Effects of High-Frequency Stimulation on Subthalamic Neuronal Activity in Parkinsonian Patients

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Background: High-frequency stimulation of the subthalamic nucleus (STN) is a neurosurgical alternative to medical treatment in levodopa-responsive forms of Parkinson disease. The mechanism of action of STN stimulation remains controversial, although an inhibition of overactive STN neurons has been postulated.

Objective: To determine the effects of high-frequency STN stimulation on the neuronal activity of STN neurons in Parkinson disease patients.

Patients: Single-unit recordings of the neuronal activity of the STN were obtained before, during, and after the application of intra-STN electrical stimulation in 15 Parkinson disease patients. Changes in firing frequency and pattern were analyzed using various combinations of stimulus frequency (range, 14-140 Hz).

Results: Stimulation at a frequency greater than 40 Hz applied within the STN significantly decreased the firing frequency and increased the burst-like activity in the firing pattern of STN neurons. An aftereffect was observed in cells that had been totally inhibited during high-frequency stimulation.

Conclusion: The beneficial effects of high-frequency stimulation result from a change in the firing pattern of cellular discharge and a blockade of the spontaneous overactivity of STN neurons.

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Six months after surgery, parkinsonian motor disability (Unified Parkinson’s Disease Rating Scale part III) was improved by 68% (range, 35%-90%) under stimulation alone and by 91% (range, 76%-98%) under stimulation and drug treatment. The levodopa-equivalent dosage was reduced by 77% (range, 23%-100%) and the severity of motor-related complications by 82% (range, 58%-100%).

NEUROSURGICAL PROCEDURE

The neurosurgical procedure was performed as previously described. The electrodes were implanted in a single session, according to stereotactic coordinates determined by preoperative magnetic resonance imaging and peroperative electrophysiological recordings. The procedure, performed under local anesthesia, after a night in the stereotactic frame, was started in the right hemisphere of patients who were awake and free of antiparkinsonian drugs for more than 12 hours. A 15-mm burr hole was drilled for access to the STN along the trajectory defined by the stereotactic coordinates of the STN and the angle of approach determined preoperatively on the basis of magnetic resonance imaging data. Five parallel stainless-steel guide tubes (length, 120.0 mm; internal diameter, 0.9 mm; and external diameter, 2.0 mm for the upper 50.0 mm of the tube and 1.5 mm for the remainder) were then inserted stereotactically to 15.0 mm above the predetermined target. Four of the tubes were arranged, at a distance of 2.4 mm, around a central tube positioned according to the stereotactic coordinates, permitting stimulation and recording from the central, anterior, posterior, medial, and lateral parts of the STN. The tubes were fixed to the frame through a specially designed mechanical interface with a hydraulic micrometer drive (David Kopf Instruments, Tujunga, Calif). The concentric exploration electrodes (model IOWVFGESE5BP4; FHC Instruments, Bowdoinham, Me) consisted of a central tungsten microelectrode for recording and an external tubing for macroelectrode stimulation. The microelectrode tip (diameter, 25 µm; and impedance, 10 megaohms) protruded 2 mm beyond the external macroelectrode stimulating shank (exposed surface diameter, 0.7 mm; and height, 1.5 mm). High microelectrode impedance was used to isolate single-unit and multunit cellular spikes from the background noise.

The electrodes were inserted into the guiding tubes connected to the ground of the electronic device, so that no Faraday shield was needed. Recording was performed along the 5 parallel trajectories from the electrodes that were inserted, using the hydraulic microdrive in 10-µm steps from 5 mm above to 10 mm beyond the predefined target. Signals were amplified (NL104 Neurolog System; Digitimer, Welwyn Garden City, Hertfordshire, England), filtered (NL125, Digitimer, 500 Hz to 5 kHz), monitored acoustically, displayed on an oscilloscope, and recorded magnetically (0-5 kHz, 8 channels [TEAC FM; TEAC Corp, Tokyo, Japan]). During the electrophysiological procedure, control stereotactic x-ray films were regularly obtained using a profile (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 Schaltenbrand and Wahren stereotactic frame.

ELECTROPHYSIOLOGICAL PROCEDURE

Extracellular unit recordings were performed in patients who were awake and at rest in the absence of any passive or active movement.

SIMULTANEOUS IPSILATERAL RECORDING AND STIMULATION

Recordings of neuronal activity were obtained simultaneously from the 5 electrodes and allowed the identification and localization of the STN (Figure 1). The progression of the de-
scent of the electrodes was stopped when at least 2 electrodes recorded neuronal activity specific to the STN over a length of 2.5 mm. Recorded neurons were included if they were well isolated and stable and could be sampled for at least 20 seconds. Cathodic monopolar stimulation, relative to the guide tube (anode), was performed through one electrode, while single neuronal activity recordings were obtained from the other electrode. The neuronal activity was recorded under different frequencies (14, 40, 80, and 140 Hz) with a constant current and pulse width (2 mA, 60 microseconds, and negative square pulses). For each condition, neuronal activity was recorded for 20 seconds before stimulation, 20 seconds with stimulation, and 20 seconds after stimulation.

A sham procedure was also performed. Stimulation (140 Hz, 60 microseconds, and 2 mA) was applied outside the STN while recording STN neuronal activity. During this procedure, the external macroelectrode stimulating shank was positioned 10 mm from the microelectrode tip, located within the STN (Figure 1).

POSTOPERATIVE ANALYSIS

Off-line analysis of the digitized recordings was performed with a CED1401 data acquisition system (Cambridge Electronic Design, Cambridge, England) and Spike 2 software (version 2.09; Cambridge Electronic Design). The stimulation artifact was eliminated from the signal by means of a double-threshold window before spike detection. The mean firing rate and interspike intervals were calculated for each cell. In accord with the method described by Kaneoke and Vitek, a discharge density histogram was constructed for each cell to determine the firing pattern, which was defined as irregular, regular, or bursting. During 14-Hz stimulation within the STN, poststimulus histograms were analyzed (length of bin, 4 milliseconds; and number of bins, 18) and expressed as a proportion of the baseline firing rate in each cell before stimulation. Statistical analysis of group values was performed using t tests. Results were considered significant at P<.05.

RESULTS

Forty STN neurons were recorded from the STN (right side) of the 15 patients. The mean±SD number of action potentials recorded per cell was 2705±1459 spikes, during a mean±SD sampling time of 89.7±36.6 seconds. The mean±SD firing rate was 36.8±14.6 Hz (range, 12.1-69.4 Hz) (Table). Three types of activity were identified: irregular (33 cells or 83% of the total STN population), burst (5 cells or 13% of the total STN population), and regular (2 cells or 5% of the total STN population).

EFFECT OF HIGH-FREQUENCY STIMULATION ON STN NEURONAL ACTIVITY

Complete recordings before, during, and after ipsilateral 140-Hz stimulation within the STN (mean±SD sampling time, 79.2±17.5 seconds) were obtained in 21 STN cells (Figure 2A). The mean firing rate of the 21 cells decreased by 77% during stimulation (P<.001) (Table). Among the 21 recorded cells, residual neuronal activity persisted in 15 of the 21 cells, 6 of which presented a modified firing pattern: 4 cells switched from an irregular to a burst pattern, 1 cell from a burst to an irregular pattern, and 1 cell from a regular to an irregular pattern. An example of the neuronal activity recording of one such subthalamic cell is shown in Figure 3. Before stimulation, the neuron presented an irregular pattern, with a mean firing rate of 28.5 Hz. Although high-frequency stimulation applied within the STN induced an artifact, residual neuronal activity could still be detected. The mean firing rate of this single cell decreased to 10.1 Hz, and the discharge pattern was modified from an irregular to a burst type. The neuronal activity of the other 6 cells was totally inhibited, with a mean±SD progressive resumption of activity 1.6±0.9 seconds (range, 0.9-2.9 seconds) after the stimulation had been stopped in 4 cells (Figure 4).

During the sham procedure, 8 neurons were recorded within the STN before, during, and after ipsilateral high-frequency stimulation (mean±SD sampling time, 71.8±9.9 seconds) applied above the STN. The neuronal activity was not modified (Table), except in 1 neuron in which firing decreased in frequency and switched from an irregular to a burst type (Figure 2).

