded. In Groups A and B, both stimulation-on and stimulation-off periods were examined. The first 20-minute period consisted of a stimulation-off period, the next 20-minute period of a stimulation-on period, and the last 20-minute period consisted of a stimulation-off period. During the stimulation-on period, the therapeutic intensity, which had been identified earlier (see Evaluation of the Behavioral Response to DBS of the STN) was used for the stimulation. These stimulus intensities ranged from 80 to 100 μA, depending on the individual rat.

Histological Assessment

On the day following motor testing, the rats were deeply anesthetized with chloral hydrate and perfused transcardially with 60 ml of saline followed by 200 ml of 4% paraformaldehyde in phosphate-buffered saline. Their brains were removed and postfixed in the same fixative for 24 hours. The fixed brains were embedded in paraffin and cut into 7-μm-thick coronal sections through the STN, pallidum, and SNc. Each section was stained with H & E prior to a standard histological examination. Sections of SNc were subjected to TH-immunohistochemical analysis. Tyrosine hydroxylase staining was performed following the modified avidin-biotin-peroxidase complex method. Anti-TH rabbit polyclonal antibody (dilution, 1:200; Affiniti Research Products, Ltd., Mannheim, UK) was applied as the primary antibody. The TH-immunoreactive neurons in the SNc were then counted, ordinarily in the section that exhibited the most discrete separation of the SNc from the ventral tegmental area by the optic tract. The ratio of TH-immunoreactive neurons on the right (treated) side to those on the left (intact) side was calculated as a percentage and compared between groups.

Statistical Analysis

The results are expressed as means ± standard errors of the means. Statistical evaluation was performed using a factorial ANOVA with the Fisher PLSD as a post hoc test. An error probability less than 0.05 was considered significant.

Results

Short-Term Behavioral Response to DBS of the STN

All rats in Groups A and B were evaluated for behavioral responses to STN stimulation 24 hours after implantation. With no or low (<40 μA) stimulation present, no behavioral changes were apparent, including any clear rotation asymmetry. As the stimulus intensity progressively increased, several stereotypical movements appeared. The characteristics of involuntary movements at various intensities are plotted in Fig. 3. With stimulation at approximately 50 μA, the rats displayed a transient left-sided dyskinetic movement on the side contralateral to the site of implantation. Generally, these occurred in the orofacial area or the forepaw and lasted for less than 1 minute. As the stimulation increased, the rats demonstrated a subtle tendency to turn slightly to the left. When the intensity of the stimulus

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was increased to approximately 120 μA, the dyskinetic movement was apparent in the left forepaw and the rats began to rotate rapidly to the left. As the stimulation intensified further, the dyskinetic movements extended to the entire body and became severe. With stimulation set at approximately 250 μA, all rats displayed generalized dyskinetic movements and tonic twisting of the body, and they often turned to lie down on their backs. The optimal therapeutic intensity for continuous stimulation was set just below the threshold for apparent dyskinetic movement in the contralateral forepaw, which ranged from 80 to 100 μA. When the rats were continuously stimulated at this therapeutic intensity, a partial diskineic movement was occasionally seen at the beginning of the stimulation, but soon disappeared. No changes in feeding, external appearance, or locomotion were noted for the following 2 weeks.

