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ARTICLE *in* JOURNAL OF NEUROSCIENCE RESEARCH · APRIL 2003

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Journal of Neuroscience Research 72:259–267 (2003)

Influence of the Frequency Parameter on Extracellular Glutamate and γ-Aminobutyric Acid in Substantia Nigra and Globus Pallidus During Electrical Stimulation of Subthalamic Nucleus in Rats

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High-frequency stimulation (HFS) of the subthalamic nucleus (STN) proves to be an efficient treatment for alleviating motor symptoms in Parkinson's disease (PD). However, the mechanisms of HFS underlying these clinical effects remain unknown. Using intracerebral microdialysis, we previously reported that HFS induces, in normal rats, a significant increase of extracellular glutamate (Glu) in the globus pallidus (GP in rats or GPe in primates) and the substantia nigra pars reticulata (SNr), whereas γ-aminobutyric acid (GABA) was increased only in the SNr. Bradykinesia can be improved by STN stimulation in a frequency-dependent manner, a plateau being reached around 130 Hz. The aim of the present study was to determine whether neurochemical changes are also frequency dependent. Electrical STN stimulation was applied at various frequencies (10, 60, 130, and 350 Hz) in normal rats. The results show that, for Glu, the amplitude of increase detected in GP and SNr is maximal at 130 Hz and is maintained at 350 Hz. No modifications of GABA were observed in GP whatever the frequency applied, whereas, in SNr, GABA increased from 60 to 350 Hz. Our results provide new neurochemical data implicating STN target structures in deep-brain-stimulation mechanisms.

Key words: deep brain stimulation; subthalamic nucleus; globus pallidus; substantia nigra pars reticulata; intracerebral microdialysis; amino acids; high-performance liquid chromatography

Chronic high-frequency deep brain stimulation (DBS) has been proposed for the thalamic ventral intermedius nucleus (Vim), the globus pallidus internus (GPi), and the subthalamic nucleus (STN) as an alternative to ablative surgery in the treatment of various movement disorders (Benabid et al., 1991, 1998; Benazzouz et al., 1993). The beneficial effects of DBS in these basal ganglia nuclei appear to be markedly frequency dependent and occur primarily if not exclusively only at high frequencies (over 100 Hz; Dostrovsky and Lozano, 2002). Indeed, high-frequency stimulation (HFS) of STN has been reported to induce clinical improvement in Parkinson's disease (PD; Limousin et al., 1995, 1998). Since 1993, several studies (Pollak et al., 1993; Krack et al., 1998; Kumar et al., 1999; Burchiel et al., 1999; Gerschlager et al., 1999; Bejjani et al., 2000; Houeto et al., 2000; Moro et al., 2000) have been conducted on the efficacy of STN stimulation in PD patients, but the issues concerning the values of electrical variables for each parkinsonian symptom have not been precisely addressed. Although DBS mimics the effects of ablation (Guridi et al., 1993; Gill and Heywood, 1997; Guridi and Obeso, 2001), its precise mechanism of action is still unknown. Because HFS appears to have the same clinical effect as a lesion, stimulations are assumed to "block" STN neurons (Benazzouz et al., 1995; Hayase et al., 1996). This was recently supported from in vitro experiments demonstrating that 1 min of extracellular stimulation at 100–250 Hz produced blockade of ongoing activity in STN neurons for 6 min (Beurrier et al., 2001). However, the current model of functional organization of the basal ganglia may not reflect the true complexity of the

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Contract grant sponsor: Institut National de la Santé et de la Recherche Médicale (INSERM); Contract grant sponsor: European Community; Contract grant number: QLK 6 CT 1999 02173, 5th PCRDT; Contract grant sponsor: French Parkinson's Disease Foundation.

