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High-Frequency Stimulation of the Subthalamic Nucleus Restores Neural and Behavioral Functions During Reaction Time Task in a Rat Model of Parkinson's Disease

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Abstract

Deep brain stimulation (DBS) has been used in the clinic to treat Parkinson's disease (PD) and other neuropsychiatric disorders. Our previous work has shown that DBS in the subthalamic nucleus (STN) can improve major motor deficits, and induce a variety of neural responses in rats with unilateral dopamine (DA) lesions. In the present study, we examined the effect of STN DBS on reaction time (RT) performance and parallel changes in neural activity in the cortico-basal ganglia regions of partially bilateral DA-lesioned rats. We recorded neural activity with a multiple-channel single-unit electrode system in the primary motor cortex (MI), the STN, and the substantia nigra pars reticulata (SNr) during RT test. RT performance was severely impaired following bilateral injection of 6-OHDA into the dorsolateral part of the striatum. In parallel with such behavioral impairments, the number of responsive neurons to different behavioral events was remarkably decreased after DA lesion. Bilateral STN DBS improved RT performance in 6-OHDA lesioned rats, and restored operational behavior-related neural responses in cortico-basal ganglia regions. These behavioral and electrophysiological effects of DBS lasted nearly an hour after DBS termination. These results demonstrate that a partial DA lesion-induced impairment of RT performance is associated with changes in neural activity in the cortico-basal ganglia circuit. Furthermore, STN DBS can reverse changes in behavior and neural activity caused by partial DA depletion. The observed long-lasting beneficial effect of STN DBS suggests the involvement of the mechanism of neural plasticity in modulating corticobasal ganglia circuits.

Keywords

deep brain stimulation; electrophysiology; striatum; motor cortex; single-unit recording

Reaction time (RT) tasks that require appropriate response to unpredictable stimuli are impaired in Parkinsonian patients, which have been attributed to both attentional and motor deficits (Evarts et al., 1981; Bloxham et al., 1987; Abbruzzese and Berardelli, 2003). In experimental animal models of Parkinson's disease (PD), rats with nigrostriatal dopamine (DA) lesions show deficits in RT tasks (Amalric and Koob, 1987; Amalric et al., 1995). Unilateral DA lesions induce an increase in RT on the side contralateral to the lesion, and a preferential response bias on the side ipsilateral to the lesion (Carli et al., 1989; Phillips and Brown, 1999). Bilateral partial DA lesions are associated with prolonged RT and a reduction in overall correct responses (Amalric and Koob, 1987; Moukhles et al., 1994).

Numerous studies have indicated that correct execution of an RT task requires coordination of the entire thalamocortical basal ganglia circuitry (Amalric et al., 1994; Baunez et al., 1995). Electrophysiological studies have identified neural responses in the basal ganglia that are associated with cue presentation, movement initiation, and movement execution (Gardiner and Kitai, 1992; Shi et al., 2004a). According to the classic model (Alexander et al., 1986; Alexander and Crutcher, 1990), DA depletion leads to disruption of cortical basal ganglia circuits, i.e., over-activation in the subthalamic nucleus (STN) and basal ganglia output structures, including the globus pallidus internal (GPi) and the substantia nigra pars reticulata (SNr). In support of this model is the observation that, following MPTP treatment in the monkey, the ratio of inhibitory/excitatory activity in the GPi is significantly decreased during passive arm movement (Boraud et al., 2000). In addition, our previous study in behaving rats showed an increased activity in the STN and SNr after DA depletion, which is in partial agreement with the classic model (Chang et al., 2006).

Deep brain stimulation (DBS) in the STN has been commonly applied to treat PD (Benazzouz et al., 1993; Benabid et al., 2000; Krause et al., 2001; Fang et al., 2006). In spite of remarkable clinical effectiveness, the mechanisms of DBS are still unclear. Although predominant inhibitory responses have been reported in the stimulation site during STN DBS (Dostrovsky and Lozano, 2002; Magarinos-Ascone et al., 2002; Filali et al., 2004; Shi et al., 2006), more complex responses have been demonstrated (Windels et al., 2000; Garcia et al., 2003; Hashimoto et al., 2003; Maurice et al., 2003). A lasting inhibition of GPi/SNr neurons was observed in anesthetized rats during STN HFS (Benazzouz et al., 1995; Tai et al., 2003), whereas variable responses were found in SNr neurons during STN DBS. In anesthetized rats, the spontaneous firing of SNr cells was either increased or decreased with a global enhancement of the firing rate in the overall population of SNr cells (Degos et al., 2005). The activity of SNr cells was decreased at low-intensity stimulation and increased at a higher intensity (Maurice et al., 2003). Moreover, nearly equal numbers of excitatory and inhibitory responses were found in SNr in a treadmill-locomotion task under behaviorally effective DBS (Shi et al., 2006). As part of the limbic circuit, the STN may also be involved in regulation of cognitive function (Hamani et al., 2004; Haegelen et al., 2009). Thus, in addition to its clear effect on PD motor deficits, STN DBS may exert a broader impact on cognitive functions (Temel et al., 2005a, 2005b, 2006).