**Motor Effects of DBS of the STN in Rats New to Long-Term Stimulation**

Rats in the control group (Group C) demonstrated circling to the right (ipsilateral to the site of the 6-OHDA injection) after amphetamine administration (6.11 ± 1.19 turns/minute). Rats with implanted electrodes that had not undergone previous continuous stimulation demonstrated a circling behavior similar to that observed in Group C before the stimulation was turned on, although the number of turns (5.23 ± 1.1 turns/minute) was slightly less than that in controls. Immediately after stimulation at the therapeutic intensity (80–100 μA), the rats stopped circling and slightly veered or slowly rotated to the opposite side (−0.51 ± 0.27 turns/minute [left rotations are expressed as minus values]) (Fig. 4 upper). Compared with the rotation observed in the control group, or compared with their own rotation when stimulation was turned off, the rotation rate of rats in Group B with stimulation was significantly low (factorial ANOVA with Fisher PLSD, p = 0.0005 and p = 0.0047) (Fig. 4 center). This response was dependent on the intensity of the stimulation. We usually started by setting the therapeutic intensity just below the threshold of apparent dyskinetic movement. When the intensity was increased above the threshold, however, the rats rotated increasingly often and rapidly in the direction opposite to the rotation observed.
Neuroprotective effects of chronic STN stimulation

Motor Effects of DBS of the STN in Rats Undergoing Long-Term Stimulation

Rats undergoing 2 weeks of continuous stimulation (Group A) displayed no typical circling during the amphetamine-induced rotation test with stimulation turned either on or off (turns/minute: on, 0.54 ± 0.26; off, 0.61 ± 0.28) (Fig. 4 lower). Both values were significantly smaller than those obtained in the control group (factorial ANOVA with Fisher PLSD, p = 0.003 and p = 0.004, respectively). Although the rats rotated toward the injected side several times after administration of amphetamine, this rotation was far from a typical amphetamine-induced rotation in that it was more like ambling than circling. With the stimulation turned on, the rats often ceased their movements, and occasionally turned slightly to the opposite side. These movements seldom attained the appearance of contralateral circling, as seen during stimulation in the animals in Group B.

Localization of DBS of the STN

To identify the location of the STN, we used extracellular microelectrode recording in all rats and targeting usually required fewer than three trajectories (mean 1.4). Although we initially attempted to implant the stimulation electrode in 17 rats in Groups A and B, the sites selected in four rats (23.5%) were unsatisfactory, as shown histopathologically (two electrodes were located too deeply in the cerebral peduncle and the other two were located posteriorly, near the SNr). These four animals were subsequently excluded from the study. The success rate for localization was 76.5% (13 of 17). In these 13 rats, the tip of the implanted electrode was shown histologically to have been situated in the STN. Figure 2B illustrates the electrode location in the STN. We found that a small lesion developed after continuous stimulation in all rats. The locations and sizes of lesions in all the rats in Groups A and B are demonstrated in Fig. 5. We consider the sizes of the lesions to be acceptable because they were only slightly larger than the diameter of the electrode. Even the rats in Group B had lesions of a similar size without continuous stimulation. The mean diameters of the lesions were the following: AP 262 ± 7 μm, ML 278 ± 27 μm, and DV 468 ± 58 μm in Group A; and AP 259 ± 35 μm, ML 263 ± 29 μm, and DV 456 ± 68 μm in Group B. Most important, most STN neurons that were located beyond the lesion showed no remarkable morphological change.

Dopaminergic Neuron Loss in the SNc

Tyrosine hydroxylase-immunoreactive neurons in the ipsilateral SNc were depleted after the 6-OHDA injection in animals in Groups B and C. In contrast, these neurons were well preserved after long-term DBS of the STN (Group A) (Fig. 6). The mean numbers of TH-immunoreactive neurons in the left and right SNc were as follows: Group A, 90.3 ± 13.8 in the left and 76.1 ± 14.1 in the right; Group B, 82.6 ± 10.6 in the left and 38 ± 8.3 in the right; and Group C, 70.3 ± 7.8 in the left and 21.5 ± 5.4 in the right. The ratio of TH-immunoreactive neurons in the right SNc (injected side) to those in the left SNc (intact side) in each group was 84.8 ± 7.6% in Group A, 43.5 ± 4.7% in Group B, and 29.3 ± 5.4% in Group C. The ratio of TH-immunoreactive neurons in the animals in Group B decreased fairly similarly to those in the control group, although the ratio was slightly higher (43.5 ± 4.7% compared with 29.3 ± 5.4%, p = 0.12 [not significant]). Meanwhile, the ratio of TH-immunoreactive neurons in Group A was much higher than that measured in other two groups. Differences between Groups A and C, as well as between Groups A and B, were statistically significant (p = 0.001 and 0.003, factorial ANOVA followed by Fisher PLSD) (Fig. 7).
The results of the present study support our hypothesis that STN stimulation can improve behaviorally evident motor abnormalities and protect nigral dopaminergic neurons from 6-OHDA–induced toxicity. Two main topics will be considered here: our experimental model and the potential neuroprotective effects of DBS of the STN.

