Impact of High-Frequency Stimulation Parameters on the Pattern of Discharge of Subthalamic Neurons

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García Liliana, Giampaolo D’Alessandro, Pierre-Olivier Fernagut, Bernard Bioulac, and Constance Hammond. Impact of high-frequency stimulation parameters on the pattern of discharge of subthalamic neurons. J Neurophysiol 94: 3662–3669, 2005. First published September 7, 2005; doi:10.1152/jn.00496.2005. In clinical conditions, high-frequency stimulation (HFS) of subthalamic (STN) neurons in Parkinson’s disease is empirically applied at ≥100 Hz (130–185 Hz), with pulses of short duration (60–100 μs) and 1- to 3-mA amplitude. Other parameter values produce no effect or aggravate the symptoms. To gain a better understanding of the mechanisms that underlie the therapeutic action of HFS, we have compared the effects of different combinations of parameter values delivered by clinical stimulators on the activity of STN neurons recorded in whole cell patch-clamp configuration in slices. We showed that none of the tested combinations of parameters silenced the neurons. Non-therapeutic combinations i.e., low-frequency pulses (10–50 Hz), even at large amplitude or width, further excited the STN neurons with respect to their spontaneous activity. In contrast, combinations in the therapeutic range (80–185 Hz, 90–200 μs, 500–800 μA) replaced the preexisting activity by spikes, time-locked to the stimuli and thus presenting a striking regularity. When increasing pulse width or amplitude in this high-frequency range, the dual effect was still present but the activity generated became more irregular. We propose that during HFS at clinically relevant parameters, STN neurons behave as stable oscillators entirely driven by the stimulation, giving an average stable STN output that overrides spontaneous activity and introduces high-frequency regular spiking in the basal ganglia network.

INTRODUCTION

Chronic high-frequency stimulation (HFS) of the subthalamic nucleus (STN) is the most widely used neurosurgical procedure to date for patients with Parkinson’s disease (Benazzouz et al. 1993; Limousin et al. 1995). HFS operates with a certain combination of parameter values: the best effects on motor behavior are obtained with regular pulses of 100–185 Hz with a narrow width (60–100 μs) and an amplitude of 1–3 mA. In contrast low-frequency stimulation (LFS) at 5–10 Hz has no effect or worsens parkinsonism (Moro et al. 2002; Rizzone et al. 2001). The mechanisms by which electrical stimulation of a structure deep in the brain reinstates motor function are still not elucidated. According to studies in humans, suffering from Parkinson’s disease and in monkeys treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), loss of dopaminergic projections to the striatum results in hyperactivity of subthalamic neurons, aberrant temporal couplings, and abnormal oscillations in cortico-basal ganglia circuits with increased coherence in the 10- to 30-frequency band (Bergman et al. 1998; Hassani et al. 1996; Levy et al. 2000, 2002; Miller and De Long 1987; Williams et al. 2002). The hyperactivity of STN in the parkinsonian state and the amelioration of motor symptoms by lesion or chemical inactivation of STN neurons led to the hypothesis that STN-HFS silences STN neurons (Aziz et al. 1991; Bergman 1990; Gill and Heywood 1997; Levy et al. 2001). In agreement with this hypothesis, extracellular recordings during HFS in patients and animal models reported a reduction of STN activity (Filali et al. 2004; Tai et al. 2003; Welter et al. 2004). The silencing hypothesis was first challenged by recordings in MPTP-treated monkeys where HFS, at parameters that decreased akinesia, changed the spontaneous irregular firing of target cells of the STN (GPe and GPi) to a high-frequency regular pattern of discharge, time-locked to the stimulation (Hashimoto et al. 2003). Using rat STN slices, we have recently shown that HFS at therapeutic frequencies generates a stable pattern of bursts of spikes time-locked to the stimulation that overrides ongoing activity (Garcia et al. 2003). Based on these observations, we suggested that the beneficial actions of HFS are related to both the suppression of the spontaneous activity and its replacement by a novel pattern of activity. If this hypothesis is correct, only STN-HFS at “therapeutic” parameters should have this dual effect. In the present study, we have varied the values of each of the parameters one at a time and determined how it modified the pattern of STN activity in slices from dopamine-depleted rats. We report that therapeutic and non-therapeutic combinations of parameters have totally different impacts on STN activity.