In the present study, a chronic, multi-channel single-unit recording technique was employed to simultaneously record neural activity from the MI, SNr and STN of rats performing RT tasks, before and after a DA lesion. Bilateral STN DBS was applied to evaluate its effects on

RT performance and associated neural responses. The goal of the present study is to examine neural mechanisms whereby STN DBS modulates motor and cognitive functions.

MATERIALS AND METHODS

Subjects

Sixteen male Sprague-Dawley rats weighing 200–250 g at the start of the experiment were used in this study. Each rat was individually housed, and all rats were maintained on a reverse 12-hr light/dark cycle (lights off from 8:00–20:00) for at least 7 days before training. Food was provided ad libitum throughout the duration of the experiment. Animals were water-deprived for 24–36 hr before the training for the RT task, and thereafter provided a small amount of water each day, to maintain at least 85% of their pre-water deprivation body weight during the experiment. All procedures were in accordance with the guidelines published in the *NIH Guide for the Care and Use of Laboratory Animals* and the principles presented in the *Guidelines for the Use of Animals in Neuroscience Research* by the Society for Neuroscience.

Surgery

Four days before surgery, rats were allowed free access to water. Animals were anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.), then mounted on a Kopf stereotaxic apparatus. Supplementary doses of ketamine/xylazine were given when necessary to maintain anesthesia. Penicillin (60,000 U, i.m.) was administered after surgery to prevent infection.

Microelectrode array implantation—All rats were implanted bilaterally into three brain areas. Eight-wire stainless steel microarrays (50- μ m diameter, Biographics Inc., Winston-Salem, NC), soldered to connecting pins on a connector (Omnetics Inc., Minneapolis, MN), were slowly lowered into primary motor cortex (MI) and substantia nigra pars reticulata (SNr). Ten-wire platinum-iridium microarrays (8 for recording and the additional 2 for stimulation) were implanted in STN to provide more efficient high frequency stimulation (HFS).

The stereotaxic coordinates used to target these structures were based on the atlas of Paxinos and Watson (1986). The stereotaxic coordinates were: for the MI, 0 mm anterior to bregma (A), 1.5 mm lateral to the midline (L), and 1.6 mm ventral to the cortical surface (V); for STN, –3.5 mm A, 2.5 mm L, and 7.2 mm V; and for SNr, –5.2 mm A, 2.0 mm L, and 7.7 mm V. Six screws were placed for anchoring the dental cement headset. Four ground wires were positioned around the lateral screws about 2 mm ventral to the cortical surface.

Preparation of the cranium and surgical recovery—In addition, two 26-gauge cannulae were implanted 2-mm above the dorsolateral striatum, at 1.0 mm A and 3.5 mm L bilaterally. These cannulae were later used for injection of the chemical lesion agent. At the end of surgery, the headstage and cannulae were embedded onto the cranium with dental acrylic cement. All rats were housed individually after surgery, and allowed to recover for at least 10 days before the behavioral testing and physiological recordings.

Bilateral 6-OHDA Lesions in the Dorsolateral Striatum

After completion of the control experiments, each rat received bilateral chemical lesions of the nigrostriatal DA system. First, two 31-gauge needles were inserted into each cannula, and the tip of each needle was extended into the brain 2 mm beyond the cannulae (4 mm ventral to the surface of cortex). A solution of 6-hydroxydopamine (6-OHDA, Sigma Aldrich, St. Louis, Missouri; dosage: 16 µg/4 µl saline with 0.1% ascorbic acid) was then infused slowly into the dorsolateral striatum. After the 8-min injection period, the injection needle was left in place for at least 2 min to prevent fluid leaking into the injection track.

Electrophysiological Recording

Neuroelectric signals were detected by the implanted microwires. Signals were passed from these wires to the head-stage assemblies, and then to a preamplifier, via three light-weight cables and a commutator. Light-weight cables connected the detachable headstage to a rotating commutator on the ceiling of the chamber, which allowed the rat to move freely about the chamber. The signals were then filtered (between 0.5 and 5 kHz, with a 6 dB cutoff) before they were sent to a multichannel spike-sorting device.

Spike activities were displayed on a computer monitor. There, all traces were continually monitored in windows with defined amplitude and duration. Spike activity and behavioral events were both recorded as timestamps at a 5-kHz sample rate. Data collection was controlled with a PC-based acquisition software Magnet (Biographics Inc., Winston-Salem, NC). The identity of clearly-sorted single units was verified by autocorrelogram and high quality, constant waveform with signal-to-noise ratio greater than 3. Spike analog waveforms were simultaneously monitored with digital storage oscilloscope (DSO) 2102 M (Link Instruments Inc.) during recording (see Supp. Info. Fig. 2). Data were then analyzed offline with Stranger (Biographics Inc.) and NeuroExplorer (Plexon Inc., Dallas, TX) software. Animal behaviors during the experimental sessions were videotaped with an imbedded time line synchronized with neuronal activity data for offline analysis.

Deep Brain Stimulation

DBS was delivered bilaterally to the STN, in a bipolar configuration. Charge-balanced pulses (60-µs stimulation pulse counterbalanced by 33% current, 180-µs opposite current) were programmed into the DS-8000 digital stimulator and DLS-100 digital linear isolator (World Precision Instrument, Inc., Sarasota, FL). This charge-balanced pulse was delivered through platinum-iridium electrodes (50 µm in diameter) and incorporated to minimize tissue damage caused by HFS. The stimulation parameters were: 130 Hz and 30–60 µA current. These parameters were adjusted below a threshold that would cause any visible behavioral responses, such as moving around or twisting of facial muscles. The stimulation pulse was triggered by a Magnet System RT Protocol program. Stimulation started 30 min after the beginning of the experimental session, and lasted for 20–30 min. During HFS, the remaining eight microwires in the bundle were used to record neural activity in the STN stimulation site.

Additional Supporting Information may be found in the online version of this article.

Data Analysis

Behavioral analysis—The RT performance data from 4 consecutive experimental sessions both before and after DA lesion were pooled together. Performance accuracy was measured by calculating the proportion of correct trials as a percentage of the total number of trials. Error response (early and late) was also calculated in the same manner, but separately. RT performance data from intact rats, partial DA-lesioned rats, and DA-lesioned with STN DBS rats were compared by Chi-square test.

Neural response analysis—Data were processed offline with the software Stranger (Biographics Inc.) and Neuro-Explorer (Plexon Inc.) for basic analysis and graphics. MatLab (Mathworks Inc. Natick, MA) and SPSS (SPSS Inc., Chicago, IL) were used for advanced statistical analysis. Data were analyzed from one representative session from each rat.

RESULTS

Behavioral and Electrophysiological Responses During the RT Task in Intact and DA-Lesioned Brains

Rats were trained to reach a 60–70% correct responses rate over four consecutive sessions. Incorrect trials were mostly due to early responses.

Behavioral changes after DA lesion—In 6/16 rats, there was no significant difference in performance following the 6-OHDA lesion (data not shown). Immunohistochemical analysis in these six rats revealed that their 6-OHDA lesions were not substantial. Thus, the data from these 6 rats were excluded from further analysis. In the remaining 10 rats, there was a profound decrease in general motor activity immediately after the lesion, and 3 of these rats could hardly perform the task. Performance gradually stabilized over 3–5 weeks, after which reproducible post-lesion results were obtained. A significant decrease in correct responses ($P < 0.001$, *Chi-square* test) was observed in all 10 rats tested (Table I).

The nature of the behavioral deficits varied among subjects. Early responses were substantially increased in 5 subjects, compared to the pre-lesion level. Late responses were increased in 3 rats, compared to the pre-lesion level. Both early and late responses were markedly increased in the remaining 2 rats (Table I). The pool-up cumulative distribution curve of RT response after dopamine depletion was shifted significantly toward longer response time (Mann-Whitney test, $P < 0.001$, see Fig. 1).

Electrophysiological changes after DA lesion—Extracellular single unit activities were recorded simultaneously from the MI, SNr and STN during the RT task. A total of 279 neurons were recorded from 10 rats in intact sessions: 108 in the MI, 98 in the SNr, and 73 in the STN. After 6-OHDA lesion, 263 neurons were recorded: 95 in the MI, 95 in the SNr, and 73 in the STN. Among all observed neural responses, there were more excitatory responses than inhibitory and biphasic responses. Neurons that exhibited excitatory, inhibitory or biphasic responses were collectively called responsive neurons, as they related to corresponding events (Fig. 2).