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Received 2 September 2002; Revised 2 December 2002; Accepted 17 December 2002

network (Parent and Cicchetti, 1997; Brown and Marsden, 1998; Parent et al., 2001), and there are several other neural systems in the vicinity that could be excited or blocked by stimulation at 60–100 Hz. At present, many other hypotheses are still under discussion, such as large-fiber-system activation (Ashby et al., 1999; Dostrovsky and Lozano, 2002; Vitek, 2002), excitation of subthalamic neurons (Hashimoto et al., 2000, 2002; Bevan and Wilson; 1999), or modification of STN cell firing pattern (Ni et al., 2001). Application of currents may activate or inactivate neurons depending on their morphology, membrane properties, distance from the electrode, orientation with respect to the electrode, discharge rate, and simulus parameters (Ranck, 1975). The applied current may influence local neurons and fibers that project from or within the region of stimulation, and further differential effects may occur in the cell body and axon of the same neuron. Although the impact of HFS on STN neurons is still unclear, it is clinically well established that frequency of STN electrical stimulation is a critical parameter for alleviating motor symptoms of PD. The beginning of improvement of bradykinesia and tremor can be observed with STN stimulation at frequencies above 50 Hz, and rigidity is improved from 33 Hz. Bradykinesia can also be worsened if a low-frequency stimulation is applied (Limousin et al., 1995, 1998; Rizzone et al., 2001; Moro et al., 2002). However, it has been clearly shown that, in advanced PD patients, chronic DBS STN at 130–185 Hz provides the greatest benefits for all symptoms. Therefore, a broad band of STN frequency stimulation seems to be efficient in treating a variety of symptoms.

The present work was designed to investigate in rats, using intracerebral microdialysis, the influence of the variation of frequency of stimulation of STN on extracellular glutamate (Glu) and γ-aminobutyric acid (GABA) in its two principal targets: globus pallidus (GP in rats or GPe in primates) and substantia nigra pars reticulata (SNr). We report here the pallidal and nigral modifications of extracellular Glu and GABA [involved in subthalamopallidal/subthalamonigral pathways and pallidosubthalamic/pallidonigral projections, respectively (Gerfen and Wilson, 1996)] obtained with four different frequencies of stimulation (10, 60, 130, and 350 Hz). The findings give some useful indications for the electrical frequency parameter in DBS of the STN and some interesting information about the possible mechanism of action of DBS STN.

MATERIALS AND METHODS

Animals

Studies were performed with male Sprague Dawley OFA rats (Iffa Credo, Les Oncins, L'Arbresle, France) weighing between 280 and 350 g and housed in a temperature-controlled environment (22°C ± 2°C) with food and water ad libitum and a 12 hr light-dark cycle. Animal care and experiments were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH publications number 80-23, revised 1978) and the French Ministry of Agriculture (authorization number 38-R1001). Two animal groups composed of 22 rats were used with unilateral microdialysis, one in GP and the other in SNr. For each group, four different frequencies of electrical stimulation of STN were used: 10 Hz (GP, n = 4; SNr, n = 3), 60 Hz (GP, n = 6; SNr, n = 6), 130 Hz (GP, n = 6; SNr, n = 7), and 350 Hz (GP, n = 6; SNr, n = 6).

Surgical Procedure

As previously described (Windels et al., 2000), rats were initially anesthetized by freely breathing (1 liter/min) a 3% halothane/air mixture (air mix 22% O_2, 78% N_2) and mounted in a stereotaxic frame (David Kopf Instruments). The dialysis probes were unilaterally (left side) lowered into the GP or the SNr, and the stimulation electrode was implanted into the STN on the same side. Stereotaxic coordinates relative to bregma (Paxinos and Watson, 1982) were GP: AP –1 mm, L 3 mm, and V 7.2 mm; SNr: AP –5.5 mm, L 2.5 mm, and V 8.4 mm; and STN: AP –3.7 mm, L 2.5 mm, and V 8.4 mm. During microdialysis experiments, body temperature was maintained at 37°C with a feedback-controlled heating pad (Harvard Apparatus, Edenbridge, United Kingdom) and anesthesia was ensured by free breathing (1 liter/min) of a 1% halothane/air mixture.