METHODS

Dopamine-depleted slices

We performed experiments in coronal STN slices from reserpine-treated (Hernandez-Lopez et al. 1996; Moody and Spear 1992) Nine-teen- to 22-day-old Wistar rats using a preparation technique that has been described in detail previously (Garcia et al. 2003). We preferred this model to the 6-hydroxydopamine-treated rats because we primarily aimed at obtaining dopamine depletion inside the STN. Also in the latter procedure, because of the time required for the degeneration of...
dopaminergic neurons, the rats were older and thus less convenient for in vitro recordings. Five experiments were also performed in 6-wk-old rats (140–160 g) to verify that the results obtained were not due to the immaturity of the preparation. We do not present these results separately since they were similar to those obtained in younger (juvenile) rats. After a 2-h recovery period, we placed the slices in a submersion-type recording chamber and perfused them (1.5–2 ml/min) at room temperature with a bicarbonate-buffered solution saturated with 95% O₂-5% CO₂ containing (in mM) 124 NaCl, 3.6 KCl, 1.3 MgCl₂, 1.25 N-[2-hydroxyethyl]piperazin-N’-2-ethanesulfonic acid (HEPES), 2.4 CaCl₂, 26 HCO₃⁻, and 10 glucose.

Stimulation and artifact suppression

The stimulating electrode used in the present study (NEX-100; Phymep, Paris) had a contact length of 500 μm and a contact area of 0.3 mm². We positioned the electrode in the middle of the STN and connected it in the monopolar configuration. We applied the stimulation between the tip of the electrode (negative pole) and a platinum wire surrounding the slice (ground). The electrical stimulation consisted of negative square current pulses the amplitude of which was displayed continuously. First we fixed the width of the pulses and then we adjusted manually their frequency. Once these two parameters were adjusted, we increased the current amplitude gradually to avoid sudden variations of the baseline. We tested different durations (60, 90, 200, 400 μs), intensities (100–1,000 μA) and frequencies (10, 50, 80, 130, 185 Hz). For a current of 500 μA, the current density delivered at the tip of the electrode was 1.6 mA/mm². However, stimulation applied in a submerged recording chamber places the exposed portion of the stimulating electrode in contact with the bath as well as with the tissue. This introduces a bath resistance (50 Ω-cm) in parallel with the tissue resistance (300–500 Ω-cm) (Nowak and Bullier 1998) and thus reduces the current seen by the tissue. We have estimated that with the NEX-100 electrode, 2/3 of the applied current leaked in the bath. In the present study, we always report the current intensity applied to the slices, though in the tissue the current was ≥3 times smaller. To verify this estimation, we conducted some experiments with a smaller tipped electrode having a contact length of 250 μm and a contact area of 0.2 mm² (RNEX-300, Phymep, France). When this electrode was positioned in the STN, its contact surface was entirely inside the 400-μm-thick slice. The application of a current two to three times weaker than in the previous experiment produced similar electrophysiological responses of the STN neurons. Although pulse frequency and pulse width stay the same whatever the electrode and recording configuration, the differences in surface area of the electrode contact and the configuration of the extracellular medium renders the comparison of pulse amplitude between in vivo and in vitro studies difficult. In patients, the electrode delivers a current of around 500 μA/mm² (3 mA across 6 mm²) (Lozano et al. 2002), i.e., approximately three times less than in the present study. However, the current density generated by a monopolar point source falls off rapidly in space. For example at a distance of 200–400 μm from the stimulating electrode, the current density generated in vivo by a current intensity of 1 mA is equal to 500–2,000 μA/cm² (Grill 2003).