The numbers of responsive neurons were reduced significantly after DA lesion in comparison with intact condition (Fig. 3). For the self-initiated nosepoke behavior, the numbers of responsive neurons were reduced in all three recording areas (from 44.4% to 26.3% in the MI, $\chi^2 = 7.213$, $P = 0.0072$; from 50.0% to 25.3% in the SNr, $\chi^2 = 12.55$, $P = 0.0004$; and from 41.1% to 24.7% in the STN, $\chi^2 = 4.469$, $P = 0.0345$). For another self-initiated behavior lever press, the numbers of responsive neurons decreased significantly in the MI (from 57.4% to 36.8%, $\chi^2 = 8.567$, $P = 0.0034$) and the SNr (53.1% to 37.9%, $\chi^2 = 4.473$, $P = 0.0344$) but not the STN (from 53.4% to 43.8%, $\chi^2 = 1.343$, $P = 0.2528$). However, these numbers were not significantly changed for the stimulus-triggered behaviors such as lever release (the rats had to respond quickly as soon as a cue tone was perceived), (in MI, $\chi^2 = 0.9357$, $P = 0.3334$; in SNr, $\chi^2 = 0.8447$, $P = 0.3581$; in STN, $\chi^2 = 0.1301$, $P = 0.7183$). In addition, about 8–10% of neurons showed responses to sensory signals such as the cued light and the cue tone, which were not altered after the DA lesion (data not shown).

Effects of STN DBS on RT Performance and Electrophysiological Responses in the Cortico-Basal Ganglia Circuit

Behavioral effect of DBS—Bilateral STN DBS improved RT performance in 6/10 rats with recognized impaired task performance (Table II). Histological examination confirmed that bilateral electrodes were correctly localized in the STN in all of these 6 rats. In the remaining four rats, the electrodes missed the target. Among these 6 rats with successful DA-lesions there were more late responses during an entire experimental session, apparent in response curve plotting. As indicated in Figure 4 as a representative example, the late response curve appeared above the correct response curve (Fig. 4A). Whereas correct responses notably increased following STN DBS, this increase continued even after DBS ceased (from 3800 to 7000 sec; Table II and Fig. 4B). In addition, in the session without DBS, the percentage of correct responses (20%) and late responses (60%) were sustained (Fig. 4C). In contrast, following STN DBS, the correct response percentage increased, and the late response percentage rapidly decreased. This effect was maintained till the termination of the session (Fig. 4D). Furthermore, the collective reaction time of all response categories was significantly lower during ($P < 0.01$) and after ($P < 0.05$) STN DBS, compared to the pre-stimulation condition (see Fig. 4E).

Effect of DBS on neuronal responses—In the DBS experiment, a total of 189 neurons were recorded from six rats. There were 62 recorded in the MI, 68 in the SNr, and 59 in the STN. In parallel with the behavioral improvement, HFS elicited a variety of changes in neural activity in these brain regions.

A cluster analysis of neural firing rate in all targeted brain regions during and after DBS revealed different patterns of response. The firing rate of each neuron was first normalized as z-scores to the baseline firing rate (pre-stimulation period). This allowed for detection of statistical significance and enabled comparison among neurons with different discharge rate.

There were four different clusters of neural firing patterns in response to STN DBS. Cluster 1 (C1 in Fig. 5A) neurons showed increased firing during stimulation; the robust responses

appeared at the onset of DBS, and gradually reduced until the end of the DBS period. Cluster 2 neurons showed moderate yet persistent firing increase during STN DBS. Cluster 3 neurons showed long-lasting, post-stimulation increase of spike activities. Cluster 4 neurons comprised the majority; these neurons exhibited substantial firing decrease during DBS, and returned to control levels after the stimulation was turned off (see Fig. 5).

Similar proportions of firing increase neurons were found between the recorded brain areas, and were generally around 20% of the recorded neural population (Table III). Firing increase responses following the cessation of DBS were most prominent in the MI (Fig. 5B), which suggests that disinhibition of MI neurons may account for improved behavioral performances observed after the cessation of STN DBS, as displayed in Figure 4.

A long-lasting neural modulation by STN DBS was also observed (Fig. 6). In one example, a STN neuron showed no obvious responses around lever press behavior before and during STN DBS. However, after the DBS was turned off, the same neuron showed a biphasic response, with an increase of firing before the lever press, and a decrease of spike activity after the lever press. These newly emerged responses lasted over the remainder of the experimental session. In addition, the baseline firing rate of this neuron increased during and after DBS, compared to the pre-stimulation period. These results suggest a long-term modulation of the cortico-basal ganglia circuit by STN DBS. A switch from non-responsive to responsive stage with DBS may signify enhanced neural coding for improved RT performances.

The number of responsive neurons in different operational behaviors increased after the cessation of DBS. There was a significant increase in the number of responsive neurons in the following brain areas and conditions: in the SNr (*Chi-square* = 6.864, *P* = 0.0088) and STN (*Chi-square* = 4.052, *P* = 0.0441) during nosepoke (in the MI, *Chi-square* = 5.2037, *P* = 0.1535); in the MI (*Chi-square* = 3.969, *P* = 0.0463) and SNr (*Chi-square* = 6.287, *P* = 0.0122) during lever press (in the STN, *Chi-square* = 2.127, *P* = 0.1447); and in the SNr (*Chi-square* = 4.080, *P* = 0.0434) during lever release (in the MI, *Chi-square* = 2.062, *P* = 0.1510, in the STN, *Chi-square* = 2.842, *P* = 0.0918). Interestingly, the SNr, a basal ganglia output site as well as a direct target of the STN glutamate projection, exhibited an increase in the number of responsive neurons during all three behavioral events (Fig. 6B).

DISCUSSION

The present study revealed a long lasting effect of STN DBS on a simple RT task, and confirmed the finding that RT performance in rats is severely impaired after partial bilateral striatal 6-OHDA lesion (Amalric and Koob, 1987; Smith et al., 2002; Temel et al., 2005b). Furthermore, these results have demonstrated a parallel neural response changes in cortico-basal ganglia loops. In general, the number of neurons responsive to operational events decreased after partial DA lesions. STN DBS, at intensities that did not induce any side effects, significantly improved RT performance and restored neural responses to different behavioral events.

The most common behavioral impact of a DA lesion in our subjects is a significant decrease in correct responses. This is reflected in the increase of either delayed or premature responses. The former may reflect akinesia signs common to PD, and the latter is likely due to either a motor weakness and postural instability which impaired the animal's ability to maintain pressure on the lever, or an impairment of cognitive functions, such as attention (Evarts et al., 1981; Bloxham et al., 1987; Moukhles et al., 1994; Amalric et al., 1995; Baunez et al., 1995; Smith et al., 2002). Our study confirmed the findings that DA lesions increased both premature and late responses in animals performing different RT tasks (Amalric et al., 1995; Temel et al., 2005b).

Impairment in RT task performance was previously reported among rats with partial DA depletion (Amalric and Koob, 1987). Interestingly, similar impairments were seen in patients with PD (Montgomery et al., 1991). Pharmacological studies suggested that RT deficits might be mediated by D2 receptors, since D2- but not D1- or D3- antagonists, could induce RT deficits similar to those observed under DA depletion conditions (Amalric and Koob, 1987; 1989; Coffin et al., 1989; Amalric et al., 1993, 1995; Smith et al., 2000).

The assertion that RT tasks are D2-mediated strengthens the rationale for applying STN DBS to improve RT performance. D2-expressing medium spiny neurons are striatopallidal projection neurons of the indirect pathway. They project to the STN, the pivotal structure that connects cortical and striatal inputs to the basal ganglia output structures. Within the STN, thalamocortical basal ganglia pathways are topographically divided into motor, limbic and association circuits (Hamani et al., 2004; Haegelen et al., 2009). This functional anatomic organization of basal ganglia circuits is the basis for the involvement of the STN in a variety of physiological functions. In addition to motor regulation, the STN also plays a significant role in multiple cognitive functions.

STN has been found to have significant impact on RT performance. It has been demonstrated that lesion of the STN increases premature responses in the RT task, while bilateral stimulation of STN in normal rats decreased the premature responses (Desbonnet et al., 2004). Previously, STN DBS has also been tested in DA-lesioned rats performing different RT tasks (Darbaky et al., 2003; Temel et al., 2005b). Among unilaterally DA-lesioned rats performing choice RT tasks, STN DBS significantly reduced contralateral neglect only in animals with mild DA lesions (Darbaky et al., 2003). Other studies applied a mild bilateral striatal DA lesion protocol, similar to the one we used in the current study, and reported a stimulation intensity-dependent effect on different behavioral contexts of choice RT tasks (Temel et al., 2005b). One of these studies showed that, at low intensity (3 μ A), STN DBS reduced premature responses, while at high intensity (30 μ A), STN DBS restored RT and motor response time, among partially and bilaterally DA-lesioned rats. Thus, the authors proposed the existence of segregated cognitive and motor circuits that can be regulated by different stimulation parameters.

Our study demonstrated that, with a simple RT paradigm, STN DBS could normalize 6-OHDA-induced deficits in RT performance. This beneficial effect was only found in rats with correct electrode placement in the STN. This finding confirmed a specific role for STN in improving RT performance. In addition, the moderate stimulation intensities that did not

cause behavioral interferences, improved both motor and cognitive functions, as demonstrated by the observed reduction in both late and premature responses.

One of the most interesting results in our study were the long-lasting effects we observed with STN DBS, that extended up to an hour after DBS was ceased. Such long-lasting effects have not been observed with major motor deficits, such as bradykinesia in PD animal models and PD patients (Chang et al., 2003; Temperli et al., 2003; Shi et al., 2004b). The long-lasting effect observed in the current study may be explained by two factors. First, the bilateral striatal lesions applied in the current study induced mild DA depletion, much less severe than the cases in PD patients and medial forebrain bundle lesion animal model of PD wherein more than 90% of DA is depleted. Second, unlike the predominant motor task focus of previous studies and clinical evaluations, the focus on reaction time in our current study takes into account both motor and cognitive elements. The effect of STN DBS on severe motor impairment may disappear quickly after DBS was ceased, yet the effect on mild motor impairment and cognitive function may indeed outlast the stimulation period.