Electrical Stimulation

A concentric stimulating bipolar electrode (outer diameter 200 μm, distance between the two poles 1 mm; Nex 100; Rhodes Medical Instruments, Woodland Hills, CA) was used. Stimuli were delivered with a World Precision Instrument (Stevenage, United Kingdom) acupulser and stimulus isolation units giving a rectangular pulse. Stimulation parameters were as follows. Pulse width and intensity were stable and corresponded to those routinely used in parkinsonian patients (60 μsec and 500 μA, respectively), and four different frequencies were tested: 10, 60, 130, and 350 Hz. At the end of each experiment, an electrical lesion was made via the stimulation electrode to check further its histological location.

Microdialysis

The microdialysis probes made in house were prepared and used as previously described (Bruet et al., 2001). Briefly, they consisted of a concentric arrangement of a stainless steel tube (outer diameter 0.4 mm; Hamilton, Bonaduz, Switzerland), polyethylene tubing (outer diameter 1.09 mm; Merck, Darmstadt, Germany), and silica tubing (outer diameter 150 μm; Merck) placed inside. The silica tubing extended beyond the distal end of the steel tube and was covered by a cuprophan tubular dialysis membane (Hospal, Lyon, France) sealed at the bottom with epoxy glue. The length of the dialysis membrane was adapted according to the small brain nuclei studied: 2 mm for GPe and 1 mm for SNr.

The perfusion liquid flowed out of the distal end of the steel tube passing proximally between the tube and the membrane. The probes were perfused with artificial cerebrospinal fluid (NaCl, 149 mM; KCl, 2.8 mM; $MgCl_2$, 1.2 mM; $CaCl_2$, 1.2 mM; glucose, 5.4 mM; pH 7.3) at a flow rate of 1 μl/min. Before implantation, each probe was tested in vitro in a standard Glu or GABA solution for determination of recovery of Glu and GABA (Tossman and Ungerstedt, 1986). After implantation, the dialysate fractions were collected at 15 min intervals. The first six

fractions were discarded to avoid effects of parenchymal disturbance so that an approximately steady-state level was reached. The following four fractions (1 hr) were then collected to appreciate basal values, and four other dialysates were collected during 1 hr of DBS STN. Poststimulation effects were evaluated by collecting the next eight fractions (2 hr). Dialysates were automatically collected with a refrigerated autosampler (Univentor, Zejton, Malta) and stored at −80°C until analysis.

Glutamate and GABA Assay

The concentrations of Glu and GABA in dialysate samples were determined by high-performance liquid chromatography (HPLC) as previously described (Donzanti and Yamamoto, 1988; Windels et al., 2000). Briefly, samples and standards were derivatized with o-phtaldialdehyde. The injection was automatically processed by a refrigerated autoinjector (SIL 10 AXL; Shimadzu) onto a 3 μm C18 reverse-phase column (100 × 4.6 mm). The mobile phase consisted of NaH_2PO_4 (0.05 M, pH 5.8) with 12% acetonitrile, and the flow rate was 1.2 ml/min delivered by a LC 10AT Shimadzu pump. Extracellular concentrations of amino acids were estimated by rationing peak areas of each amino acid and their respective external standard (Analytical Software Class LC10; Shimadzu). The running time for each determination was 30 min.

Histology

At the end of the experiment, animals were sacrificed under deep halothane anesthesia by decapitation. Coronal 20 μm tissue sections were cut at −20°C using a microtome cryostat (Microm HM 500; Microm, Heidelberg, Germany) at the pallidal, nigral, and subthalamic levels (Paxinos and Watson, 1982). Cresyl violet staining was performed to check the correct location of microdialysis probes and stimulation electrode. All animals presenting internal bleeding around the dialysis probes or electrodes were eliminated as well as animals with misplaced microdialysis probes or stimulation electrode.

Drugs

All drugs were purchased from Sigma (St. Louis, MO), except for o-phtaldialdehyde, which purchased from Merck, and sodium dihydrogen phosphate (NaH_2PO_4) and β-mercaptoethanol, which were purchased from Fluka (Buchs, Switzerland).

Statistical Analysis

The mean value obtained in the four dialysates collected before DBS STN was used as the baseline. Results are expressed as percentage of variation from this baseline value. For each determination of Glu and GABA concentration values and for each structure studied, Mann-Whitney tests were performed. