The impact of high frequency stimulation parameters on the pattern of discharge of subthalamic neurons

Liliana Garcia¹, Giampaolo D’Alessandro³, Pierre-Olivier Fernagut¹, Bernard Bioulac¹ and Constance Hammond²

¹Laboratoire de neurophysiologie (Centre National de la Recherche Scientifique UMR 5543), Université de Bordeaux 2, 146 rue Léo Saignat, 33076 Bordeaux Cedex, France, ²Institut de Neurobiologie de la Méditerranée (Institut National de la Recherche Médicale U 29), 163 route de Luminy, BP 13, 13273 Marseille Cedex 9, France and ³School of Mathematics, University of Southampton, Southampton SO17 1BJ, England

Corresponding author:
Dr C. Hammond
INMED Inserm, BP 13
163 route de Luminy
13273 Marseille Cedex 09 France
hammond@inmed.univ-mrs.fr
tel: 33 4 91 82 81 00
Fax: 33 4 91 82 81 01

Figures: 5; Table: 1

Number of pages: 24

Running head: STN-HFS imposes a regular pattern
Abstract

In clinical conditions high frequency stimulation (HFS) of subthalamic (STN) neurons in Parkinson’s disease is empirically applied at 100 Hz or above (130-185 Hz), with pulses of short duration (60-100 µs) and 1-3 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 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 pre-existing 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.

Key words: HFS, subthalamic nucleus, return map, in vitro
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 (2; 29). HFS operates with a certain combination of parameter values: the best effects on motor behaviour 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 (39; 43). 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 10-30 frequency band (3; 21; 27; 28; 35; 49). 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 (1; 4; 17; 26). In agreement with this hypothesis, extracellular recordings during HFS in patients and animal models reported a reduction of STN activity (13; 45; 47). 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 (20). 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 (14). 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.

**Materials and Methods**

*Dopamine-depleted slices*

We performed experiments in coronal STN slices from reserpine-treated (22; 38) 19-22-d old Wistar rats using a preparation technique that has been described in detail previously (14). 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 six weeks old-rats (140-160 g) in order 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 2hr 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, France) 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 platinium wire surrounding
the slice (ground). The electrical stimulation consisted of negative square current pulses whose amplitude 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 to 1000 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) (40) 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 at least 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) (30) 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 \mu m from the stimulating electrode, the current density generated \textit{in vivo} by a current intensity of 1 mA is equal to 500 - 2000 \mu A/cm\textsuperscript{2} (18).

Artifacts in current-clamp recordings had a longer duration than that applied (60 to 400 \mu s) 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 (36). 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 3-15 mV amplitude, surmounted or not by evoked spikes (see Fig. 1D). We did not suppress this rebound in order 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.

\textit{Recordings}

Recorded STN cells were located at a distance of 100-400 \mu m 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 M\Omega) contained (in mM): 120 Kgluconate, 10 KCl, 10 NaCl, 1 CaCl\textsubscript{2}, 1.1 or 10 EGTA, 10 HEPES, 0 or 2 MgCl\textsubscript{2}, 2 MgATP and 0.5 NaGTP, pH 7.2-7.3. We recorded only one cell per stimulated STN to avoid post-effects 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\textsuperscript{+} current (7; 10) (compare Fig. 1, C and D left).
Mathematical analysis

We analysed 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 histograms of the inter-spike intervals (ISIs), burst durations and inter-burst intervals (IBIs). We have also plotted the power spectrum and the autocorrelation function of the trace and the ISI return map. A return map or Poincaré map illustrates the serial dependence of ISIs. It consists of a point plot, where each point corresponds to a spike. The abscissa and ordinate of each point are, respectively, the ISI preceding and following the corresponding spike. The 45° line indicates the position of identical values. For a strictly periodic signal of period $T$ the points of the ISI return map have all the same coordinates, $(T,T)$. If, instead, a spike of a regular spike train is missing, then the return map has points at coordinates $(T,T)$, $(T,2T)$ and $(2T,T)$ (see Fig. 5). To detect slow variations in the spiking frequency within a single burst we have also plotted the ISI as a function of the time of occurrence of the spike within a burst. This is also a point plot, but in this case the horizontal coordinate of each point is the time of occurrence within the burst while the vertical coordinate is the interval with the previous spike (examples in Fig 1, C and D). Finally, we have computed a set of “synchronisation indices”: we define the synchronisation index of order $j$ as the percentage of ISIs equal to $j$ times the stimulation interval within a range of ± 1ms. For example, in a neuron with stimulation indices $n_1 = 30\%$
and \( n_2 = 60\% \), 30\% of the ISIs are equal to the stimulation interval while 60\% are equal to twice the stimulation interval. Clearly in this example where at least 90\% of the ISIs are locked to the external stimuli, the dynamics is very much driven by the stimulation. The sum of the synchronisation indices of order \( j \) and their distribution provides a good quantitative measure of whether the neuron is or is not driven by the external stimulation.

**Results**

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

**HFS at therapeutic parameters**

Figure 1 shows the typical response of STN neurons to HFS of the STN with pulses at frequency and duration values commonly used in clinical applications, 130 Hz and 90 \( \mu \)s. Before stimulation, the neuron had a spontaneous bursting activity (Fig. 1, A and C). The interval between spikes (ISI) increased within the burst (Fig. 1C left). Therefore the plot of the ISI as a function of the time of occurrence of the spikes within the bursts gave a graph where the points were on a curve sloping upwards (see methods, Fig. 1C right). HFS (550 \( \mu \)A) had a dual effect: (i) it forced STN neurons to discharge bursts of evoked spikes and thus (ii) totally blocked their spontaneous activity (Fig. 1 B, D). Both these effects lasted the duration of HFS and reversed at the end of HFS after a period of post-HFS silence (not shown). During bursts, all spikes were evoked by a stimulus: the synchronisation indices for the recordings in Fig. 1 D are \( n_1 = 5\% \), \( n_2 = 88\% \), \( n_3 = 4\% \). The first spikes inside bursts were evoked at 130 Hz but then “missed” one stimulus out of two or three stimuli and were thus separated by an interval that was twice or three times that of the stimulus signal as shown by
the plot of the ISI as a function of the time of occurrence of the spike within bursts (Fig. 1D right). If an interval $T$ regularly separated the spikes inside bursts, the graph would consist of a single horizontal line at height $T$. Here, some points had a vertical coordinate equal to $T$ (7.7 ms), but most of them had it equal to $2T$ (15.4 ms), $3T$ (23.1 ms) or $4T$ (30.8 ms). In this example, 88% of the intra-burst spikes were synchronized to one out of two stimuli (Fig. 1, D and E). Bursts, unlike spikes, did not appear at predictable times and among neurons, the periods of silence between bursts were highly variable (table 1 and Fig. 1F left). Bursts had also highly variable durations between cells (table 1, Fig. 1F right). In summary, HFS at 130 Hz generated a new pattern of STN activity consisting of spikes time-locked to the stimulation and organized in bursts. This HFS-driven activity totally replaced the spontaneous one.

**Variation of pulse frequency**

Low frequency stimulation (LFS) of the STN at 10 Hz evoked a completely different response compared to 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: $103 \pm 56$ ms) (Fig. 2). These HFS-evoked spikes were intermingled with the spontaneous ones when spontaneous activity had a frequency higher than 10 Hz. In the opposite case, spontaneous spikes disappeared during LFS (Fig. 2, A and C). This observation is confirmed by the synchronisation indices: $n_1 = 29\%$, $n_2 = 19\%$, $n_3 = 4\%$ for 2B (top), indicating that only 52% of the ISIs where synchronous with the stimulation, while $n_1 = 93\%$, $n_2 = 1\%$, $n_3 = 0\%$ for 2D (top). In this latter case, nearly all recorded spikes are exactly synchronous with the external stimulation. Moreover, the depolarisation of the membrane by high intensity pulses at 10 Hz increased the probability of spontaneous firing during LFS (Fig. 2A, bottom trace). Therefore LFS at 10 Hz had a simple excitatory effect: it evoked single spikes at 10 Hz or its sub-harmonics (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 traces). Increasing smoothly the frequency of stimulation up to 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 Hz 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 1000 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, evolved with pulse amplitude. HFS at 130 or 185 Hz had no effect or evoked single spikes for intensities up to 300 µA (Fig. 3, A1 and B1, 300 µA). When we progressively increased the pulse amplitude up to 500 µA, spontaneous spikes and evoked single spikes disappeared and were replaced by bursts of evoked spikes (Fig. 3A1 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 one out of 1-2 or 1-3 stimuli (Fig 3, A2 and B2, synchronisation indices \( n_1 = 42\% \), \( n_2 = 47\% \), \( n_3 = 2\% \), and \( n_4 = 11\% \), \( n_5 = 68\% \), \( n_3 = 12\% \), \( n_4 = 3\% \), respectively). At amplitudes larger than 500 µA,
HFS still evoked bursts but with a far less regular structure. Either the intra-burst temporal structure was disorganized, with, for example, spikes only present at the beginning and end of membrane depolarisations (Fig. 3, A1 and B1, 600 and 700 µA) or the cell depolarised 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, intra-burst spikes were evoked one out of 1-4 or 2-7 stimuli in this cell (Fig 3 A3, B3, synchronisation indices \( n_1 = 11\% \), \( n_2 = 61\% \), \( n_3 = 2\% \), \( n_4 = 6\% \), and \( n_1 = 1\% \), \( n_2 = 15\% \), \( n_3 = 18\% \), \( n_4 = 14\% \), \( n_5 = 8\% \), \( n_6 = 7\% \), \( n_7 = 5\% \), 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. 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 (intra-burst frequency defined as the inverse of the average ISI) (Fig. 4, A1 and B1, 90 µs). At these parameters, the HFS-evoked bursting activity was stable in the sense that bursts had a regular temporal structure with most of the spikes evoked one out of 1-2 or 2-4 stimuli (Fig 4, A2 and B2, synchronisation indices \( n_1 = 10\% \), \( n_2 = 80\% \) and \( n_3 = 4\% \), and \( n_1 = 12\% \), \( n_2 = 69\% \), \( n_3 = 9\% \) and \( n_4 = 2\% \), respectively). Figures 3A2 and 4A2 are not identical though studying the same set of HFS parameters because they were obtained from two different populations of STN cells, one being much larger than the other. At widths larger than 300 µs,
HFS still evoked bursts but with different characteristics. Either the intra-burst temporal structure was disorganized with interruptions of spiking (Fig. 4 B1, 400 µs) or long trains of spikes replaced bursts (Fig. 4 A1, 400 µs). At such durations, most of the spikes were evoked in this cell every 2-5 or 2-7 stimuli, respectively (Fig. 4 A3, B3, synchronisation indices \( n_1 = 3\% , n_2 = 41\% , n_3 = 22\% , n_4 = 9\% , n_5 = 4\% , n_6 = 2\% \) and \( n_1 = 3\% , n_2 = 66\% , n_3 = 17\% , n_4 = 5\% , n_5 = 3\% , n_6 = 2\% \), 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 decreasing the pulse width.

The ISI signatures of STN neurons according to stimulation parameters

The return maps of figure 5 summarize the results of the present study. To construct the return maps of figures 5A1,2 and 5B1,2 we used the ISIs between all recorded spikes. For figures 5C1,2 and 5D1,2 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 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 Figure 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 (44) 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 µs for 5B1 and 400 µs 5B2), 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, while 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 (12). In contrast, in response to 130 Hz 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 towards 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 up to twenty 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 (80-185Hz), short duration (90-200 µs) and moderate supra-threshold 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 depletion) during high frequency and long-term stimulation of intranuclear afferents. Also, HFS depresses subthreshold voltage-dependent currents underlying intrinsic pacemaker activity (6). 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 (11).

**HFS-evoked spikes have a regular pattern**

HFS-evoked spikes have a totally different pattern during HFS-driven bursts than during bursts recorded in vitro upon membrane hyperpolarization in control STN slices (8; 9) 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), are 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 (46), during a conditioned movement (16; 48) or in the parkinsonian state (5; 41) 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. (31) and Lee et al. (24) performed in vitro experiments similar to ours but used very short periods of stimulation (10-60s or 0.1-2 s, respectively). They report excitation of STN neurons (24) or a first period of bursting activity before silence (31). 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 silence STN neurons (13; 45; 47; 50). 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 (14; 24; 25) whereas similar experiments in vivo showed no effect in the STN (45; 47) or inhibition of STN (13) 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 (6; 13; 14; 24; 45; 47).

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 (39; 43) 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 (19). 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 (20). Also, HFS of the STN activate some SNr neurons in control rats (32). 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 (14) thus suggesting asynchronization of HFS-driven bursts. Also, somatic recordings may not be representative of the axonal output since axons are more susceptible to extracellular stimulation than somas as they are activated with pulses of shorter width (23; 33; 34; 40; 42). 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 (37) proposed in their modelling 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 (15).

Acknowledgments

We wish to thank P Burbaud and D. Guelh for helpful discussions. Funding was provided by Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale (C.H.) and Fondation de France (C.H.). POF present address: UCLA, department of neurology, Reed neurological research centre B117, 710 Westwood plaza, Los Angeles, CA 90095-1769.