In parallel with these long-lasting behavioral improvements, we observed corresponding changes in neural activity. These changes also lasted about an hour after the termination of DBS. Such closely correlated, long-term effects on both behavioral and neural responses suggest an abnormal and plastic cortico-basal ganglia circuit that is sensitive to behaviorally-effective STN DBS. Previous studies in the slice preparation have demonstrated HFS-induced neural plasticity in STN neurons; both LTP and LTD can be induced by HFS of the STN (Shen et al., 2003). These STN HFS-induced neural plasticity mechanisms may support the long-term changes in neural responses observed in the present study. These long-lasting changes in neural activity include baseline firing rate changes, as well as specific behavioral event-related neural responses.

An additional result from our study was that the number of neurons responding to operational behavioral events decreased in all the recorded brain areas after partial DA lesion, alongside a concurrent worsening of RT performance. These observations likely reflect a disrupted neural coding in the cortico-basal ganglia circuit via DA depletion. Such disruption was more evident during nose-poke and lever press events, and did not appear during lever release. Interestingly, nosepoke and lever press belong to self-initiated behavioral events, while the lever release is a response triggered by external cues. These results are in agreement with previous findings that self-initiated movements are more greatly affected by PD than externally triggered motor actions (Sabatini et al., 2000). Furthermore, lever release is associated with the speed of RT. This dissociation of changes in neural firing rate and RT speed suggests that other forms of neural coding may be responsible for speed changes. On the other hand, rate coding may play a significant role in the attention process during nosepoke and lever press events.

Another main finding of our study is that STN DBS improved RT performance and restored behavioral event-related neural responses with specific characteristics (Fig. 6B). Among all recorded brain areas, SNr neurons exhibited the most significant increase in event-related neural responses, across all three behaviors (nosepoke, lever press and lever release). In MI neurons, a significant increase in event-related neural responses only occurred during lever

press. In STN neurons, an increase in event-related neural responses only occurred during nosepoke. These results demonstrate a partial restoration of rate coding of specific behavioral events, and, as the SNr is the main output site of the basal ganglia, they suggest that STN DBS exerts profound and long lasting therapeutic effects on the information outflow of the basal ganglia.

The motor cortex exhibited prominent long-term responses to STN DBS in terms of containing the largest portion of cluster 3 neurons in Figure 6. It is documented that effective DBS could also restore appropriate task-dependent recruitment of cerebral activity (Grafton et al., 2006), and normalize the reduced metabolism in cortical motor areas (Davis et al., 1997; Fukuda et al., 2001). In addition, STN DBS can induce resonate anti-dromic activation of cortical circuits and suppress slow wave and beta band oscillations (Li et al., 2007; Gradinaru et al., 2009), suggesting a modulation of cortical activity which may contribute to the therapeutic effect of STN DBS. The long lasting cortical responses found in the present study provide additional supporting evidence for the role of cortex in mediating DBS effects. Unlike the tentative mechanism of ‘disrupting the neural circuit’ during advanced PD motor symptoms (Lozano et al., 2002), the effects of STN DBS in the current study seems to restore, rather than disrupt, the cortico-basal ganglia circuit affected by partial DA depletion.

In addition, our current study has revealed more prominent firing increase responses to STN DBS, both during and after stimulation (especially in MI), than observed in our previous studies. Previously, STN DBS during a treadmill locomotion task induced predominantly inhibitory responses in STN stimulation site (Shi et al., 2006). Conflicting results have been reported in regard to neural responses of basal ganglia output sites to STN DBS. Both increase and decrease in firing rate in the SNr have been elicited by STN DBS depending on the stimulation intensity in anesthetized rats (Degos et al., 2005). On the other hand, predominant excitatory responses were found in the GPi when behaviorally effective STN DBS was delivered (Hashimoto et al., 2003). Our studies in behavioral rats showed nearly equal proportions of excitatory and inhibitory responses in the SNr during behaviorally effective DBS (Shi et al., 2006). However, the STN DBS induced inhibitory responses in the SNr dramatically increased when the rat performed a limb use asymmetry (LUA) task (Shi et al., 2005). Similarly, task-dependent neural responses to DBS have also been reported in human patients with PD (Grafton et al., 2006; Campbell et al., 2008). Such behavioral task-dependent neural responses to DBS suggest that this stimulation fuels a dynamic process that regulates ongoing behavioral performances, by integrating environmental inputs and internally-generated motivation.

This study also provides additional evidence for context-dependent behavioral and neural responses to DBS. Unlike the results observed with DBS during two types of motor performance, the forced treadmill locomotion vs. self-initiated rearing movement in LUA, the observations made with the RT task add a cognitive dimension to the behavioral context. Specifically, increased neural activity was induced by STN DBS in all recorded brain areas, and these results are in sharp contrast to the prevalent inhibitory responses observed with STN DBS and basic motor tasks (Shi et al., 2005, 2006). This overall difference between neural responses observed in this and our previous basic motor studies may also be

attributed to the differences in DA lesion severity. The relatively mild striatal lesions performed in this study may preserve more DA function, which may account for the higher prevalence of excitation.

In conclusion, this exploratory study demonstrated that bilateral high frequency stimulation in the STN can improve impaired RT performance caused by DA depletion. These findings suggest that motor deficits, and possibly associated cognitive deficits, can be corrected with HFS applied in the STN. Our electrophysiological recordings concurrent to RT performance suggest that both network regulation in the cortico-basal ganglia circuit and task-dependent dynamic modulation can be the basis for therapeutic mechanisms associated with DBS treatment. Our view is that DBS in this study may restore and sustain for a time a background pattern of activity established by recurrent activity within the cortical striatal looping system. Such distributed activity states, perhaps molded from modules or circuits mediating of working memory function, may be needed to normalize the cognitive aspect of function needed for proper RT performance.

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Fig. 1.
The cumulative distribution of RT in 10 rats before and after successful DA depletion. Correct and late responses are summed up together in the distribution. Grey and dark lines indicate the result during the pre-lesion (naïve) and post-lesion (PD) sessions, respectively. Marked right-shift of the cumulative frequency distribution curve can be seen after DA depletion.

Fig. 2.

Examples of typical neural responses to different behavioral events. Raster and peri-event histograms during RT tasks are displayed for each neuron. Panels **A–D** and **E** representatively illustrate neural response (excitatory and inhibitory) to following events: cuelight, nosepoke, lever press, tone, and lever release, respectively. Panel **F** shows two typical neurons with biphasic responses (excitatory/inhibitory or inhibitory/excitatory).

Fig. 3.

A summary of neural responses from different brain regions before and after successful DA-lesion. The percent of responsive neurons in MI, SNr and STN brain regions during nosepoke (top), lever press (middle) and lever release (bottom) behavior were shown in the figure. Blank and grey bars indicate data from rats under intact (naive) and lesioned (PD) conditions. Dopamine lesion significantly reduced neuronal responses on nosepoke in all three brain areas, on lever-press in two out of three areas, while no changes were found in

any brain area for the lever-release. *, **, and ***: *Chi-square* test, $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

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Fig. 4.

An example of the beneficial effect of DBS on RT performance. **A:** Cumulative RT performance was shown in frequency curves plotted over time. Dopamine lesion alone induced a marked elevation of late responses. **B:** Changes of RT performance with DBS (stimulation on) and without DBS (stimulation off). Correct responses notably increased following STN DBS, an effect which continued even after DBS ceased. The plots in **(C)** and **(D)** illustrate the percentage of correct, early, and late performance in the same rat as in **(A)** and **(B)**, respectively. **E:** Mean and standard error of RTs in the same session before, during

and after DBS. Mean RT was significantly decreased during and after DBS. *, **: $P < 0.05$, $P < 0.01$; respectively, ANOVA followed by Duncan's post-hoc test.

Fig. 5.

Types and examples of neural responses to DBS. **A:** Colorcoded image plot of neuronal firing patterns (z-scores over the baseline) revealed by cluster analysis. Warm colors (red to yellow) represent elevated firing rates, as indicated by z-scores bigger than 2. Conversely, cold colors (blue to cyan) represent lowered firing rates. Each horizontal line represents the one neuron. C1 through C4 represent four categories of neural firing patterns with distinct features. **B:** Rearrangement of clusters into each brain areas. Firing patterns were sorted by recording region (MI, STN and SNr). Most cluster 1 neurons were found in STN. **C:**

Representative examples from each cluster. This time histogram plot revealed that cluster 1 neurons showed sharp and strong response to DBS, especially at the first several minutes; cluster 2 neurons showed mild response that lasts through the DBS period; cluster 3 neurons gradually increase their firing rates after DBS; cluster 4 neurons showed either inhibitory or no response at all to DBS. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Fig. 6.

