Subthalamic Stimulation and Neuronal Activity in the Substantia Nigra in Parkinson’s Disease

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Maltête D, Jodoin N, Karachi C, Houeto JL, Navarro S, Cornu P, Agid Y, Welter ML. Subthalamic stimulation and neuronal activity in the substantia nigra in Parkinson’s disease. J Neurophysiol 97: 4017–4022, 2007. First published April 25, 2007; doi:10.1152/jn.01104.2006. High-frequency stimulation of the subthalamic nucleus (STN) is an effective treatment for severe forms of Parkinson’s disease (PD). To study the effects of high-frequency STN stimulation on one of the main output pathways of the basal ganglia, single-unit recordings of the neuronal activity of the substantia nigra pars reticulata (SNr) were performed before, during, and after the application of STN electrical stimulation in eight PD patients. During STN stimulation at 14 Hz, no change in either the mean firing rate or the discharge pattern of SNr neurons was observed. STN stimulation at 140 Hz decreased the mean firing rate by 64% and the mean duration of bursting mode activity of SNr neurons by 70%. The SNr residual neuronal activity during 140-Hz STN stimulation was driven by the STN stimulation. How the decrease in rate and modification of firing pattern of SNr-evoked neural activity, during high-frequency STN stimulation, contribute to the improvement of parkinsonian motor disability remains to be elucidated.

INTRODUCTION

High-frequency stimulation of the subthalamic nucleus (STN) improves motor symptoms of Parkinson’s disease (PD) (Limousin et al. 1998), but the effects of high-frequency stimulation on neuronal activity in the basal ganglia remain controversial. Inhibition of STN neurons has been observed during high-frequency stimulation in normal rats and rats with 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal dopaminergic pathway (Shi et al. 2006; Tai et al. 2003), in primates rendered parkinsonian (Meissner et al. 2005), and in PD patients (Filali et al. 2004; Welter et al. 2004). Contradictory effects on the neuronal activity in the basal ganglia output nuclei, however, have been reported. Whereas neurons are mostly activated in the globus pallidus of parkinsonian primates by stimulation of the STN (Hashimoto et al. 2003), inhibition of neuronal activity has been observed in the substantia nigra pars reticulata (SNr) in rat models of parkinsonism (Maurice et al. 2003; Shi et al. 2006; Tai et al. 2003). An increase in the SNr neuronal activity has been recently reported during high-frequency STN stimulation in PD patients (Galati et al. 2006). In the present study, we describe the perioperative effects of low- and high-frequency electrical stimulation of the STN on the neuronal activity of the SNr in patients with PD.

METHODS

Neurosurgical procedure

Eight patients with PD (ages: 50 ± 12 yr; disease duration: 16 ± 5 yr) underwent surgery for bilateral implantation of electrodes in the STN. The neurosurgical procedure was performed as previously described (Bejjani et al. 2000), in patients awake, at rest, and free from antiparkinsonian treatment for ≥12 h. The leads were implanted in a single operation, according to stereotactic coordinates determined by preoperative magnetic resonance imaging and peroperative electrophysiological recordings. Five coaxial leads [a central tungsten recording microelectrode and an external tube for macrostimulation (FHC Instruments, Bowdoinham, ME)] were lowered stereotactically to 5 mm above the predetermined target, in 10-μm steps along five parallel trajectories using a hydraulic microdrive (David Kopf Instruments, Tujunga, CA). Four of the leads were arranged, at a distance of 2 mm, around a central lead positioned according to the stereotactic coordinates, permitting stimulation and recording from the central, anterior, posterior, medial, and lateral parts of the STN and SNr. The microelectrode tip (diameter: 25 μm; impedance: 10 MΩ) protruded 5 mm beyond the macroelectrode (exposed surface diameter: 0.7 mm; length: 1.5 mm, surface area: 6.0 mm²). Signals were amplified (NL104 Neurolog System, Digitimer, Eastleigh, UK), filtered (NL125, 500 Hz to 5 kHz), monitored acoustically, displayed on an oscilloscope, and recorded magnetically (bandwidth: 0–5 kHz, eight channels TEAC FM). During the electrophysiological procedure, control profile stereotactic X-ray films were regularly obtained, right–left projections, with the short X-ray radiological device of the Leksell stereotactic frame to check the electrode trajectories and depth. This device consists of X-ray–visible fiducial markers and a cassette holder affixed to the stereotactic frame itself. Before starting the surgical procedure, the direction of the X-ray unit and the position of the stereotactic frame were adjusted to allow superimposition of the values of the right and left Z- and Y-scales of the fiducial markers at the level of the target. Trajectory reconstruction was performed on sagittal and frontal maps of a digitized Schatelbrand and Wahren (1977) stereotactic frame.

Electrophysiological procedure

Extracellular single-unit recordings were performed in awake but immobile patients at rest, and obtained simultaneously from the five leads used to identify and localize the STN and then the SNr (Hutchison et al. 1998). Electrode descent was stopped when at least two of them recorded neuronal activity characteristic of the SNr (Benazzouz et al. 2000; Hutchison et al. 1998). Nigral recorded neurons were included if they were well isolated, stable (signal-to-
Off-line analysis

Off-line analysis was performed with a CED 1401 data-acquisition system and Spike2 software (Version 5; Cambridge Electronic Device, Cambridge, UK) (Welker et al. 2004). Spikes were discriminated from noise and stimulation artifacts on the basis of their form and amplitude, using the wavemark template-matching utility and the double-threshold window discriminator of Spike2 software, and the mean firing frequency and pattern of discharge of SNr neurons were calculated.

noise ratio ≥ 2:1, maximum amplitude change: 30%), and could be sampled for ≥ 60 s. Cathodal monopolar stimulation was performed through the macroelectrode of one electrode localized in the STN. Ipsilateral single-cell recordings were made through the microelectrode tip of another electrode in the SNr. To ensure the correct positioning of the stimulating electrode into the STN, the coordinates of the STN were first determined by recording single-neuron activity (Hutchison et al. 1998), then the stimulating macroelectrode was positioned at the same coordinates. Nigral neuronal activity was recorded for 20 s before, during, and after STN electrical stimulation at 14 and 140 Hz; current and pulse width were constant (2 mA, 60 μs, cathodal square pulses, voltage: 2 V). This protocol, accepted by INSERM, was approved by the local ethics committee; all patients signed an informed written consent.

FIG. 1. Effects of high-frequency (140-Hz) stimulation within the subthalamic nucleus (STN) on the spontaneous activity of a substantia nigra pars reticulata (SNr) single neuron. Spikes (8 event markers, top trace) were discriminated from the stimulus artifact with the wavemark template-matching utility and double-threshold window discriminator (Spike2 software), and the mean firing frequency and pattern of discharge of SNr neurons were calculated.

RESULTS

Spontaneous SNr neuronal activity

Thirty-five cells were recorded from the SNr of the eight patients. The number (mean ± SD) of action potentials recorded per cell was 3,310 ± 1,527, over a period of 110.9 ± 53.5 s. The mean firing rate was 35.0 ± 14.3 Hz (range: 13.7–77.8 Hz). Two modes of discharge were identified (Kaneoke and Vitek 1996): regular (27 cells) and irregular (eight cells). Thirty-three cells of 35 discharged some bursts (S value ≥ 3) with 27% of spikes with a bursting mode activity for a relatively limited duration (17.0 ± 13.2% of time). Twenty SNr cells were recorded during ipsilateral STN stimulation with 14- and/or 140-Hz stimulation (Table 1). Eight SNr cells were recorded with the posterior (n = 5) or medial electrode (n = 3) during STN stimulation with the central electrode; 12 cells were recorded with the central electrode while stimulating with the STN electrode placed medial (n = 5), posterior (n = 5), or anterior (n = 2).