EFFECT OF MODIFYING THE FREQUENCY OF STIMULATION ON STN NEURONAL ACTIVITY

The effect of modifying the frequency of stimulation was studied in 11 STN neurons (mean±SD sampling time, 122.6±53.5 seconds) (Figure 5 and Figure 6). Before stimulation, the mean±SD firing rate of the 11 neurons was 31.7±16.4 Hz (range, 12.0-56.9 Hz), with an irregular (9 cells), burst (1 cell), or regular (1 cell) pattern. A 14-Hz stimulation in the STN (60 microseconds and 2 mA) did not significantly change the mean firing rate (10 cells) (P=.31) or firing pattern, except in 1 cell that fired irregularly before stimulation and displayed burst activity during and after stimulation (Figure 5). Peristimulus histograms showed an inhibitory period following the pulse of stimulation in 6 cells, with a mean±SD rebound of activity 32.6±8.5 milliseconds after the stimulation (Figure 6). No inhibitory period during the peristimulus period was detected in 3 cells, and a poststimulus excitatory period was detected in 1 cell. A 40-Hz stimulation (60 microseconds and 2 mA) decreased the mean firing rate of 8 cells by 38% (P<.002), with no change in the firing pattern, except for 1 cell in which activity was regular before stimulation and irregular during and after stimulation (Figure 5). When the frequency of stimulation was increased to 80 Hz, there was an 81% reduction in the mean firing rate of 7 cells (P<.002). Total inhibition of firing was observed in 1 cell, and in 3 cells, the firing pattern switched from regular (1 cell) or irregular (2 cells) before stimulation to burst activity during stimulation.

# Table

<table>
<thead>
<tr>
<th>Frequency of Stimulation</th>
<th>Before Stimulation</th>
<th>During Stimulation</th>
<th>After Stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>140 Hz</td>
<td>38.1±14.5</td>
<td>6.7±7.1†</td>
<td>36.0±15.9</td>
</tr>
<tr>
<td>Intra-STN (n=21)</td>
<td>38.1±14.5</td>
<td>6.7±7.1†</td>
<td>36.0±15.9</td>
</tr>
<tr>
<td>Sham procedure (n=8)</td>
<td>40.4±11.8</td>
<td>35.6±18.0</td>
<td>41.2±14.4</td>
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<tr>
<td>80 Hz (n=7)</td>
<td>40.9±13.4</td>
<td>5.9±5.5†</td>
<td>40.3±9.9</td>
</tr>
<tr>
<td>40 Hz (n=8)</td>
<td>41.9±12.1</td>
<td>25.8±8.4†</td>
<td>41.6±13.1</td>
</tr>
<tr>
<td>14 Hz (n=10)</td>
<td>29.2±14.9</td>
<td>26.9±14.6</td>
<td>30.1±14.7</td>
</tr>
</tbody>
</table>

*Values are mean±SD and expressed in Hertz. †P<.002 compared with before stimulation.
The recording of neuronal activity within the STN performed during neurosurgery in PD patients showed that high-frequency stimulation in the STN resulted in a reduction in the frequency of neuronal activity and a change in the pattern of cellular discharge. The validity of the results would be questionable if stimulation had not been performed strictly within the limits of the STN or if the unavoidable stimulation artifact masked persistent cellular activity. Several lines of evidence suggest that this was not the case, however: (1) We consider that the recording microelectrodes were confined to the STN, as the neuronal activity recorded during the neurosurgical procedure was similar to that previously described in parkinsonian monkeys and in PD patients. This result is consistent with the marked postoperative improvement in motor disability observed in the patients, as previously reported in parkinsonian monkeys and in PD patients. (2) A short latency response (<3 milliseconds) cannot be excluded, as observed in the internal globus pallidus (GPi) of monkeys rendered parkinsonian, during high-frequency stimulation of the STN. It seems unlikely, however, that the stimulation artifact could have masked persistent cellular activity, because a high-frequency stimulation that was efficacious in the STN did not change the frequency of cellular discharge recorded by the electrode placed within the STN when applied outside the structure (Figure 2). Moreover, the mean firing rate of neurons recorded in the STN was not modified when the frequency of the concomitant stimulation was less than 40 Hz (Figure 5).

To our knowledge, this study is the first direct demonstration of a reduced frequency of neuronal discharge during high-frequency stimulation within the