Artifacts in current-clamp recordings had a longer duration than that applied (60–400 μs) due to membrane capacitance, but all the same much shorter than that of spikes (1–2 ms). A sample and hold electronic device (built in the laboratory) synchronized with the stimulator allowed the removal of artifacts (Minzly et al. 1993). This artifact suppressor held the recorded signal at the level before the current pulse and restored it a few microseconds later. After the suppression procedure, stimulation artifacts appeared in recordings as positive capacitative rebounds of 3- to 15-mV amplitude, surmounted or not by evoked spikes (see Fig. 1D). We did not suppress this rebound to visualize the stimuli. Comparison of recording traces before and after artifact suppression showed that the sample-and-hold procedure did not consistently affect spike recordings.
Recordings

Recorded STN cells were located at a distance of 100–400 μm from the central stimulating electrode. We used an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA) to perform whole cell recordings in current-clamp mode. The electrodes (8–12 MΩ) contained (in mM) 120 Kgluconate, 10 KCl, 10 NaCl, 1 CaCl₂, 1.1 or 10 EGTA, 10 HEPES, 0 or 2 MgCl₂, 2 MgATP, and 0.5 NaGTP, pH 7.2–7.3. We recorded only one cell per stimulated STN to avoid posteffects of long-term stimulation (1–2 h). Current output was displayed on a four-channel chart recorder (Gould Instruments, Longjumeau, France), digitized (Digidata, Axon Instruments), stored on a computer using axoscope software (Axon Instruments) and videotaped for subsequent off-line analysis. We differentiated spikes evoked by the stimulation from the spontaneous ones on the following basis: they were always triggered by artifacts and were not preceded by the spontaneous slow depolarization known to result from the persistent Na⁺ current (Beurrier et al. 2000; Bevan et al. 1999) (compare Fig. 1, C and D, left).

Mathematical analysis

We analyzed the recordings in a semi-automatic manner using purpose-written software in Yorick. The software detected automatically spikes and bursts. Spikes are a region of the trace with high variation and were identified by the time of occurrence of the maximum. The software identified bursts as plateaus of the lower envelope of the trace: it considered all regions of the envelope above a certain threshold as bursts and the corresponding spikes as part of the same burst. We inspected all the traces visually to ensure the correct identification of all their features. In those cases where the default threshold parameters identified spikes or bursts incorrectly, we chose other sets of parameters. To ascertain the nature of the output activity of the STN neurons, for each recording, we have produced smooth variation and were identified by the time of occurrence of the maximum spikes and bursts. Spikes are a region of the trace with high

![Table 1](https://www.jn.org/content.github.org/94/6/3664.full.pdf)

**Table 1. Quantitative parameters of the STN bursting pattern in response to HFS at the indicated parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>130 Hz</th>
<th>185 Hz</th>
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<tbody>
<tr>
<td>Fixed Duration 90 μs</td>
<td>400 μA</td>
<td>700 μA</td>
</tr>
<tr>
<td>Mean intraburst ISI, ms</td>
<td>12 ± 6</td>
<td>21 ± 6</td>
</tr>
<tr>
<td>Mean interburst intervals, ms</td>
<td>2022 ± 1350</td>
<td>330 ± 387</td>
</tr>
<tr>
<td>Mean burst duration, ms</td>
<td>656 ± 339</td>
<td>341 ± 409</td>
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<tr>
<td>Number of cells</td>
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<td>20</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>130 Hz</th>
<th>185 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed Intensity 400 μA</td>
<td>90 μs</td>
<td>400 μs</td>
</tr>
<tr>
<td>Mean intraburst ISI, ms</td>
<td>15 ± 5</td>
<td>21 ± 10</td>
</tr>
<tr>
<td>Mean interburst intervals, ms</td>
<td>1925 ± 153</td>
<td>582 ± 610</td>
</tr>
<tr>
<td>Mean burst duration, ms</td>
<td>546 ± 202</td>
<td>453 ± 496</td>
</tr>
</tbody>
</table>

Values are means ± SD. STN, subthalamic neuron; HFS, high-frequency stimulation; ISI, interspike interval.

RESULTS

In dopamine-depleted slices (n = 166 cells), 76% of STN cells discharged spontaneously in single-spike mode (2–10 Hz, see Figs. 2–4) and 24% in bursting (Fig. 1A) or mixed (single spikes and bursts, not shown) mode.
Variation of pulse frequency

Low-frequency stimulation (LFS) of the STN at 10 Hz evoked a completely different response compared with 130 Hz. In all STN neurons tested (n = 57), 10 Hz stimuli either had no effect (at low intensity) or evoked single spikes at 10 Hz (mean ISI: 103 ± 56 ms). These HFS-evoked spikes disappeared during LFS (Fig. 2, A and C). This observation is confirmed by the synchronization indices: n1 = 29%, n2 = 19%, n3 = 4% for 2B (top), indicating that only 52% of the ISIs where synchronous with the stimulation, while n1 = 93%, n2 = 1%, n3 = 0% for Fig. 2D (top). In this latter case, nearly all recorded spikes are exactly synchronous with the external stimulation. Moreover, the depolarization of the membrane by high-intensity pulses at 10 Hz increased the probability of spontaneous firing during LFS (Fig. 2A, bottom). Therefore LFS at 10 Hz had a simple excitatory effect: it evoked single spikes at 10 Hz or its subharmonics (Fig. 2, B and D), and it did not block their spontaneous activity (sometimes even increased it), even with high-intensity (200% above the threshold) and long duration (400 μs) pulses (Fig. 2A-D, bottom). Increasing smoothly the frequency of stimulation ≤185 Hz at constant pulse duration (90 μs) and amplitude (200-1000 μA) progressively changed the HFS-evoked response from evoked single spikes to the dual effect described above. At 50 Hz, HFS induced STN neurons to discharge single spikes at 50 Hz or doublets-triplets or trains of spikes at 50 Hz (n = 52). The percentage of each type of response was roughly 30% (data not shown). In contrast, from 80 to 185 Hz, HFS had consistently its dual effect in 85-90% of the tested cells: the generation of a new activity consisting in spikes time-locked to the stimulation and organized in bursts together with the suppression of spontaneous activity (n = 99; Fig. 1B).

Variation of pulse amplitude

We increased the pulse amplitude from 100 to 1,000 μA and kept its duration constant (90 μs). LFS at 10 Hz, as shown in Fig. 2, evoked single spikes at 10 Hz provided that the product of the pulse amplitude and duration was above the spiking threshold. Further increasing the LFS pulse amplitude never switched the pattern of the response to bursting mode. In contrast the activity evoked by HFS at 80, 130, and 185 Hz, evoked with pulse amplitude. HFS at 130 or 185 Hz had no effect or evoked single spikes for intensities ≤300 μA (Fig. 3, A1 and B1, 300 μA). When we progressively increased the pulse amplitude to 500 μA, spontaneous spikes and evoked single spikes disappeared and were replaced by bursts of evoked spikes (Fig. 3, A1 and B1, 500 μA). At these parameters, HFS-evoked bursting activity was stable in the sense that bursts had a regular temporal structure with most of the spikes evoked 1 of 1–2 or 1–3 stimuli (Fig. 3, A1 and B1, synchronization indices n1 = 42%, n2 = 47%, n3 = 2%, and n1 = 11%, n2 = 68%, n3 = 12%, n4 = 3%, respectively). At amplitudes >500 μA, HFS still evoked bursts but with a far less regular structure. Either the intraburst temporal structure was disorganized, with, for example, spikes only present at the beginning and end of membrane depolarizations (Fig. 3, A1 and B1, 600 and 700 μA) or the cell depolarized and long trains of spikes replaced bursts (Fig. 3, B1, 700 μA). In the former case, interruption of spiking revealed a plateau of 30–35 mV amplitude. At such high intensities, intraburst spikes were evoked one out of 1–4 or 2–7 stimuli in this cell (Fig. 3 A3, B3, synchronization indices n1 = 11%, n2 = 61%, n3 = 2%, n4 = 6%, and n1 = 1%, n2 = 15%, n3 = 18%, n4 = 14%, n5 = 8%, n6 = 7%, n7 = 5%, respectively), and we thus observed many more ISIs outside the displayed range than with lower intensities (see legend). We re-obtained a stable HFS-evoked bursting state by decreasing the pulse amplitude.

Variation of pulse width

We increased stimulus duration and kept constant its intensity (400–500 μA). LFS at 10 Hz, as already shown in Fig. 2, evoked single spikes at 10 Hz whatever the stimulus duration, as long as the product of pulse amplitude and duration was above the spiking threshold. Further increasing the LFS pulse amplitude never switched the pattern of the response to bursting mode. In contrast the activity evoked by HFS at 80, 130, and 185 Hz, evoked with pulse amplitude. HFS at 130 or 185 Hz had no effect or evoked single spikes for intensities ≤300 μA (Fig. 3, A1 and B1, 300 μA). When we progressively increased the pulse amplitude to 500 μA, spontaneous spikes and evoked single spikes disappeared and were replaced by bursts of evoked spikes (Fig. 3, A1 and B1, 500 μA). At these parameters, HFS-evoked bursting activity was stable in the sense that bursts had a regular temporal structure with most of the spikes evoked 1 of 1–2 or 1–3 stimuli (Fig. 3, A1 and B1, synchronization indices n1 = 42%, n2 = 47%, n3 = 2%, and n1 = 11%, n2 = 68%, n3 = 12%, n4 = 3%, respectively). At amplitudes >500 μA, HFS still evoked bursts but with a far less regular structure. Either the intraburst temporal structure was disorganized, with, for example, spikes only present at the beginning and end of membrane depolarizations (Fig. 3, A1 and B1, 600 and 700 μA) or the cell depolarized and long trains of spikes replaced bursts (Fig. 3, B1, 700 μA). In the former case, interruption of spiking revealed a plateau of 30–35 mV amplitude. At such high intensities, intraburst spikes were evoked one out of 1–4 or 2–7 stimuli in this cell (Fig. 3 A3, B3, synchronization indices n1 = 11%, n2 = 61%, n3 = 2%, n4 = 6%, and n1 = 1%, n2 = 15%, n3 = 18%, n4 = 14%, n5 = 8%, n6 = 7%, n7 = 5%, respectively), and we thus observed many more ISIs outside the displayed range than with lower intensities (see legend). We re-obtained a stable HFS-evoked bursting state by decreasing the pulse amplitude.

**FIG. 2.** Low-frequency stimulation (LFS, 10 Hz)-evoked response with increased pulse amplitude (A and B) or duration (C and D). A and C: spontaneous (spont) single-spike (s) activity of the 2 STN neurons before stimulation and distributions of the respective spontaneous ISIs. A: responses to 10-Hz pulses of constant width (90 μs) and increased amplitude (300-1,500 μA) from top to bottom. B: distributions of ISIs during 10-Hz pulses of 500 μA (top) or 1,000 μA (bottom) amplitude (constant duration, 90 μs) in the same cells (n = 10). C: responses to 10-Hz pulses of constant amplitude (500 μA) and increased duration (60–400 μs) from top to bottom. D: distributions of ISIs during 10-Hz pulses of 90 μs (top) and 400 μs (bottom) duration (constant amplitude, 500 μA) in the same cells (n = 10). a, the 10-Hz stimuli; a, positive rebounds of artifacts; e, evoked spike; s, spontaneous spike; Traces in A and C are from 2 different cells.
threshold. In contrast, activity evoked by HFS at 80, 130, and 185 Hz evolved with pulse duration. HFS with pulses of 60 μs duration mostly evoked single spikes (Fig. 4, A1 and B1, 60 μs). When we increased the duration to 90 μs, it generated bursts of evoked spikes with a mean frequency of 65 ± 12 Hz (intraburst frequency defined as the inverse of the average ISI; Fig. 4, A2 and B2, 90 μs). At these parameters, the HFS-evoked bursting activity was stable in the sense that bursts had a regular temporal structure within a burst, at the specified pulse amplitude, averaged over n similar experiments, A1 and B1: spontaneous single-spike activity before stimulation (spont). From top to bottom, responses to 130- or 185-Hz stimuli of 300 to 700 μA amplitude. *, bursts showing spikes at their beginning and end; ···, long train of evoked spikes. **· · · · · · 60 mV. A2-B3, ISI as a function of the time of occurrence within a burst during HFS at 130 Hz and 500 μA (A2, n = 80) or 700 μA (A3, n = 20) and during HFS at 185 Hz and 500 μA (B2, n = 100) or 700 μA (B3, n = 59). Some ISIs were outside the displayed range: <1% for A2 and B3, 3% for A3 and 9% for B3.

At such durations, most of the spikes were evoked in this cell every 2−5 or 2−7 stimuli, respectively (Fig. 4, A3 and B3, 90 μs). Synchronization indices n1 = 41%, n3 = 22%, n4 = 9%, n5 = 2%, and n1 = 3%, n3 = 66%, n5 = 17%, n4 = 5%, n6 = 3%, n6 = 2%, respectively). ISIs outside the displayed range were much more numerous than with shorter pulse durations (see legend). We re-obtained a stable HFS-evoked bursting state by increasing pulse width.

**FIG. 4.** Variation of the HFS-evoked response with increased pulse amplitude. All electrophysiological recordings are from the same STN neuron. High-frequency stimuli were applied at constant frequency (130 or 185 Hz) and duration (90 μs). For each frequency, left columns (A1 or B1) show recording traces and right columns (A2, A3 or B2, B3) the intrabursts ISIs as a function of time of occurrence within a burst, at the specified pulse amplitude, averaged over n similar experiments. A1 and B1: spontaneous single-spike activity before stimulation (spont). From top to bottom, responses to 130- or 185-Hz stimuli of 300 to 700 μA amplitude. *, bursts showing spikes at their beginning and end; ···, long train of evoked spikes. **· · · · · · 60 mV. A2-B3, ISI as a function of the time of occurrence within a burst during HFS at 130 Hz and 500 μA (A2, n = 80) or 700 μA (A3, n = 20) and during HFS at 185 Hz and 500 μA (B2, n = 100) or 700 μA (B3, n = 59). Some ISIs were outside the displayed range: <1% for A2 and B3, 3% for A3 and 9% for B3.

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**Fig. 4.** Variation of the HFS-evoked response with increased pulse amplitude. High-frequency stimuli were applied at constant frequency (130 or 185 Hz) and amplitude (400 μA). Left (A1, B1): recording traces from 2 different STN neurons; right (A2, A3 or B2, B3), the ISIs as a function of the time of occurrence within a burst, at the specified pulse width, averaged over n similar experiments. A1 and B1: spontaneous single-spike activity before stimulation (spont). From top to bottom, responses to 130- or 185-Hz pulses of 60- to 400-μs duration. *, burst with spikes at its beginning and end; ···, long depolarized train of evoked spikes. **· · · · · · 60 mV. A2-B3: ISI as a function of the time of occurrence within a burst during HFS at 130 Hz and 90 μs (A2, n = 10) or 400 μs (A3, n = 10) and during HFS at 185 Hz and 90 μs (B2, n = 15) or 400 μs (B3, n = 13). Some ISIs were outside the displayed range: <1% for A2 and B2, 2% for A3 and 3% for B3.

**Fig. 5.** Variation of the HFS-evoked response with increased HFS parameters. The return maps of Fig. 5 summarize the results of the present study. To construct the return maps of Fig. 5, A1, A2, and B1, B2, we used the ISIs between all recorded spikes. For Fig. 5, C1, C2, and D1, D2, we used the ISIs between spikes belonging to the same bursts (intraburst ISIs). In the absence of stimulation, the spontaneous firing pattern consisted of isolated
FIG. 5. ISIs return maps. A: STN spontaneous activity in single spike (A1) and bursting (A2) mode. B: STN activity during LFS at 10 Hz and pulse width of 90 ms (B1) or 400 ms (B2). STN activity during HFS at 130 Hz (C1) and 185 Hz (D1) in optimal conditions (90 μs, 500 μA; C2 and D2, respectively) and in the case of overstimulation (90 μs, 800 μA; C1 and D1). Averages over 225, 71, 44, 43, 80, 20, 100, and 59 recordings and percentage of “out of range ISIs” equal to 3, 2, 1, 3, <1, 1, <1, and 2%, respectively, from left to right, top to bottom.