Effects of low-frequency electrical stimulation of the subthalamic nucleus on spontaneous substantia nigra pars reticulata neuronal activity

Stimulation at 14 Hz in the STN did not significantly modify the mean firing rate of 16 SNr neurons (Table 1). The firing pattern was not modified in 14 cells (regular, n = 11; irregular, n = 3), one cell switched its firing pattern from irregular to regular, and one cell from regular to irregular (Table 1). Fourteen SNr cells displayed some bursts (S value ≥ 3); the mean percentage of spikes with a bursting mode activity was unchanged before and during 14-Hz STN stimulation (Table 1). The mean duration of bursting mode activity tended to decrease, although the difference was not significant (Table 1). PSTHs showed an inhibitory period of 9.6 ± 2.2 ms after the stimulation pulse in five cells (Fig. 2). No inhibitory period during the poststimulus period was detected in seven cells and a poststimulus excitatory period of 25.0 ± 3.8 ms was detected in four cells (Fig. 2). The responses of SNr cells to 14-Hz STN stimulation was similar whatever the spatial relationship of the stimulating and recording electrodes: 1) in the five SNr cells with an inhibitory period, three were recorded with the central electrode during STN stimulation with the medial (n = 2) or
Effects of high-frequency electrical stimulation of the subthalamic nucleus on spontaneous substantia nigra pars reticulata neuronal activity

STN stimulation at 140 Hz induced a 67% decrease in the mean neuronal firing rate of 16 SNr neurons (range: −32 to −100%) and a 34% increase in the mean firing rate of one SNr cell (Table 1). Before 140-Hz STN stimulation, nine cells displayed a regular activity and eight an irregular activity. There was no change in the firing pattern in 10 cells (regular, n = 7; irregular, n = 3), five cells switched from irregular to regular firing pattern, and two cells from regular to bursting firing pattern (P = 0.39, Table 1). There was a 51% decrease in the percentage of the spikes contributing to bursts and a 70% decrease in the mean duration of bursting mode activity during 140-Hz STN stimulation (Table 1, Fig. 3). In all SNr cells, PSTHs showed a three-phase response with an inhibition (0–2 ms), excitation (2–4 ms), and inhibition (4–7 ms) sequence after stimulation pulse (Fig. 4). Fifteen cells were inhibited with a persistent neuronal activity 2–4 ms after the stimulation.

TABLE 1. Effects of subthalamic (STN) stimulation on the ipsilateral neuronal activity of the substantia nigra pars reticulata (SNr)

<table>
<thead>
<tr>
<th></th>
<th>14-Hz STN Stimulation (n = 16)</th>
<th>140-Hz STN Stimulation (n = 17)</th>
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<tr>
<td></td>
<td>Before</td>
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<td></td>
<td>Before</td>
<td>During</td>
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<tr>
<td>Frequency, Hz</td>
<td>27.2 ± 9.8</td>
<td>25.6 ± 8.5</td>
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<tr>
<td>Mean S index</td>
<td>4.1 ± 1.9</td>
<td>4.3 ± 1.6</td>
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<tr>
<td>% Duration, S ≥ 3</td>
<td>14.3 ± 12.8</td>
<td>8.2 ± 6.1</td>
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<tr>
<td>% Spikes, S ≥ 3</td>
<td>23.5 ± 19.6</td>
<td>18.4 ± 14.5</td>
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<td>Discharge pattern</td>
<td>(Kaneoke and Vitek 1996)</td>
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<td>Regular</td>
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<td>Irregular</td>
<td>4</td>
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<td>Bursting type</td>
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Values are means ± SD. *See METHODS. *P < 0.05 when compared to prestimulation values.

posterior (n = 1) electrode, and two were recorded with the posterior (n = 1) or medial (n = 1) electrode during STN stimulation with the central electrode; 2) in the four SNr cells with an excitatory period, two were recorded with the central electrode during STN stimulation with the medial (n = 1) or posterior (n = 1) electrode and two were recorded with the posterior (n = 1) or medial (n = 1) electrode during STN stimulation with the central electrode; and 3) in the seven SNr cells with no change, three were recorded with the central electrode during STN stimulation with the posterior electrode and four were recorded with the posterior (n = 1), medial (n = 2), or anterior (n = 1) electrode during STN stimulation with the central electrode.

Effects of high-frequency electrical stimulation of the subthalamic nucleus on spontaneous substantia nigra pars reticulata neuronal activity

STN stimulation at 140 Hz induced a 67% decrease in the mean neuronal firing rate of 16 SNr neurons (range: −32 to −100%) and a 34% increase in the mean firing rate of one SNr cell (Table 1). Before 140-Hz STN stimulation, nine cells displayed a regular activity and eight an irregular activity. There was no change in the firing pattern in 10 cells (regular, n = 7; irregular, n = 3), five cells switched from irregular to regular firing pattern, and two cells from regular to bursting firing pattern (P = 0.39, Table 1). There was a 51% decrease in the percentage of the spikes contributing to bursts and a 70% decrease in the mean duration of bursting mode activity during 140-Hz STN stimulation (Table 1, Fig. 3). In all SNr cells, PSTHs showed a three-phase response with an inhibition (0–2 ms), excitation (2–4 ms), and inhibition (4–7 ms) sequence after stimulation pulse (Fig. 4). Fifteen cells were inhibited with a persistent neuronal activity 2–4 ms after the stimulation.

FIG. 2. Poststimulus time histograms (PSTHs) for 14-Hz stimulation in the STN (4-ms bins). PSTHs were reconstructed for a 15-s period during a 14-Hz STN stimulation with of 72-ms duration (18 bins of 4 ms each, 278 sweeps), and shown as a proportion of the baseline neuronal activity in each cell (PSTHs reconstructed for a 15-s period before STN stimulation, horizontal dashed line). Solid black line represents a cell with an excitatory period after each stimulus pulse; the coarse dashed black line represents a cell with an inhibitory period after each stimulus pulse; and the fine dashed black line a cell without an inhibitory period after each stimulus pulse. *Values below the lower limit. #Values above the higher limit of the PSTH confidence intervals obtained without 14-Hz STN stimulation.

FIG. 3. Effects of 140-Hz STN stimulation on SNr neuronal activity. A and B: discharge of a cell before (A) and during (B) 140-Hz STN stimulation; as a sequence of spikes (top trace) and instantaneous frequency of discharge (Inst. Frequency, bottom trace). Middle continuous trace: bursting discharge detected by the Poisson surprise analysis (S value = 3). This neuron showed a reduction in the mean firing rate (39.7 Hz before vs. 10.2 Hz during 140-Hz stimulation) and in bursting discharges (mean percentage of spikes: 69.9% before vs. 0% during 140-Hz stimulation, mean percentage of duration: 55.2% before vs. 0% during 140-Hz stimulation).
This study shows that high-frequency stimulation within the STN in PD patients reduces the frequency and changes the pattern of neuronal firing in the SNr. Several lines of evidence suggest that these results are robust. 1) The recording microelectrodes were localized within the SNr because the neuronal activity recorded during the neurosurgical procedure was similar to that previously described in animal models of parkinsonism (Benazzouz et al. 2000; Tai et al. 2003) and PD patients (Hutchison et al. 1998). 2) The electrode used for high-frequency stimulation was located within the STN as shown by stereotactic X-rays taken during the operation. 3) It is unlikely that the stimulus artifact could have masked the response of SNr neurons to STN stimulation: the artifact was short (1 ms, Fig. 1) and the time-locked excitatory response observed in animals or PD patients was maximal 2 to 4 ms after the stimulation pulse (Galati et al. 2006; Shi et al. 2006); the mean firing rate of neurons recorded in the SNr was not modified when the STN was stimulated at 14 Hz (Table 1).

These results are in line with those obtained in rats rendered parkinsonian in which the SNr neuronal activity has been shown to predominantly decrease with low STN stimulation intensity (Maurice et al. 2003; Shi et al. 2006; Tai et al. 2003). However, these results are different from those recently reported by Galati et al. (2006), where an increase in the SNr neuronal firing rate during STN high-frequency stimulation was found. Two main reasons could explain these differences. 1) The volume of stimulated tissue in our experiment was smaller than that in the Galati et al. study because both intensity and surface of stimulation were smaller (2 vs. 2–3 V and 2.4 vs. 6.0 mm²). Because the effective electric field volume was smaller, the electrical stimulation was necessarily more focal, thus modulating the activity of a more restricted neuronal population. Furthermore, changes in neuronal activity could be dependent on the position and orientation of the neuron and its axon with respect to the electrode and the stimulation parameters. In a computational model of the thalamocortical cell body and axon, it has been proposed that subthreshold high-frequency electrical stimulation for direct activation of the thalamocortical relay neurons caused suppression of intrinsic firing activity in both the soma and axon, whereas suprathreshold stimulation induced suppression of intrinsic firing in the soma but efferent output at the stimulus frequency in the axon (McIntyre et al. 2004). More recently, a computational model constructed to elucidate the effects of STN stimulation on the neuronal activity of the internal segment of the globus pallidus (GPI) in monkeys has shown similar results for the subthalamopallidal neuron with an inhibition of the intrinsic firing in the GPi (Miocinovic et al. 2006). 2) The duration of stimulation was different in the two studies: short stimulating periods (20 s) were used in our study, whereas 30 min of stimulation were used in the Galati et al. study. One explanation could be that high-frequency STN stimulation first decreases SNr neuronal activity, as shown in this study, but that neuronal activity is reversed when electrical stimulation is maintained for several minutes, thus leading to neuronal activation. Such an inversion of electrophysiological effects during high-frequency STN stimulation on the SNr neuronal activity could result from neuronal plasticity, either through a direct effect implicating pulse, decreased (n = 10) or similar (n = 5) to the neuronal activity obtained before stimulation (Fig. 4A). In two SNr cells, a significant increase in the neuronal activity 2–4 ms after the stimulation pulse was induced by 140-Hz STN stimulation (Fig. 4B). In four of the five SNr cells with an inhibitory period during 14-Hz STN stimulation and recorded during 140-Hz stimulation, an inhibition was also observed during 140-Hz STN stimulation (not shown). In the two SNr cells excited by 140-Hz STN stimulation, no excitatory or inhibitory periods were noted during 14-Hz STN stimulation (not shown). In all but two cells, the neuronal activity recorded during 140-Hz STN stimulation was time-locked to the stimulation pulse with the presence of periodic spiking at 7.14-ms intervals, corresponding to a frequency of 140 Hz (Fig. 4).

**FIG. 4.** PSTHs and cross-correlograms for 140-Hz stimulation in the STN. A: example of SNr cell with an inhibitory response. B: example of SNr cell with time-locked excitatory and inhibitory responses. PSTHs were reconstructed for a 15-s period before (left) and during (right) a 140-Hz STN stimulation of 7-ms duration (7 bins of 1 ms each, 2,100 sweeps). Cross-correlograms were calculated for a 20-s period before (left) and during (right) 140-Hz STN stimulation (300 ms, 1-ms bin width). Gray line represents the mean firing frequency (Hz). *Values under the lower limit. #Values above the higher limit of the PSTH confidence intervals obtained without 140-Hz STN stimulation.
the subthalamonic pathway or through an indirect effect implicating the activation of indirect inputs to the SNr. These electrophysiological changes observed in the SNr over time could explain, at least in part, the time course of the clinical effects observed in parkinsonian patients during STN stimulation. Whereas rigidity or tremor is known to disappear within seconds after the onset of high-frequency STN stimulation (Krack et al. 2002), improvement of bradykinesia is delayed for several minutes, hours, or even days (Krack et al. 2002).

