Electrophysiological and metabolic evidence that high-frequency stimulation of the subthalamic nucleus bridles neuronal activity in the subthalamic nucleus and the substantia nigra reticulata

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ABSTRACT **High-frequency stimulation (HFS) of the subthalamic nucleus (STN) has been shown to produce a dramatic alleviation of motor symptoms in patients with advanced Parkinson's disease. Its functional mechanism, however, remains obscure. We used extracellular recording and in situ cytochrome oxidase (CoI) mRNA hybridization to investigate the effects of HFS of the STN on neuronal activity of the STN and the substantia nigra reticulata (SNr) in normal rats and rats with 6-hydroxydopamine (6-OHDA) lesion of the substantia nigra compacta (SNc). To allow detection of spikes and analysis of firing activity, artifacts recorded during stimulation were scaled down using a template subtraction method. In both normal and lesioned rats, the activity of a majority of STN neurons was inhibited during stimulation. In the SNr, HFS also induced an inhibition of the activity of a majority of neurons in normal and lesioned rats. In situ hybridization histochemistry confirmed these results in that it showed a significant decrease in levels of CoI mRNA expression in the STN and SNr in both normal and lesioned rats during stimulation. These data afford an interesting insight into the functional mechanism of deep brain stimulation and support the hypothesis that HFS exerts an inhibitory influence on STN neuronal firing.—Tai, C.-H., Boraud, T., Bezard, E., Bioulac, B., Gross, C., Benazzouz, A. Electrophysiological and metabolic evidence that high-frequency stimulation of the subthalamic nucleus bridles neuronal activity in the subthalamic nucleus and the substantia nigra reticulata. *FASEB J.* 17, 1820–1830 (2003)**

Key Words: · *Parkinson's disease* · *extracellular recordings* · *basal ganglia* · *cytochrome oxidase*

High-frequency stimulation (HFS) of the subthalamic nucleus (STN) is now considered the most effective neurosurgical therapy for Parkinson's disease. This relatively recent treatment has been derived from extensive experimental research. Studies of rat and non-human primate models have shown that the death of dopaminergic cells in the substantia nigra pars compacta (SNc) modifies the neuronal firing pattern of STN neurons and can induce an augmentation of their firing activity (1–6). Other research in MPTP-treated monkeys has shown that parkinsonian motor symptoms are considerably alleviated by lesion (7, 8) or HFS (9, 10) of the STN. Since HFS of the STN was first tested in humans, numerous reports have shown a dramatic alleviation of motor symptoms in patients presenting severe parkinsonism (11–14). Since both lesion and HFS of the STN appear to have similar clinical effects, it has been suggested that HFS of the STN may modify the activity of the basal ganglia by exercising an inhibitory effect on STN neurons. Exactly how has still not been clearly determined.

In the single-unit extracellular recording studies so far reported the effects of HFS of the STN could only be studied once stimulation was over, since stimulus artifacts masked underlying neuronal activity. These data showed that immediately after the cessation of HFS, STN neurons tested were underactive in the normal rat (15); in the SNr, after the cessation of stimulation, 94% of neurons were inactive in the normal rat and 90% in 6-hydroxydopamine (6-OHDA) -lesioned rats (15, 16). These in vivo results have been confirmed by intracellular recording of STN slices (17). A recent study using in situ hybridization of cytochrome oxydase has now also shown a decrease in the metabolic activity of STN neurons in response to HFS of the STN (18). Biochemical studies using microdialysis , however, have shown that HFS of the STN induced a significant increase in extracellular glutamate and GABA levels in the ipsilateral SNr in normal rats (19). The mechanism that is put into action by HFS of the

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STN may well be more complex than at first imagined. It is important to clarify the situation.

The study we present investigates the changes induced in STN and SNr neuronal activity during HFS of the STN in normal rats and in rats with 6-OHDA lesions of the SNc using *1)* single-unit extracellular recording and *2)* measurement of the expression of cytochrome oxidase subunit I (CoI) mRNA.

MATERIALS AND METHODS

Experiments were carried out on 35 adult male Wistar rats weighing 260–480 g. Twenty-three rats were used for electrophysiological recordings of STN (n=12/23) and SNr (n=11/23) neurons and 12 rats were used for the metabolic study. Animals were housed at constant temperature and humidity under a 12 h light/dark cycle with free access to food and water. All experiments were carried out in accordance with the guidelines laid down in the European Communities Council Directive of 24 November 1986 (86/609/EEC).

6-Hydroxydopamine lesion of the SNc

The protocol used for the unilateral intra-nigral microinjection of 6-OHDA has been described in a previous report (20). Eight micrograms of 6-OHDA was dissolved in 0.01% ascorbic acid (Sigma, Paris, France) diluted with 4 μL of normal saline and stored in the dark at 4°C. Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.), mounted in a stereotactic frame (Unimecanique, Paris, France), and pretreated with desipramine hydrochloride (25 mg/kg, i.p.) (Sigma, Paris, France) to protect noradrenergic neurons. After a 30 min interval, a hole was drilled above the substantia nigra and a stainless steel cannula connected by a polyethylene catheter to a Hamilton microsyringe driven by an infusion pump (Harvard Apparatus, Edenbridge, Kent, UK) was inserted to the depth indicated in Paxinos and Watson's stereotactic brain atlas (21): A –5.0, L 2.2, D 7.5. Five minutes later, a total volume of 4 μL of 6-OHDA was infused over 8 min. The cannula was then left in place for 10 min before being slowly withdrawn. Rats were tested for rotational behavior 7–10 days after surgery by subcutaneous injection of apomorphine (0.05 mg/kg, s.c.). Only rats that showed consistent turning (>20 turns in 5 min) toward the side contralateral to 6-OHDA lesion were considered to have severe SNc lesion. These rats were retained for the electrophysiological and metabolic tests carried out 4 to 5 wk later.

Electrical stimulation

A concentric bipolar stimulating electrode, tip diameter 200 μm (Phymep, Paris, France), was inserted stereotactically into the STN at an anterior inclination of 20° from the vertical on a sagittal plane. Stimuli were delivered by an isolated stimulator (Neurolog system, Digitimer, UK). The stimulation parameters used in this study (frequency, 130 Hz; pulse width 60 μs; current intensity 400 μA) were similar to those used in human stimulation (14, 22), the only exception being current intensity, which was adjusted for the rat (16, 18, 20). The intensity of 400 μA is considered to be below the threshold of the dyskinetic movements and contralateral rotations. Lower frequencies (1 Hz, 10 Hz, and 50 Hz) were also tested. For electrophysiological observation, the STN was stimulated for 10 s; for 45 min for quantification of CoI mRNA expression.

Extracellular recording

Rats were anesthestized with urethane (1.6 g/kg, i.p.) and placed in a stereotactic frame (Unimecanique, Paris). The skull and dura mater overlying the STN and SNr were then carefully removed and single-unit extracellular recordings performed in the STN and SNr before (120–180 s), during (10 s) and after (120–180 s) electrical stimulation of the STN. The glass microelectrode (WPi, Inc., Hertfordshire, UK), impedance 5–15 mOhms, containing 1% Pontamine sky blue dye dissolved in 3 M NaCl was inserted vertically to the level shown in Paxinos and Watson's rat brain atlas to correspond to that of the STN or the SNr. The stereotactic coordinates used were STN (A 3.2–3.8 mm from bregma, L 2.2–2.4 mm, H 7.5–8.2 mm) and SNr (A 5.0–5.2 mm from bregma, L 2.2–2.5 mm, H 7.5–8.5 mm). Extracellular neuronal activity was amplified with a preamplifier (Neurolog system, Digitimer, UK), displayed on an oscilloscope and stored in a computer equipped with a Powerlab interface (AD Instruments, Charlotte, NC, USA). The signal was collected unfiltered. Only neuronal activity with a signal-to-noise ratio > 3:1 was recorded and used for further investigation.

At the end of each session, the last recording site used was marked by electrophoretic injection (Iso DAM 80, WPi) of Pontamine sky blue dot through the micropipette at a negative current of 20 μA for 20 min.

Artifact downscaling

At the beginning of each recording session, the same glass microelectrode was used to record 10 templates of 10 s of background noise and stimulation artifacts in the recorded structure (STN or SNr) without any superimposed neuronal activity. These templates were then stored and used, once the recording protocol was over, to scale down the stimulation artifacts that interfere with our recordings. This allowed us to recover the neuronal signal recorded during the actual period of stimulation. The subtraction method we adopted is similar to the procedure described by Hashimoto et al., (23), using Matlab software (Matlab release 12, The Mathworks, Natick, MA, USA). The 10 templates were synchronized and averaged; the resulting mean template was synchronized with each recording of STN and SNr neuronal activity made during this session and subtracted. This procedure does not completely eliminate artifacts but reduces considerably their duration and amplitude. It thus lengthens significantly the period available for spike detection and analysis of neuronal activity, as shown in **Fig. 1**. We observed a certain variability in the shape of the stimulus artifact from one session to another, depending on the different electrical properties of individual micropipette electrodes. The average duration of each artifact after template subtraction was 1.50 ± 0.07 ms (mean±SE). At a stimulation frequency of 130 Hz, the interpulse interval is 7.7 ms. This meant that in the case of a 10 s stimulation, 8.05 s became available for analysis (80.5%).

Since artifacts are reduced but not completely eliminated, it could be objected that certain spikes could nonetheless be masked by residual artifacts. If we take the example of a stimulation at 130 Hz, the standard frequency used for human stimulation, one could postulate that STN neurons driven at this frequency should fire after a very short fixed latency interval and that the residual artifact could consequently mask these spikes, which would not be counted. This objection does not take into account the fact that even if this were the case, the "missing" spikes could be tracked further down the line. We know that SNr neurons are driven by monosynaptic glutamatergic inputs from the STN and that there is a mean latency period of 4.4 ms between the stimulus

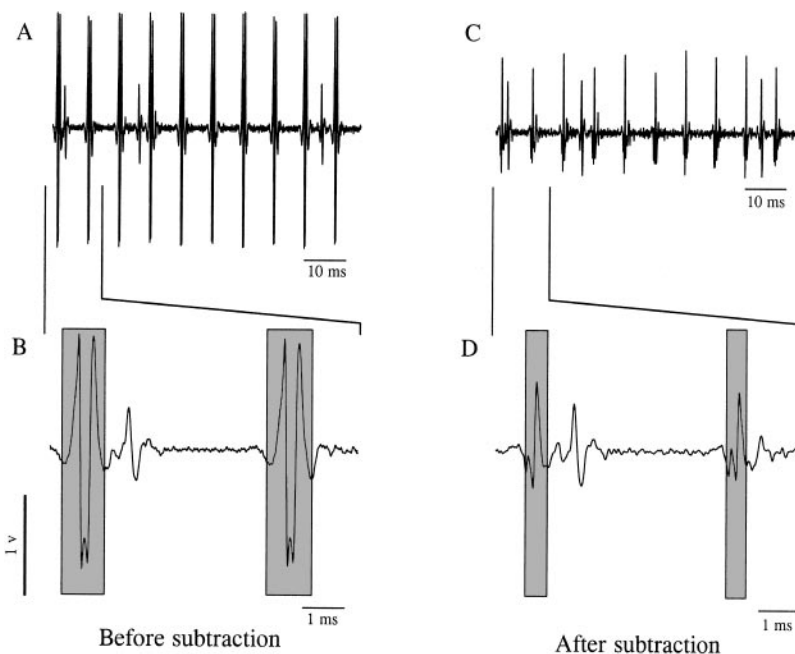

Figure 1. Stimulation artifact downscaling by the template subtraction method. Representative example of an STN neuron recorded during 130 Hz STN stimulation, before template subtraction (*A, B*) and after (*C, D*). Note the marked decrease in amplitude and duration of the artifact after subtraction.

pulse and the onset of SNr activity (24). Since the duration of this latency is threefold the duration of the mean residual stimulus artifact, the spikes that could have been masked on the STN recording should not be masked by residual artifacts on the SNr recording.