Neural responses and task-dependent modulation with DBS. **A:** Examples of peri-event rasters and histograms for a neuron recorded in the STN, before, during, and after stimulation. There is no meaningful modulation of neural activity with lever press in the pre- and during-stimulation periods. However, a consistent response to lever press was observed following STN HFS. Note that the baseline firing rate (before lever press) is increased during and post-stimulation. **B:** Neural responses related to nosepoke, lever press and lever release behavior in the MI, SNr and STN before (blank bars) and after DBS (grey bars). A global increase of neuronal responses were observed after DBS. *, **: *Chi-square test*, $P < 0.05$ and $P < 0.01$, respectively.

TABLE I

RT Performance Before and After 6-OHDA Lesions

Classes	Rat ID	Naïve					DA-lesioned				
		Early (%)	Correct (%)	Late (%)	Total trials		Early (%)	Correct (%)	Late (%)	Total trials	
late↑	D11	17.85	70.20	11.95	1272		20.16	41.45 ^a	38.39 ^a	883	
	D15	27.96	64.86	7.18	1198		14.75 ^a	44.24 ^a	41.01 ^a	485	
	D19	37.25	55.72	7.03	1380		39.46	34.53 ^a	26.01 ^a	466	
Early↑	D17	15.46	69.16	15.38	1203		35.12 ^a	46.02 ^a	18.86	880	
	D20	30.37	59.73	9.90	1485		51.91 ^a	38.81 ^a	9.29	603	
	D21	7.30	76.21	16.49	1219		66.94 ^a	33.06 ^a	0.00	496	
both↑	D24	20.84	71.24	7.92	1238		51.43 ^a	48.16 ^a	0.41 ^a	245	
	D25	12.82	68.66	18.52	1388		66.11 ^a	29.95 ^a	3.94 ^a	838	
	D16	21.28	74.88	3.85	1222		35.34 ^a	42.05 ^a	22.61 ^a	578	
	D26	13.78	79.95	6.27	1132		42.56 ^a	43.59 ^a	13.85 ^a	321	

^a Chi-square test, $P < 0.001$, compared with corresponding class under naïve condition. Numbers in the table were pooled data from four consecutive sessions.

TABLE II

The Beneficial Effect of Bilateral STN DBS on RT Performance

Rat	Period	Correct (%)	Early (%)	Late (%)
D15	Pre-stimulation	12 (30.0)	2 (5.0)	26 (65.0)
	During-stimulation	46 (66.7) ^c	10 (14.5)	13 (18.8) ^c
	Post-stimulation	52 (59.8) ^b	19 (21.8) ^a	16 (18.4) ^c
D16	Pre-stimulation	29 (38.2)	36 (47.4)	11 (14.5)
	During-stimulation	49 (59.8) ^b	23 (28.0) ^a	10 (12.2)
	Post-stimulation	88 (59.1) ^b	44 (29.5) ^b	17 (11.4)
D20	Pre-stimulation	7 (25.0)	16 (57.1)	5 (17.9)
	During-stimulation	4 (30.8)	8 (61.5)	1 (7.7)
	Post-stimulation	88 (62.0) ^c	34 (23.9) ^c	20 (14.1)
D21	Pre-stimulation	14 (43.8)	18 (56.2)	0
	During-stimulation	52 (80.0) ^c	13 (20.0) ^c	0
	Post-stimulation	46 (50.5)	45 (49.5)	0
D25	Pre-stimulation	16 (37.2)	27 (62.8)	0 (0)
	During-stimulation	7 (22.6)	17 (54.8)	7 (22.6) ^b
	Post-stimulation	67 (41.9)	44 (27.5) ^c	49 (30.6) ^c
D26	Pre-stimulation	11 (36.7)	16 (53.3)	3 (10.0)
	During-stimulation	39 (65.0) ^a	10 (16.7) ^c	11 (18.3)
	Post-stimulation	53 (52.0)	16 (15.7) ^c	33 (32.4) ^a

The numbers of responses and percentage are presented. a, b, and c: *Chi-square* test, $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, compared with the ratio before stimulation.

TABLE III

The Number and Percentage of Neurons in Different Firing Pattern Categories in Each Brain Region Following Stimulation

Brain areas	Response type	During stimulation (%)	Post-stimulation (%)
MI	Increase	13 (20.97)	26 (41.9) ^a
	Decrease	14 (22.6)	3 (4.8) ^b
	No response	35 (56.4)	33 (53.2)
SNr	Increase	12 (17.6)	13 (19.1)
	Decrease	4 (5.9)	5 (7.4)
	No response	52 (76.5)	50 (73.5)
STN	Increase	14 (23.7)	13 (22.0)
	Decrease	8 (13.6)	3 (5.1)
	No response	37 (62.7)	43 (72.9)

^{a,b} *Chi-square test*, $P < 0.05$ (*Chi-square* = 6.322) and $P < 0.01$ (*Chi-square* = 8.248), respectively, compared with counts during stimulation.