spikes (5A1) or bursts (5A2). In both cases, the points that represent the ISIs were clustered around the diagonal. In the former case, this indicates that the spikes were approximately periodic within a single recording, but different recordings contained spikes with different firing periods. In the latter case, an analysis of the ISIs as a function of the time of occurrence within the burst (Fig. 1, A and C) indicated that the ISIs varied slowly within a single burst. Therefore in the case of Fig. 5A2, the points within a single burst move up and down the diagonal as the ISI slowly changes. The ISI patterns referred to as ISIs signatures (Szucs et al. 2005) were totally different under LFS and HFS. In response to 10-Hz stimulation (Fig. 5B), the firing pattern was periodic irrespective of the width of the stimulation pulse (100 ms for B1 and 400 ms for B2) but with many intermingled spontaneous spikes, thus giving rise to the characteristic triangular structure of the return map. The triangular pattern can be understood by considering a succession of periodic spikes P (evoked at 10 Hz) of period T (100 ms) with an intermingled random spontaneous spike S at a random interval R after a periodic spike. Spikes in a periodic train each give rise to a point with coordinates (T, T) on the return map, whereas the spontaneous spike S and its two neighboring periodic spikes give rise to three points with coordinates (T, R), (R, T – R), and (T – R, T) that lie on the three sides of a triangle with vertex (T, T). Different spontaneous spikes will happen at different intervals R after a periodic spike: therefore their corresponding points in the return map cover the three sides of the triangle (Faure et al. 2000). In contrast, in response to 130- and 185-Hz stimulations the ISIs were concentrated at approximately integer multiples of 7.7 or 5.4 ms (intervals between HFS pulses), and the return maps showed distinct clusters around these values with a decreasing probability toward higher values. This indicates that for parameter values close to the therapeutic ones (Fig. 5, C1 and D1), the firing pattern followed the stimulation with most of the spikes evoked every second stimulation pulse. When we increased the stimulation intensity (Fig. 5, C2 and D2) the firing pattern, while still being dominated by the stimulation period, became more irregular and the return map showed spikes separated by ≈20 times the stimulation period.

**DISCUSSION**

Our extensive study of the input-output properties of STN neurons shows conclusively that HFS does not silence the STN neurons. On the contrary, their spontaneous activity is replaced completely by a regular bursting pattern at what we have called “therapeutic parameters,” i.e., high-frequency (80–185 Hz), short-duration (90–200 μs), and moderate suprathreshold intensity pulses. Outside this range, the spontaneous activity is either not suppressed or replaced by an irregular bursting pattern. The fact that a regular firing pattern, albeit at the level of the single neuron, can be observed only in a therapeutic range of parameter values suggests that the role of HFS is not only to suppress pathological STN activity but also to introduce high-frequency regular spiking in the basal ganglia network.

There are several interpretations for the absence of spontaneous spikes inside and between HFS-driven bursts. Spontaneous and evoked afferent synaptic responses (EPSPs and IPSPs) may decrease due to synaptic plasticity (receptor desensitization or transmitter deactivation) during high-frequency and long-term stimulation of intranuclear afferents. Also, HFS depresses subthreshold voltage-dependent currents underlying intrinsic pacemaker activity (Beurrier et al. 2001). Between bursts, periods of hyperpolarization that last 0.3–2 s prevent the generation of spontaneous and evoked spikes. We propose that HFS produces regular bursts by resetting the STN somatic membrane in a reproducible region of its parameter space where burst-generating currents undergo voltage-dependent activation or de-inactivation with appropriate kinetics (Elson et al. 1999).

**HFS spikes have a regular pattern**

HFS-evoked spikes have a totally different pattern during HFS-driven bursts than during bursts recorded in vitro on membrane hyperpolarization in control STN slices (Beurrier et al. 1999; Bevan et al. 2002) or at resting membrane potential in dopamine-depleted slices. Spontaneous spikes inside spontaneous bursts are spaced by highly variable ISIs, whereas HFS-evoked spikes inside HFS-evoked bursts, have a high-frequency (60–80 Hz), time-locked to the stimulation, spaced by intervals that are multiples of the inter stimuli interval and thus evoked at predictable times (see Fig. 5). The pattern of HFS-evoked spikes may also differ from that during bursts recorded in slow-wave sleep (Urbain et al. 2000), during...
a conditioned movement (Georgopoulos et al. 1983; Wichmann et al. 1994), or in the parkinsonian state (Bergman et al. 1994; Perier et al. 2000) in the rat and monkey, but a precise analysis of the structure of these activities is lacking.