Analysis of electrophysiological data

The baseline spontaneous activity of each STN and SNr neuron was analyzed before stimulation with a spike discriminator and mean firing rates were calculated. Firing rates of normal rats were then compared with those of 6-OHDA lesioned rats, using Student's *t test.* Firing patterns were analyzed using the method developed by Kaneoke and Vitek (25) as described (26) and compared using a Chi-square test, also described (5, 6). To analyze mean firing rates of STN and SNr neurons during stimulation, artifacts were first downscaled and the residual signal filtered with a second order Butterworth filter (300–3000 Hz). We considered that a neuron responded to stimulation and changed its activity if its firing rate varied by >20% from the mean baseline firing rate. Firing frequency before and during STN stimulation was compared using Wilcoxon's signed rank test. Too few spikes were recorded during the 10 s of stimulation to allow analysis of the firing pattern for this period.

Tissue preparation

Immediately after death, brains were removed from the skull, frozen by rapid immersion in isopentane (–45°C), and stored at –80°C. They were later cut into coronal 20 μm slices at –20°C using a cryostat (Leica, Germany), thaw mounted on to gelatin-double-coated slides, and stored at –80°C.

Histological examination of electrode location

Cresyl violet staining was used to determine the location of the Pontamine sky blue dots marking the recording sites in each structure and the tip of the stimulating electrode (**Fig. 2*A–B***). Only those brains in which the location of the stimulating and the recording electrodes was clearly visible were used for data analysis.

Assessment of nigro-striatal lesion

To assess the level of dopamine loss, we measured dopamine transporter (DAT) expression in the striatum of the rats that had received unilateral 6-OHDA treatment. For radiolabeling of [^{125}I](E)-N-(3-iodoprop-2-enyl)-2β-carboxymethyl-3β-(4′-methylphenyl) nortropane (PE2I), we used the stannyl precursor to identify dopaminergic nerve endings. This method of radiolabeling has been described (27). After purification,

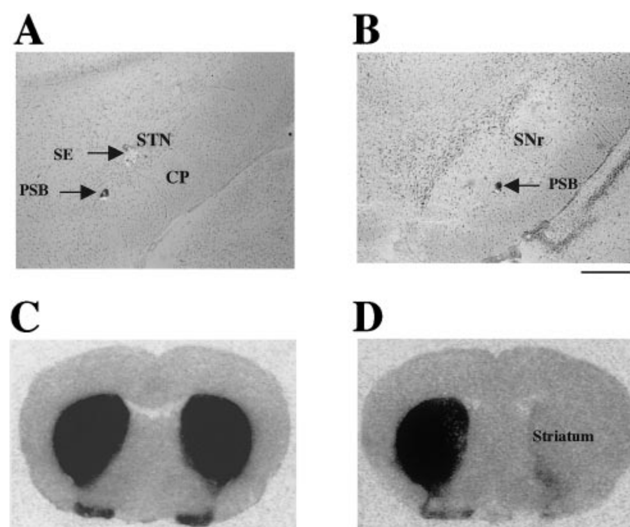

Figure 2. Photomicrographs of cresyl violet stained sections showing a typical example of the Pontamine sky blue dots (PBS) marking the location of the last neuron recorded on an electrode track in the STN (*A*) and SNr (*B*). *C, D)* Digitized autoradiographic images showing striatal PE2I binding to dopamine uptake sites in normal rats (*C*) and in rats with unilateral 6-OHDA lesion of the SNc (*D*).

[^{125}I] PE2I was obtained in a no-carrier-added form with a specific activity of 2000 Ci/mmol and stored in ethanol at –20°C, a temperature at which it remains stable for 1 month. Sections were incubated for 90 min at 25°C with 100 pM [^{125}I] PE2I in pH 7.4 phosphate buffer (in mM: Nah_2PO_4 10.14, NaCl 137, KCl 2.7, and KH_2PO_4 1.76) as described previously (27). Adjacent sections were incubated with 100 μM cocaine (Sigma) to define nonspecific binding. After incubation, all sections were washed twice for 20 min in phosphate buffer at 4°C and dried at room temperature. They were then exposed to β radiation-sensitive film (Hyperfilm β-max; Amersham Pharmacia Biotech, Buckingamshire, UK), together with calibrated [^{125}I]microscales (Amersham) in X-ray cassettes, for 3 days for autoradiographic assessment of the radioactivity bound to regions of interest. At the end of this period, only rats presenting a 90–100% DAT loss in the striatum were retained for data analysis (Fig. 2*C–D*).

In situ hybridization histochemistry

In situ hybridization was performed as described (27) using an oligonucleotide probe designed to recognize CoI mRNA (5′-AGT GGC AGA TGT AAA GTA GGC TCG GGT GTC TAC ATC TAG GCC TAC-3′) (18). Two picomoles of the oligonucleotide was tailed by the isotope at 37°C in a mixture containing 10 μL sterile water, 12.5 μL reaction buffer (sodium cocodylate 120 mM and dithiothreitol 100 mM), and 2 μL terminal deoxynucleotide transferase (all reagents DuPont/NEN) with 7 μL of ^{35}S-dATP (82.5μCi, NEN). After 60 min of incubation, the labeled probe was purified using Bio-spin chromatography columns (Bio-Rad, Hercules, CA, USA) centrifuged at 1100 *g* for 4 min (Z382K, Hermel). 1 M dithiothreitol (5*volume of eluted probe) was then added to eluted ^{35}S-dATP-labeled probe. One microliter of this solution was counted by a liquid scintillation counter (Tricarb, 1500 Packard) to assess the efficiency of labeling. Sections were allowed to hybridize at 42°C for 18 h with 150 μL of hybridization solution (50% formamide, 4× standard sodium citrate (SSC), 10% dextran sulfate, 10 mM dithiothreitol and labeled probe up to a final concentration of 3×10^6cpm/mL). They were then thoroughly washed for 30 min at room temperature in 1 × SSC, 30 min at 55°C in 1 × SSC, and 10 min at 55°C in 0.1 × SSC. Once dehydrated and fully air-dried, both slides and autoradiographic microscale standard (Amersham Pharmacia, Orsay, France) were exposed to β-max Hyperfilm (Amersham Pharmacia, Orsay, France) for 14 days at 4°C. Control experiments showed that specific hybridization signal was eliminated by unlabeled probe in excess and by pretreatment of the slides with RNase A (20 μg/mL).

Analysis of autoradiographs

The striatum was localized for DAT analysis and the STN and the SNr for CoI mRNA analysis, using the stereotactic coordinates given in Paxinos and Watson's rat brain atlas (21). The expression of CoI mRNA was also measured in a control region, the hippocampus. The optical density of these areas was then measured with an image analysis system (Densirag V. D2.99, Biocom, Les Ulis, France), as described previously (27). Three sections per structure of each animal were analyzed by an examiner blind to experimental conditions. The three results were then averaged for each animal and converted to amount of radioactivity bound by comparison to the standards. Mean radioactivity bound and SEM were calculated for each group. Data are expressed in fmol/mg of tissue equivalent.

RESULTS

Electrophysiological recordings of STN neurons

Spontaneous activity

A total of 84 STN neurons were recorded to test the effect of high-frequency stimulation; 46 were recorded in normal rats (n=6) and 38 in 6-OHDA lesioned rats (n=6). A Student's *t test* showed no significant difference (P=0.150) in the mean firing rate of these neurons between normal (15.22±1.29 spikes/s) and lesioned rats (12.73±1.04 spikes/s). There was, however, a significant change in discharge pattern (X^2=94.21, df=1.00, P<0.001) (**Fig. 3**). In normal rats, the majority of neurons presented a regular pattern of activity (Fig. 3*A, D*). In lesioned rats the majority of cells presented an irregular or bursting pattern (Fig. 3*B–D*).

Response of STN neurons to HFS of the STN

Four different types of response were observed in normal and in lesioned rats during HFS of the STN. **Figure 4*A–D*** shows a representative example of each type of response. Results are summarized in **Table 1** and Fig. 4*E–F* and **Fig. 5**.

Normal rats A significant decrease in firing rate (72%) or even a complete inhibition of activity (15%) was observed in the great majority (87%, n=40) of STN neurons recorded during the 10 s of stimulation. Mean firing rate was 15.26 ± 1.44 spikes/s before stimulation and 7.43 ± 1.19 spikes/s during stimulation (Wilcoxon's signed rank test, P<0.001). Only 4% (n=2) of the 46 neurons tested increased their mean firing rate and 9% (n=4) presented no modification. The response to stimulation was frequency dependent; the higher the frequency of stimulation, the higher the percentage of neurons presenting an inhibitory response (0% at 1 Hz, 13% at 10 Hz, 36% at 50 Hz, 87% at 130 Hz stimulation) (Fig. 5). This phenomenon was even more marked for the rare excitatory responses observed (0% at 1 Hz, 0% at 10 Hz, 0% at 50 Hz, 4% at 130 Hz).

6-OHDA rats In lesioned animals, we also observed a significant decrease in firing rate (61%) or a total inhibition of activity (16%) in the clear majority of STN neurons tested (77%, n=29). Mean firing rate was 13.6 3 ± 1.21 spikes/s before stimulation and 2.99 ± 1.02 during stimulation (Wilcoxon's signed rank test, P<0.001). The percentage of neurons presenting a significant increase in firing rate during stimulation (11.05±2.95 vs. 32.65±0.35 spikes/s, Wilcoxon Signed Rank test, P<0.001) rose, however, to 5%. Eighteen percent showed no modification (9.50±2.17 vs. 9.87±2.31 spikes/s). As can be seen from Fig. 4*E–F*, the inhibitory and the excitatory effect induced by HFS were both more pronounced in lesioned vs. normal rats. The decrease/increase in firing rate during stimulation was accentuated in lesioned rats.

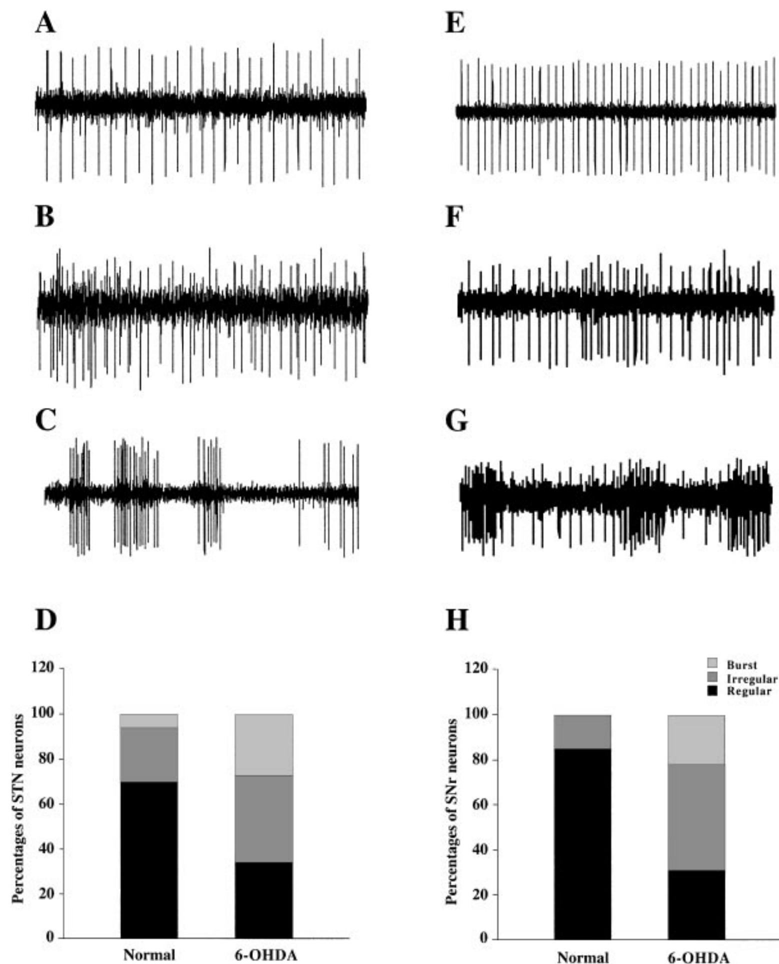

Figure 3. Single unit extracellular recordings of STN (left column) and SNr (right column) neurons in normal rats (*A, E*) and in rats with 6-OHDA lesion of the SNc (*B, C, F, G*). The histograms (*D, H*) show the prevalence of the different types of discharge pattern observed in STN (*D*) and SNr (*H*) neurons. Note that the majority of neurons recorded in the STN (*A*) and the SNr (*E*) of normal rats present a regular pattern. In lesioned rats the majority of STN and SNr neurons present either an irregular (*B* and *F*, respectively) or bursty pattern (*C* and *G*, respectively).

Electrophysiological recordings of SNr neurons

Spontaneous activity

A total of 75 SNr neurons were recorded, 38 in normal rats (n=6) and 37 in 6-OHDA lesioned rats (n=5). No significant difference was observed (Student's *t test*, P=0.414) in mean firing rate between normal (17.65±1.36 spike/s) and lesioned rats (19.41±1.65 spike/s). There was, however, a notable change in the discharge pattern (X^2=87.32, df=1.00, P<0.001) (Fig. 3). In normal rats, the spontaneous activity of these neurons was characterized by a tonic regular discharge pattern (Fig. 3*E, H*). In lesioned rats the discharge pattern became irregular or "bursty" (Fig. 3*F–H*).

Response of SNr neurons to HFS of the STN

Three types of response were noted during HFS of the STN in both normal and lesioned rats: complete inhibition, partial inhibition and no change in firing activity. No excitatory response was observed in SNr neurons. **Figure 6*A–C*** shows a representative example of each type of response and results are summarized in **Table 2** and Fig. 6*D–E*.

Normal rats During the stimulation period, 53% of SNr neurons presented partial and 3% complete inhibition. Mean firing rate decreased significantly from 17.71 ± 1.97 spikes/s before stimulation to 8.62 ± 1.16 spikes/s during stimulation (Wilcoxon's signed rank test, P<0.001). A considerable percentage (44%) showed no modification of firing rate. Mean firing rate was 17.58 ± 1.90 spikes/s before and 15.34 ± 1.58 spikes/s during stimulation.

6-OHDA rats The percentage of SNr neurons whose activity was either partially or completely inhibited during HFS of the STN rose from 56% in normal rats to 84% in lesioned animals (partially 73%, completely 11%). Mean firing rate decreased significantly from 20.88 ± 1.81 spikes/s to 7.48 ± 1.18 spikes/s (Wilcoxon's signed rank test, P<0.001). Sixteen percent did not modify their firing rate (mean firing rate 11.77 ± 2.35 spikes/s before and 11.00 ± 2.31 spikes/s during stimulation). The percentage of neurons showing no modification of their activity decreased from 44% in normal rats to 16% in lesioned animals.

CoI mRNA expression in STN and SNr neurons

The level of CoI mRNA expression in rats that had undergone 6-OHDA lesion of the SNc (n=4) was significantly higher (Student's t test, P<0.05) in the STN (**Fig. 7*A***) and the SNr (Fig. 7*B*) than the level observed in normal control rats (n=4). HFS of the STN

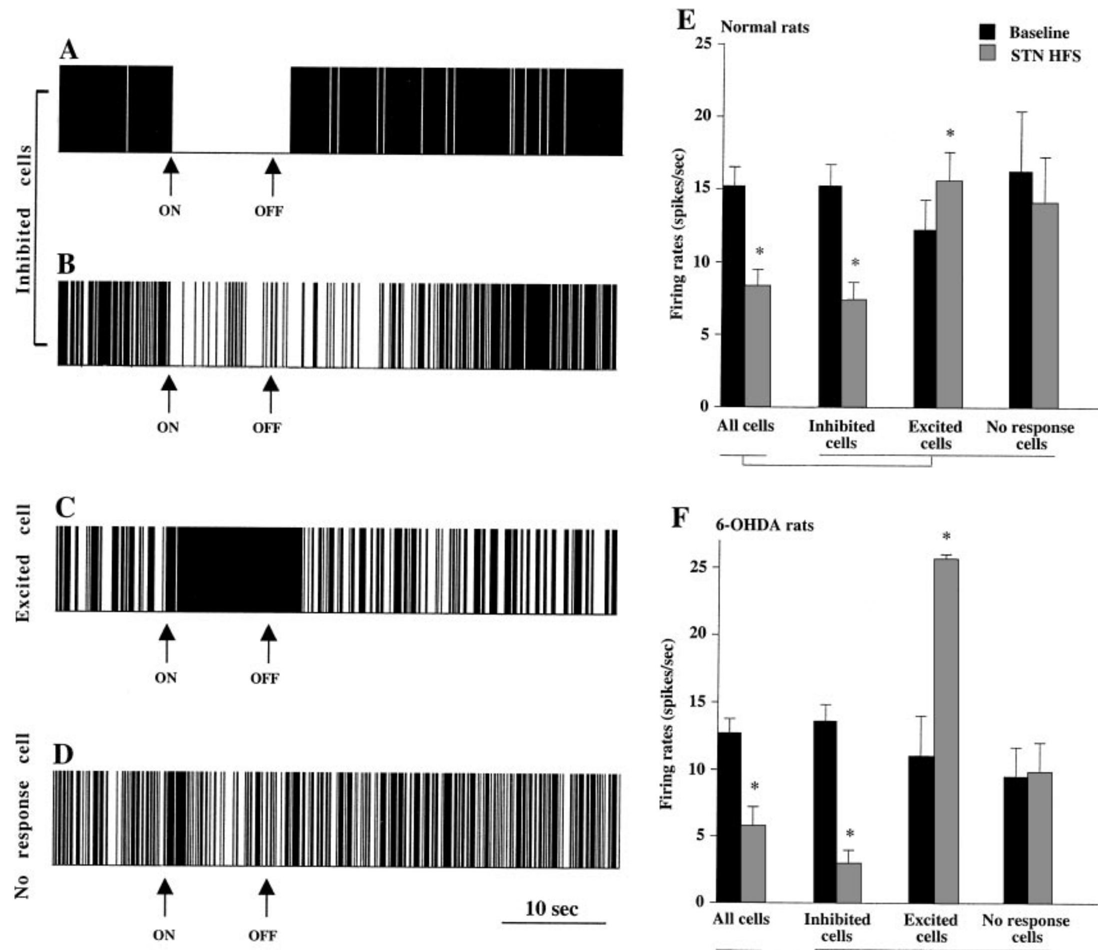

Figure 4. Left: Raster displays representing a typical example of each of the four types of response observed in STN neurons during HFS of the STN: complete inhibition (*A*), partial inhibition (*B*), excitation (*C*), and no change (*D*). Right: histograms showing the mean firing rate in response to HFS of the STN in the total population of STN neurons recorded and in each of the three subcategories of response (inhibited, excited, no response), in normal rats (*E*) and in rats with 6-OHDA lesion of the SNc (*F*). An asterisk indicates a significant difference between the mean firing rate before HFS and the mean firing rate during HFS (*$P<0.05$).

induced a significant decrease in CoI mRNA levels (Student's *t* test, $P<0.05$) in the STN (Fig. 7*A*) and the SNr (Fig. 7*B*) in normal ($n=4$) and lesioned ($n=4$) rats. These data were consistent with electrophysiological results. In the hippocampus, used as a control region, HFS of the STN did not modify (Student's *t* test, $P=0.52$) the level of CoI mRNA expression in either normal ($n=4$) or 6-OHDA lesioned ($n=4$) rats, whatever the considered subregion of the hippocampus.

TABLE 1. *Distribution of the different types of response of STN neurons to HFS of the STN (130 Hz frequency, 60 μs pulse width, 400 μA intensity) in normal rats and in rats with 6-OHDA lesion of the SNc*

	Inhibition			
	Complete	Partial	Excitation	No change
Normal rats (n = 46)	7 (15%)	33 (72%)	2 (4%)	4 (9%)
6-OHDA rats (n = 38)	6 (16%)	23 (61%)	2 (5%)	7 (18%)

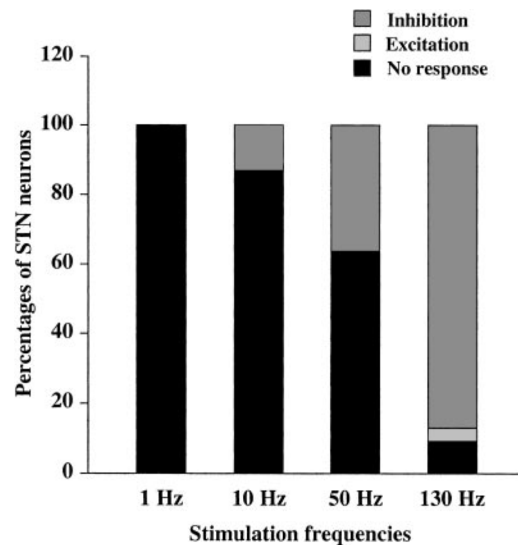

Figure 5. Histograms showing the percentage of STN neurons responding to each frequency of stimulation. Note the progressive augmentation of the percentage of neurons presenting an inhibitory response as the frequency of stimulation increases.

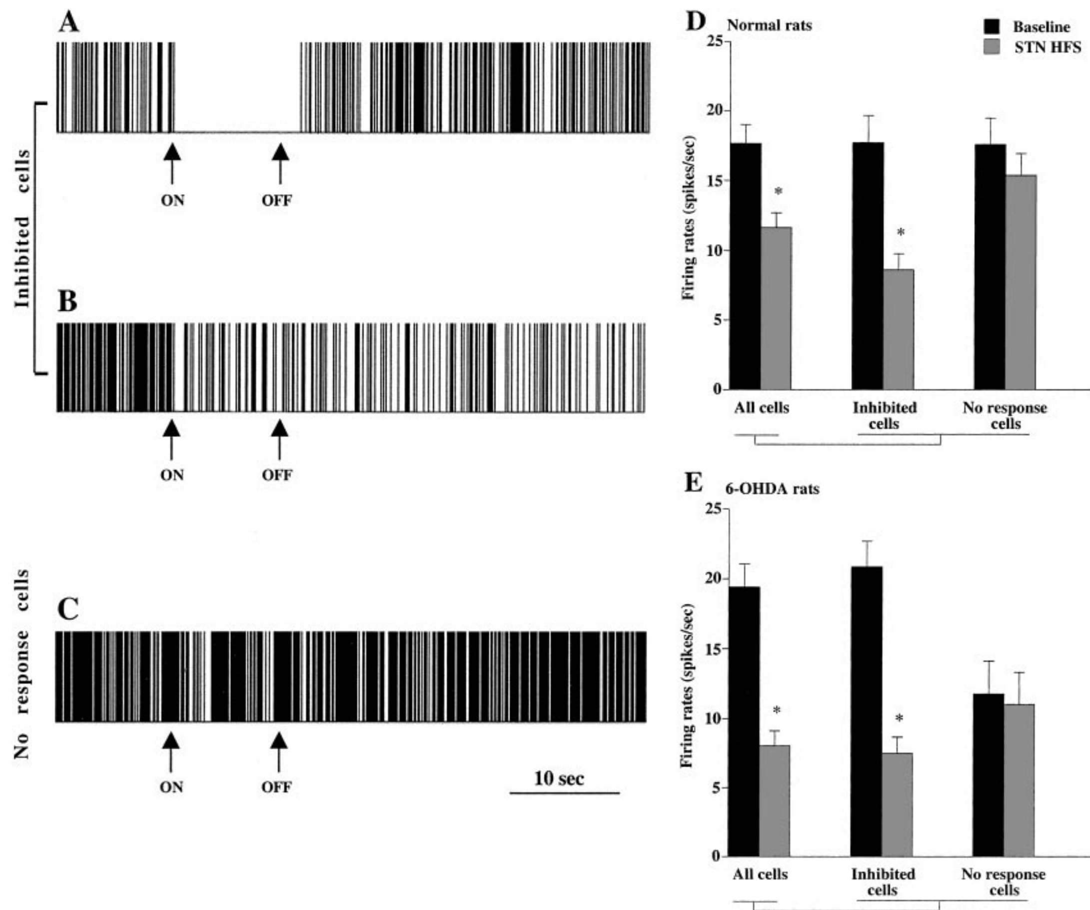

Figure 6. Left: Raster displays representing a typical example of each of the three types of response observed in SNr neurons during HFS of the STN: complete inhibition (*A*), partial inhibition (*B*), and no change (*C*). Right: Histograms showing the mean firing rates in response to HFS of the STN in the total population of SNr neurons recorded and in each of the two subcategories of response (inhibited, no response) in normal rats (*D*) and rats with 6-OHDA lesion of the SNc (*E*). An asterisk indicates a significant difference between the mean firing rate before HFS and the mean firing rate during HFS ($^*P<0.05$).