**Figure 2.** Effects of high-frequency (140 Hz, 60 microseconds, and 2 mA) stimulation on neuronal activity. Top (applied within the subthalamic nucleus [STN]): A, Mean firing rate of 21 cells. B, Distribution of the firing pattern of these cells. Bottom (applied above the STN): C, Mean firing rate of 8 cells. D, Distribution of the firing pattern of these cells.
STN in PD patients. These results are in accord with those obtained in the rat, in which neuronal activity decreased in the SNr and entopeduncular nucleus and increased in the globus pallidus and superior colliculus after high-frequency stimulation of the STN. That high-frequency stimulation inhibits STN neurons is further supported by the observation that muscimol, a γ-aminobutyric acid agonist that acts at the level of cell bodies, causes local inhibition of neuronal activity and a concomitant amelioration of parkinsonism when infused in the STN of PD patients. The inhibition could result from a direct effect on STN neurons, because high-frequency stimulation of slices of rat STN blocked burst and tonic neuronal activity. This effect was independent of synaptic transmission and was suggested to have resulted from a reduction of intrinsic voltage-gated currents, leading to an interruption of the spontaneous activity of STN neurons. If these results...
obtained in vitro can be transposed in vivo, the beneficial effects of high-frequency STN stimulation in PD patients could be explained by a direct inhibition of glutamatergic neurons in the STN (essentially composed of Golgi type 1 cells with axons that are myelinated proximal to the cell body), as also suggested by the decrease in cytochrome oxidase subunit I messenger RNA levels induced by STN high-frequency stimulation in rat STN neurons. The same results with 40-Hz (8 cells) and 80-Hz (7 cells) stimulation. Bottom: D, Distribution of the firing pattern of cells before, during, and after stimulation. E and F, The same results with 40-Hz and 80-Hz stimulation.

Figure 5. Effects of changes in the frequency of stimulation on the firing rate of subthalamic nucleus (STN) neurons. Top: A, Application of 14-Hz stimulation within the STN, with a mean firing rate of 10 cells before, during, and after stimulation. B and C, The same results with 40-Hz (8 cells) and 80-Hz (7 cells) stimulation. Bottom: D, Distribution of the firing pattern of cells before, during, and after stimulation. E and F, The same results with 40-Hz and 80-Hz stimulation.

This does not exclude the possibility of a synaptic inhibition such as excitation of myelinated fibers that comes from the external pallidum and would cause inhibition by releasing \(\gamma\)-aminobutyric acid. This has been suggested to occur in the GPi and in the SNr. The time course of the poststimulus inhibitory period observed during a 14-Hz stimulation in the STN observed in 6 of 10 cells could support this hypothesis (Figure 6). Last, a retrograde activation of the axon terminals of neurons in the external pallidum that would cause \(\gamma\)-aminobutyric acid inhibition of STN neurons can also be envisaged. These interpretations are not in line with the following: (1) the increase in glutamate concentrations observed in the globus pallidus and SNr of rats during STN high-frequency stimulation, although the increase was maximal 45 to 60 minutes following high-frequency stimulation (whereas, the duration of stimulation was 20 seconds in our procedure), and (2) the increase in firing rate of GPi neurons observed in monkeys rendered parkinsonian during STN high-frequency stimulation, although this result was obtained by using higher intensities of stimulation (>1.8 V) compared with our results (lower intensities of stimulation inducing a decreased firing rate in almost 40% of GPi neurons). We conclude that, in PD patients, STN high-frequency stimulation results in a decrease in the spontaneous STN neuronal activity, although an increased neuronal activity within the first milliseconds following the stimulation artifact cannot be excluded.

Besides local neuronal inhibition, STN stimulation induced a change in the pattern of firing in most, but not all, cells (Figure 2). The change in this firing pattern should be interpreted with caution, as the firing frequency was concomitantly reduced. Indeed, the likelihood of occurrence of a burst becomes greater as the interval between bursts increases. It cannot, therefore, be excluded that the change in firing pattern is a direct consequence of the decreased firing frequency. A change in the firing pattern has been observed in GPi neurons of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine–treated monkeys following administration of dopamine agonists and during STN high-frequency stimulation, however. We suggest that, during STN high-frequency stimulation, a change from an irregular to a burst firing pattern in STN neurons may contribute to the improvement of parkinsonian motor signs.

In conclusion, the present study suggests that high-frequency stimulation induces an inhibition of neuronal activity in the STN and a change in the firing pattern. The relative contributions of inactivation and changes in the firing pattern of STN neurons to the antiparkinsonian effects of high-frequency stimulation still need to be determined.
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Figure 6. Peristimulus (left) and poststimulus (right) histograms for 14-Hz stimulation (4-millisecond bins) of the subthalamic nucleus. Left: The gray bars represent the artifact period (duration, 3 milliseconds). A, One cell with an inhibitory period following each stimulus pulse. B, One cell without an inhibitory period following each stimulus pulse. C, One cell with an excitatory period following each stimulus pulse. Right: The smoothed poststimulus histograms are shown as a proportion of the baseline firing rate in each cell (the dotted line represents the 20-second baseline firing rate before stimulation).
REFERENCES