**Comparison of HFS-evoked firing patterns in the STN in vitro and in vivo**

Margarinos-Ascone et al. (2002) and Lee et al. (2004) performed in vitro experiments similar to ours but used very short periods of stimulation (10–60 s or 0.1–2 s, respectively). They report excitation of STN neurons (Lee et al. 2004) or a first period of bursting activity before silence (Margarinos-Ascone et al. 2002). However, comparison with our results is rendered difficult by the absence of precise analysis of the relationship between stimuli and recorded spikes. Extracellular recordings in vivo, in patients, in MPTP-treated monkeys or 6-OHDA-treated rats, all report that STN-HFS (100–140 Hz) totally silences STN neurons (Filali et al. 2004; Tai et al. 2003; Welter et al. 2004; Wu et al. 2001). During LFS also, results obtained with extracellular (in vivo) and intracellular (in vitro) recording techniques are discordant. Thus STN-LFS (1–10 Hz) evoked IPSPs, EPSPs, and spikes in STN neurons in slices in vitro (Garcia et al. 2003; Lee et al. 2003, 2004), whereas similar experiments in vivo showed no effect in the STN (Tai et al. 2003; Welter et al. 2004) or inhibition of STN (Filali et al. 2004) activity. In these extracellular studies, LFS-evoked spikes should have been recorded. Stimulus artifacts have a long duration (around 2 ms) in studies relying on extracellular recordings, in particular when the stimulation is close to the recording site and thus probably mask short-latency (1 ms) action potentials evoked by direct stimulation of nearby cell bodies or axons. As a result, experimentalists record only the silencing effect of HFS, i.e., the absence of spikes between artifacts that we also recorded in the present study. This silencing effect persists for several seconds or minutes once HFS is stopped and is thus recorded alone at the end of HFS (Beurrier et al. 2001; Filali et al. 2004; Garcia et al. 2003; Lee et al. 2004; Tai et al. 2003; Welter et al. 2004).

**Physiological relevance of the HFS-driven new bursting pattern**

Studies on the impact of electrical parameters settings on the clinical signs of Parkinson’s disease, bradykinesia, rigidity, and tremor (Moro et al. 2002; Rizzone et al. 2001) indicated that the most beneficial effects are obtained at high frequencies and that voltage is the most critical factor to obtain adequate alteration in STN activity. Effectively, we have shown here that stimulations below and above 50–80 Hz act differently on the spontaneous activity and the firing pattern of STN neurons, provided that the product of the pulse amplitude and duration was largely above spiking threshold. The relevance of the new STN bursting activity imposed by HFS at therapeutic parameters is under question: does it propagate to target neurons and have a role by itself or does it represent a regular output with a lack of information content (Grill et al. 2004). Recordings in target nuclei during STN-HFS showed that at least some of the spikes evoked by HFS in STN neurons propagate to and excite target neurons. In awake MPTP-monkeys, HFS at parameters that ameliorated the motor signs of monkeys generated an excitation in the GPe and GPi time-locked to the stimuli (Hashimoto et al. 2003). Also, HFS of the STN activate some SNr neurons in control rats (Maurice et al. 2003). However, HFS-evoked spikes recorded in target neurons do not appear to be organized in bursts or trains. One explanation could be that STN neurons fire in unsynchronized bursts thus giving a continuous high-frequency STN output. Sequential recordings of different STN neurons in the same slice showed that STN neurons respond with bursts of different durations and frequencies to the same HFS (Garcia et al. 2003), thus suggesting asynchronization of HFS-driven bursts. Also somatic recordings may not be representative of the axonal output because axons are more susceptible to extracellular stimulation than somas as they are activated with pulses of shorter width (Holsheimer et al. 2000; McIntyre and Grill 2002; McIntyre et al. 2004; Nowack and Bullier 1998; Ranck 1975). Thus HFS may depolarize first the STN efferent axons and generate there a regular tonic activity that propagates to the terminals whereas it evokes bursts of regularly spaced spikes in STN somas. Montgomery and Baker (2000) proposed in their modeling study that the therapeutic effect of HFS results from driving neurons at higher and perhaps more importantly regular frequencies. The high frequency and the regularity of HFS both clearly shape the output of the stimulated STN neurons. HFS generates, with a high degree of temporal precision, high-frequency spikes in the upper gamma range. We propose that the signature of the pattern imposed by HFS complements its silencing action and is instrumental in the consequences of HFS in bringing the basal ganglia circuit to operate (Garcia et al. 2005).

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