DISCUSSION

Our results show for both the normal and the lesioned animal a partial or even total cessation of electrical activity in a clear majority of STN and SNr neurons during stimulation. Parallel metabolic analysis also shows that STN and SNr both present a decrease in the level of CoI mRNA expression in response to HFS of the STN.

TABLE 2. *Distribution of the different types of response of SNr neurons to HFS of the STN (130 Hz frequency, 60 μs pulse width, 400 μA intensity) in normal rats and in rats with 6-OHDA lesion of the SNc*

	Inhibition			
	Complete	Partial	Excitation	No change
Normal rats (n = 38)	1 (3%)	20 (53%)	0 (0%)	17 (44%)
6-OHDA rats (n = 37)	4 (11%)	27 (73%)	0 (0%)	6 (16%)

Methodological considerations

The stimulation parameters used in this study were similar to those used in parkinsonian patients (12, 14), the only exception being current intensity, which was adjusted for the small size of the rat STN (16, 18, 20). Although the present work has been done in anesthetized rats, the intensity of 400 μA was chosen according to earlier work performed on awake animals (18). This current intensity is below the threshold of the abnormal dyskinetic movements and contralateral rotations.

To verify that the changes in the metabolic activity are specific to the effect of HFS of the STN, the level of CoI mRNA expression was measured in a control region, the hippocampus, which is not involved in the cortico-basal ganglia-thalamo-cortical loop. Quantitative analysis showed that HFS of the STN did not modify the level of CoI mRNA expression in the hippocampus of either normal or 6-OHDA lesioned rats.

STN and SNr neuronal activity in lesioned rats

Lesion of the SNc did not significantly change the firing rate of STN neurons but did induce a drastic

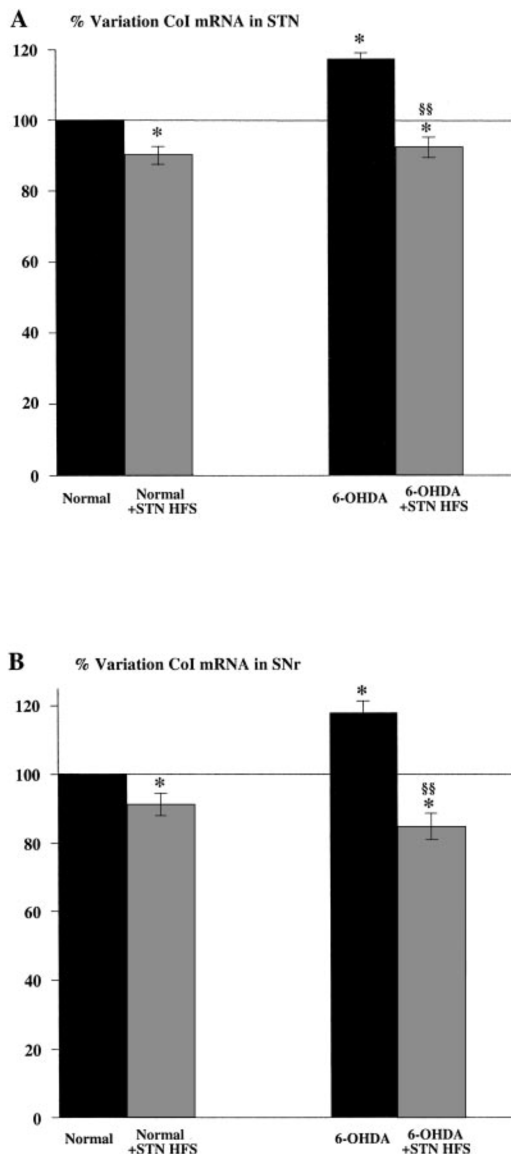

Figure 7. Histograms showing the effect of HFS of the STN on CoI mRNA levels in the STN (*A*) and the SNr (*B*) of normal rats and rats with 6-OHDA lesion of the SNc. Results are expressed as a percentage ± SEM of normal values. *Comparison (Student's *t test* $P<0.05$) between the normal group and each of the 3 other groups. §Comparison (Student's *t test* $P<0.05$) between the 6-OHDA group and the 6-OHDA + HFS group.

change in their firing pattern. Whereas in normal rats most neurons presented a regular pattern of activity, in lesioned animals the majority fired in bursts. These data confirm previous reports, which have likewise shown that the principal replicable effect of SNc lesion on STN neuronal activity is a change in firing pattern (3, 5, 6, 28, 29). A burst-firing pattern is more efficient than a regular pattern at the same firing frequency (30); a burst pattern could therefore translate a functional hyperactivity. It has already been shown that 6-OHDA lesion of the SNc induces an increase in the level of CoI mRNA expression in the rat (29); the metabolic data we report confirm these results. If STN neurons are functionally hyperactive in Parkinson's disease, it is logical that an inhibition of this hyperactivity should induce an alleviation of motor symptoms. The same would be true for SNr neurons, which also present an irregular or bursting pattern in the pathological situation and likewise show an increase in the level of CoI mRNA expression after 6-OHDA lesion of the SNc.