Reference List


Figure 1: Typical STN activity during HFS at 130 Hz. A, Control spontaneous bursting activity before stimulation. B, HFS (applied for 30 min) at constant pulse frequency (130 Hz), duration (90 µs) and amplitude (550 µA) evoked a stable bursting activity. C, expanded traces of a spontaneous burst from A (*) (left) and a spontaneous spike (s) inside the burst (middle, ●). ISI as a function of the time of occurrence within a burst averaged over 89 spontaneous bursts (right). D, expanded traces of an HFS-evoked burst from B (*), an HFS-evoked spike (e) inside the burst (middle, ●). ISI as a function of the time of occurrence within a burst averaged over 47 evoked bursts (right). E, Histogram of the intra-burst inter-spike intervals (ISIs) and average spike correlation function. F, distribution of inter-bursts intervals (IBI) and of burst durations. Analyses in E-F were performed from bursts evoked by HFS in the cell in B. a: positive rebounds of artifacts. The dotted line indicates -60 mV.
Figure 2: LFS (10 Hz)-evoked response with increased pulse amplitude (A, B) or duration (C, D). A, C spont: spontaneous single-spike (s) activity of the two 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 (300-1500 µA) from top to bottom. B, Distributions of ISIs during 10 Hz pulses of 500 µA (top) or 1000 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). Black dots under recordings indicate the 10 Hz stimuli. a, positive rebounds of artifacts; e, evoked spike; s, spontaneous spike; Traces in A and C are from two different cells.
Figure 3: 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 the left columns (A₁ or B₁) show recording traces and the right columns (A₂–A₃ or B₂–B₃) the intra-bursts ISIs as a function of time of occurrence within a burst, at the specified pulse amplitude, averaged over n similar experiments. A₁, 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. Dotted line indicates -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 B₂, 3% for A₃ and 9% for B₃.
Figure 4: Variation of the HFS-evoked response with increased pulse width. High frequency stimuli were applied at constant frequency (130 or 185 Hz) and amplitude (400 µA). For each frequency the left columns (A1, B1) show recording traces from two different STN neurons and the right columns (A2-A3 or B2-B3) the ISIs as a function of time of occurrence within a burst, at the specified pulse width, averaged over n similar experiments. A1, 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 depolarised train of evoked spikes. Dotted line indicates -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.
Figure 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 µs (B₁) or 400 µs (B₂). STN activity during HFS at 130 Hz (C) and 185 Hz (D) in optimal conditions (90 µs, 500 µA) (C₁ and D₁ respectively) and in the case of overstimulation (90 µs, 800 µA) (C₂ and D₂). Averages over 225, 71, 44, 43, 80, 20, 100, 59 recordings and percentage of “out of range ISIs” equal to 3%, 2%, 1%, 3%, <1%, 1%, <1%, 2%, respectively from left to right, top to bottom.
Table 1: Quantitative parameters of the STN bursting pattern in response to HFS at the indicated parameters.

<table>
<thead>
<tr>
<th></th>
<th>130 Hz</th>
<th>185 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fixed duration 90 µs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400 µA</td>
<td>12 ± 6</td>
<td>11 ± 5</td>
</tr>
<tr>
<td>700 µA</td>
<td>21 ± 67</td>
<td>33 ± 115</td>
</tr>
<tr>
<td><strong>Mean intra-burst ISI (ms)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400 µA</td>
<td>2022 ± 1350</td>
<td>809 ± 1042</td>
</tr>
<tr>
<td>700 µA</td>
<td>330 ± 387</td>
<td>964 ± 1381</td>
</tr>
<tr>
<td><strong>Mean inter burst intervals (ms)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400 µA</td>
<td>656 ± 339</td>
<td>308 ± 330</td>
</tr>
<tr>
<td>700 µA</td>
<td>341 ± 409</td>
<td>746 ± 878</td>
</tr>
<tr>
<td><strong>Mean burst duration (ms)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400 µA</td>
<td>540 ± 1001</td>
<td>540 ± 1001</td>
</tr>
<tr>
<td>700 µA</td>
<td>540 ± 1001</td>
<td>540 ± 1001</td>
</tr>
<tr>
<td><strong>Number of cells</strong></td>
<td>80</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>130 Hz</th>
<th>185 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fixed intensity 400 µA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90 µs</td>
<td>15 ± 5</td>
<td>12 ± 6</td>
</tr>
<tr>
<td>400 µs</td>
<td>21 ± 10</td>
<td>18 ± 106</td>
</tr>
<tr>
<td><strong>Mean intra-burst ISI (ms)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90 µs</td>
<td>1925 ± 153</td>
<td>1938 ± 1244</td>
</tr>
<tr>
<td>400 µs</td>
<td>582 ± 610</td>
<td>691 ± 1033</td>
</tr>
<tr>
<td><strong>Mean inter burst intervals (ms)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90 µs</td>
<td>546 ± 202</td>
<td>509 ± 375</td>
</tr>
<tr>
<td>400 µs</td>
<td>453 ± 496</td>
<td>540 ± 1001</td>
</tr>
<tr>
<td><strong>Mean burst duration (ms)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90 µs</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>400 µs</td>
<td>10</td>
<td>13</td>
</tr>
</tbody>
</table>