Experimental Model for Long-Term DBS of the STN

In this study we applied long-term DBS to the STN in a rat model that was designed to permit observation of awake animals during continuous stimulation of the STN. Two aspects of our model are unique. The first was the technique used to identify the location of the STN. In previous studies, investigators only used stereotactic methods according an atlas; however, placing the tip of the stimulating electrode precisely in the STN is technically difficult: the horizontal extension of the STN is at most 200 μm and the distance between the two contacts of the stimulating electrode in the STN is 100 μm. We therefore used extracellular microelectrode recording in combination with conventional stereotaxy to provide targeting guidance. The recording electrode was inserted along a horizontal line with the STN located between the ZI and the cerebellar peduncle. Firing of neurons in the STN could be distinguished fairly easily because the STN demonstrated high-frequency, irregular firing patterns, whereas the ZI and the cerebellar peduncle were relatively silent. This method was very helpful for accurate targeting in this experiment. In fact, more than half of the attempts at localization were unsuccessful without microelectrode recording in our pilot study, whereas after using electrophysiological guidance the success rate for localization virtually improved to 76.5% (13 of 17 rats).

Second, a special consideration was given to optimal stimulation parameters. Benazzouz and colleagues studied electrophysiologically the firing rates of neurons in the globus pallidus and SNr in response to DBS of the STN in anesthetized rats. The parameters of stimulation examined in that study were a frequency of 1 to 1000 Hz, a pulse width of 60 μsec, and an intensity ranging between 100 and 1000 μA. These authors concluded that most SNr neurons that were tested (91%) presented significant suppression of neuron activity in response to high-frequency stimulation delivered to the STN with parameters of 130 Hz frequency and 300 μA intensity. In other electrophysiological or microdialysis studies, similar parameters were used (frequency 130 Hz, pulse width 60 μsec, and intensity range 300–1000 μA). Considering these data, we initially assumed that a high frequency such as 130 Hz should be used; its effectiveness has been proven in animal studies and seen clinically in patients with PD. With regard to the optimal stimulation intensity in the awake rat, however, we could not obtain guidance from previous reports. In a preliminary study, we initially used an intensity greater than 300 μA, but saw a high incidence of severe and extensive dyskinetic movements. We assumed that these dyskinetic movements resembled abnormal involuntary movements, which were sometimes provoked by inappropriate stimulation in humans. Furthermore, we assumed that some rats receiving such high-intensity stimulation exhibited severe destruction in the STN and surrounding tissues. We concluded that a difference existed between the stimulation intensities, causing behavioral changes in awake rats, and intensities that cause changes that are only apparent electrophysiologically in the anesthetized rat. We therefore decided to use a relatively low intensity, which was determined by the essential absence of behavioral phenomena caused by stimulation (80–100 μA). In fact, with continuous stimulation at this “therapeutic intensity,” no rat exhibited changes in feeding, external appearance, or locomotion during 2 weeks of observation. Furthermore, all rats in Group B exhibited an immediate decrease in amphetamine-induced circling in response to stimulation at this therapeutic intensity. Thus, we believed that the stimulus intensity that we selected was suitable for our study. Altogether, our model of DBS of the STN enabled us to evaluate behavioral changes during long-term stimulation and to analyze the motor and neuroprotective benefits conferred by long-term stimulation. We believe that this model will prove to be useful for further studies related to DBS, including treatment in combination with neurotrophic factors or neuronal transplantation, or possibly in assessing new treatments for epilepsy.