Effect of STN stimulation on STN neuronal activity

HFS of the STN is now well accepted as a therapy for the severe motor symptoms that characterize Parkinson's disease. This procedure was first investigated as a reversible treatment that could mimic the clinical effects of ablative surgery (7, 9, 10, 14, 22). The probability that it bridles neuronal activity in the stimulated structure has already been suggested by electrophysiological in vivo (16, 20) and in vitro (17) studies reporting an inhibition of the neuronal activity of the STN and its efferent structures after HFS. Since artifacts masked the recorded signal, the actual period of stimulation could not be analyzed in these reports. This methodological difficulty has now been overcome with the template subtraction method, which permits analysis of the stimulation period despite the existence of stimulation artifacts. The data we report in the present study confirm the inhibitory effect of HFS of the STN on the neuronal activity of the STN in both normal rats and rats with 6-OHDA lesion of the SNc. They show for the first time that this effect is induced not only after, but during, the actual period of stimulation. These electrophysiological observations are corroborated by metabolic data showing a decrease in CoI mRNA expression in normal and 6-OHDA lesioned rats, which would indicate that HFS of the STN decreases the metabolic activity of STN neurons. This concords with results recently published by Salin et al. (18). The bridling effect of HFS has also been demonstrated in the GPi. Boraud et al. (31) reported that HFS of the GPi provoked a decrease in the firing rate of GPi neurons in the MPTP-treated monkey; more recently, Dostrovsky et al. (32) have shown that GPi microstimulation inhibits neuronal firing in the GPi of human parkinsonian patients. These various reports constitute serious evidence that HFS causes a depression of neuronal activity.

The mechanism underlying this depression, however, remains a mystery. Three hypotheses have been advanced that could explain why (as in our study), the application of HFS induces a partial or total inhibition of STN neuronal firing. It has more than once been proposed that this phenomenon could be due to a depolarization block of STN neurons (9, 16, 33). The results we report in the present study argue, however, against this hypothesis. Certainly HFS of the STN induced a complete inhibition of activity in 15% of STN neurons but no increase in firing rate was observed before activity decreased. The fact that the firing rate of >60% of STN neurons decreased but was not blocked

during stimulation is also incompatible with this hypothesis.

It has been suggested that HFS may activate GABAergic inhibitory afferents (16, 20, 34). The STN is known to receive abundant GABAergic inputs from the external globus pallidus (GPe, homologue of the rodent GP); electrical stimulation could massively activate the axons of these projections, inducing substantial GABA release, which would result in a depression of STN neuronal activity. The results we present do not contradict this hypothesis since they show that even at low frequency, an inhibitory effect was observed in a small population of STN neurons. The frequency-dependent nature of the inhibitory effect of HFS is, moreover, evident in these data. This analysis is endorsed by clinical results showing that STN stimulation in parkinsonian patients is also frequency dependent and that bradykinesia and tremor showed no improvement at frequencies below 50 Hz (14, 35). The fact that a minority of STN cells (4% in normal and 5% in 6-OHDA rats) was excited by HFS is compatible with the hypothesis of an activation of afferent projections. The afferents activated in this case would be predominantly the glutamatergic projections from the cortex reported by Fujimoto and Kitai (36) and those from the parafascicular nucleus of the thalamus described by Canteras et al. (37) and Mouroux et al. (38). Data obtained in chronaxie studies, which show that axons are more excitable than cell bodies, also support this hypothesis (39–41).

A third mechanism we could consider concerns the pacemaker properties of the subthalamic neuronal membrane. It is possible that HFS exercises a direct effect on this membrane (17, 42). A recent in vitro study using intracellular recordings has shown that after cessation of HFS the spontaneous activity of tonic and bursting STN neurons is blocked. This silencing effect does not require Ca^{2+}-dependent transmitter release and is mediated by a blockade of voltage-gated currents, in particular, the persistent Na^{+} and the L- and T-type Ca^{2+} currents that are responsible for the intrinsic spontaneous discharge modes of STN neurons (17). It is highly probable that the abnormal neuronal activity observed in the STN of 6-OHDA-lesioned rats finds its source in a serious dysfunction of afferent terminals. An imbalance in the GABAergic, glutamatergic, and dopaminergic inputs arriving in complete disorder in the STN could render STN neurons incapable of performing their pacemaker duties. HFS would to a certain extent suppress the abnormal activity of STN neurons, resulting in a re-equilibrium in the neuronal activity of motor circuit. Our results, which show that HFS has more effect on STN neurons in lesioned rats, would endorse this hypothesis.

Effect of STN stimulation on SNr neuronal activity

Not only did HFS of the STN inhibit STN neurons, it induced an inhibition of neuronal activity in the SNr, its principal efferent structure in the rat. These data can be compared with previous studies in the 6-OHDA rat model of parkinsonism showing that lesion of the STN induces a decrease in the firing activity of SNr neurons (43). Our metabolic data likewise showed a decrease in the level of CoI mRNA expression in the SNr in both normal and 6-OHDA lesioned animals. The inhibitory effect of STN HFS on SNr neurons could be explained by the fact that the SNr receives glutamatergic inputs from the STN and that HFS of the STN results in an inhibition of STN excitatory outputs to the SNr. Another possibility could be that HFS of the STN may antidromically activate GP neurons inducing the stimulation of the pallidonigral pathway, which results in inhibition of nigral firing. This hypothesis is supported by a recent microdialysis study showing that STN-HFS induces an increase of GABA levels in the SNr of normal and 6-OHDA lesioned rats (19, 44).

The decrease in GAD67 gene expression induced in the SNr of the 6-OHDA lesioned rat by HFS of the STN reported by Salin et al. (18) is similar to that found in the MPTP-treated monkey after subthalamotomy (45) and consistent with our results. It has been shown that microinjection of a GABA agonist, muscimol, in the STN provokes a decrease in the firing activity of SNr neurons in the rat (46) and reverses parkinsonian motor symptoms in human patients (47). If we apply these data to the classic model of basal ganglia function, this could imply that HFS decreases the tonically inhibitory effect on the motor thalamic nuclei, resulting in a reactivation of motor cortical areas (48). Previous studies showing that HFS of the STN increases neuronal activity in the ventrolateral and ventromedian nuclei of the thalamus (20, 49) would support this supposition. A positron emission tomography study that measured regional cerebral blood flow in parkinsonian patients has also shown that the significant improvement in motor performance induced by HFS of the STN is accompanied by an increase in neuronal activity in the supplementary motor area, the dorsolateral prefrontal, and the cingulate cortices (50).

In conclusion, the electrophysiological and metabolic data we report indicate that HFS of the STN inhibits STN neuronal activity during stimulation, thus deactivating SNr neurons. These results should shed a little more light on the mechanism underlying the clinical improvement that HFS of the STN generates in the treatment of Parkinson's disease. FJ

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