The reduced SNr firing rate observed in PD patients during high-frequency STN stimulation could result from a direct inhibition of STN neurons leading to a decreased activity of the excitatory subthalamonic glutamatergic pathway (Filali et al. 2004; Meissner et al. 2005; Shi et al. 2006; Welter et al. 2004). The observation that high-frequency STN stimulation decreases the expression of messenger RNA for subunit I of cytochrome oxidase in neurons of the rat STN is compatible with this hypothesis (Tai et al. 2003). Local synaptic inhibition in the STN might also occur by excitation of myelinated fibers projecting from the external segment of the globus pallidus (GP) or antidromic activation of axon terminals in the GP by release of inhibitory γ-aminobutyric acid (GABA) (Benazzouz et al. 2000; Shi et al. 2006; Tai et al. 2003). Finally, neurons in the STN that project to the GP might be activated (Hashimoto et al. 2003), thereby inducing a release of inhibitory GABA in the SNr (Windels et al. 2005). The fact that injection of bicuculline, a GABA antagonist, in the SNr induces a disappearance of this STN stimulation-induced inhibition is in line with this hypothesis (Maurice et al. 2003).

Beside the global reduction in the neuronal activity of the SNr during STN stimulation, we observed that SNr neuronal activity during STN high-frequency stimulation constituted three components: inhibition (0–2 ms)/excitation (2–4 ms)/inhibition (4–7 ms) after the stimulation pulse (Fig. 4), with a periodic spiking corresponding to the frequency of stimulation (Fig. 4). These three components of the SNr neuronal response to STN stimulation could result from the alternate activation or inhibition of inhibitory and excitatory afferents to the SNr. The excitatory component could result from the orthodromic activation of subthalamonic axons and consequent release of glutamate in the SNr (Boulet et al. 2006), with an oscillatory activity driven by the stimulation, as previously reported in STN neurons in rats in vitro (Garcia et al. 2005) and in the SNr in parkinsonian patients (Galati et al. 2006). The two inhibitory components could result both from the reduction of glutamatergic excitatory afferents from the STN, by inhibition of the STN neuronal activity (Filali et al. 2004; Meissner et al. 2005; Shi et al. 2006; Tai et al. 2003; Welter et al. 2004) and/or the antidromic activation of the GABAergic pallidionigral fibers (Maurice et al. 2003; Windels et al. 2005). This leads to a regularization of the SNr neuronal activity (this study), in agreement with the results obtained in the SNr of rats treated with neuroleptics (Degos et al. 2005), which might help improve parkinsonian symptoms. A regularization of the spontaneous discharge of SNr cells has also been reported during STN lesioning in animal models of PD (Tseng et al. 2001). In the GPI, the other main basal ganglia output nucleus connected to the STN (Alexander 1994), during STN stimulation in primates rendered parkinsonian, an activation with a complex electrophysiological response is also observed, with four consecutive components of inhibition and excitation, and a regularization of neuronal activity (Hashimoto et al. 2003). However, when the intensity of STN stimulation is low, about a third of GPI neurons shows a decrease in neuronal firing rate (Hashimoto et al. 2003).

In parkinsonian patients, high-frequency STN stimulation induced a decrease in the spontaneous neuronal activity and a change in the firing pattern of SNr neurons, one of the main output pathways of the basal ganglia. However, the mechanisms underlying this neuronal inhibition downstream from STN, the neurosurgical target of choice in the treatment of PD, is still controversial. Irrespective of whether this initial neuronal inhibition is reversed during long-term, high-frequency STN stimulation and the increased irregular and bursting activity in the SNr that is characteristic of parkinsonism (Hutchison et al. 1998; Wichmann et al. 1999) is normalized or interrupted by STN high-frequency stimulation remain to be demonstrated. Finally, how these modifications contribute to the improvement of parkinsonian motor disability remains to be elucidated.

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