Neuroprotective Effects of DBS of the STN

We placed the DBS electrode into the STN before delivery of the 6-OHDA insult; therefore, considerable controversy surrounds the justification of the experimental design for neuroprotection, with regard to the timing of treatment. Nevertheless, this “pretreatment design” has been used often in previous research on neuroprotection, mainly because the neurotoxic process of 6-OHDA could be completed in the very early phase after administration. Consequently, further evaluation should be performed using a more sophisticated experimental design; however, it is significant
ly important to demonstrate neuroprotective effects in this commonly used design as the first step.

In this study, the neuroprotective effect on dopaminergic neurons in the SNc due to long-term DBS of the STN was demonstrated by the circling behavior of the rats and the immunohistochemical data. Although some reports have presented similar immunohistochemical results after STN lesioning by kainic acid or ibotenic acid,7,26,27 our study is the first to demonstrate these data as they relate to DBS of the STN in awake hemiparkinsonian rats. In rats that underwent electrode implantation in the STN without continuous stimulation (Group B), nearly as many SNc neurons were lost as a result of the striatal 6-OHDA injection as in control animals, whereas in rats that received STN stimulation continuously for 2 weeks (Group A) the SNc neurons were protected from this insult. Although a small lesion developed after continuous stimulation, the size of the lesion was similar to that in the noncontinuously stimulated rats in Group B, in which the loss of dopaminergic neurons was equivalent to that in controls, and we therefore concluded that long-term stimulation was necessary to protect the SNc neurons. The results of the amphetamine-induced rotation test correlated well with the immunohistochemical findings. We assumed that the reason why rats in Group A did not display typical circling behavior after amphetamine administration, irrespective of stimulation during the test, was that the SNc dopaminergic neurons on the treated side were sufficiently preserved to prevent or minimize a drug-induced abnormality in rotation. On the other hand, the rats in Group B displayed typical circling behavior before the stimulation was given. Apparently, their SNc dopaminergic neurons were compromised to an extent, causing rotation asymmetry. Acute stimulation immediately reversed the asymmetry. This result paralleled the clinical observation that DBS of the STN can improve major motor symptoms in patients with PD immediately after stimulation. Recently, Meissner and associates17 showed similar changes in amphetamine-induced rotation behavior after DBS of the STN in awake rats. In addition, by using microdialysis they showed increased dopamine metabolism in the striatum during acute stimulation. Thus, an immediate response in Group B of our study can be explained by an immediate increase in striatal dopamine after stimulation.

Although controversy persists as to whether DBS inhibits its neuronal activity in the stimulated structure and, consequently, decreases output from it, our results fit a hypothesis that DBS decreases output from the STN that is directed to the SN. We offer one possible explanation of our results for both motor improvement and neuroprotection of SNc dopaminergic neurons in the following paragraph and Fig. 8.

In PD or in 6-OHDA–induced nigrostriatal degeneration, the neurons in the STN and the SNr are hyperactive, whereas the neurons in the SNc are strongly suppressed by GABAergic control from the SNr.20 Major STN output pathways include glutaminergic excitatory fibers to the SNr, with some fibers reaching the SNc through the SNr.22 With the hyperactivity of the STN suppressed by DBS of the STN, excessive excitation of the SNr is suppressed and SNc neurons are activated as a result. Residual dopaminergic neurons in the SNc then release dopamine to the striatum, causing an immediate improvement in rotation symmetry. This is supported by microdialysis studies in which increased striatal dopamine metabolism after STN DBS has been demonstrated.5,17,18,23,30 In some clinical studies decreases in the dose of levodopa were possible after initiating DBS of the STN in patients with PD,14,32 implying increased dopamine metabolism. On the other hand, excessive glutamate exerts neurotoxic effects via the N-methyl-D-aspartate receptor, especially when intracellular metabolism is suppressed in target cells.21,28 After injection of 6-OHDA, metabolism in the SNc neurons is thought to be strongly suppressed by the retrograde neurotoxic effects of 6-OHDA, reflecting damage to the mithochondrial electron transport system. In this circumstance, excessive glutamate from an excessively activated STN will have a neurotoxic effect, resulting in further degeneration of dopaminergic neurons in the SNc. With DBS of the STN, hyperactivity in the STN is suppressed and excessive glutamate output is thus reduced. Furthermore, stimulation decreases GABAergic input to the SNr and therefore suppression of SNc neuronal activity by the SNr is decreased, increasing neural activity and intracellular metabolism in the SNc. The excessive glutamate is relatively less neurotoxic and dopaminergic neurons in the SNc are preserved from further degeneration.

Some recent reports have concluded that DBS of the STN does not suppress neuronal activities in the internal segment of the globus pallidus or in the SNr, and might not increase dopamine metabolism in the striatum.13,33,34 Vitek33 and Hashimoto, et al.,31 have mentioned that benefits from DBS of the STN cannot be simply explained by excitation or inhibition and that alteration of neuronal firing patterns should be considered. Hilker and colleagues13 recently re-

Fig. 8. A schematic diagram showing the hypothesis for the neuroprotection granted by STN stimulation. Black arrows represent inhibitory connections and white arrows represent excitatory connections. Upper: Before stimulation, neurons in the STN and the SNr are hyperactive, whereas neurons in the SNc are suppressed by GABAergic control from the SNr. Excessive glutamate from an excessively activated STN has a neurotoxic effect, resulting in further degeneration of dopaminergic neurons in the SNc. Lower: After stimulation, the hyperactivity of neurons in the STN is suppressed, excessive excitation in the SNr is suppressed, and SNc neurons are activated as a result. Residual dopaminergic neurons in the SNc then release dopamine to the striatum, causing an immediate improvement in rotation symmetry. Because excessive glutamate output from the STN is reduced and intracellular metabolism in the SNc is increased, glutamate is relatively less neurotoxic, and dopaminergic neurons in the SNc are preserved from further degeneration.
ported that according to a positron emission tomography study in which a dopamine D2/3 receptor ligand, [11C]raclopride, was used, patients with PD who were treated with DBS of the STN showed no significant difference between stimulation-on and stimulation-off conditions. If DBS of the STN does not suppress output from the STN, motor improvement and neuroprotective effects are difficult to explain in terms of neurotransmitters and consideration of other mechanisms is required. The effects of acute and continuous stimulation on neurotransmitters may differ. Rather than reflecting a decrease in glutamate neurotoxicity, neuroprotection in the SNc might simply involve a decrease in 6-OHDA uptake by nerve terminals in the striatum, which is induced by unknown effects of DBS of the STN. To determine the true mechanism, further studies are necessary. Microdialysis evaluation of various neurotransmitters during long-term stimulation in our model therefore should be of great interest.

Conclusions

The present study showed immunohistologically and behaviorally that DBS of the STN could prevent further degeneration and dysfunction of SNc neurons in a rat model of PD. This finding may have important implications for the further development of neuroprotective therapeutic strategies. One hypothesis that could explain neuroprotection would involve suppression of glutamate input to the SNc from a hyperactivated STN. Further studies including microdialysis may cast important light on this issue. In addition, the rat model of long-term DBS of the STN used in the current study is reasonably convenient and highly useful for evaluation during continuous stimulation in awake rats. We believe that this model can contribute importantly to future experiments concerning DBS of the STN.

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Manuscript received May 13, 2003. Accepted in final form December 17, 2003. This study was supported in part by the Uehara Memorial Foundation. Portions of this work were presented at the annual meeting of the Congress of Neurological Surgeons, Denver, Colorado, October 2003.

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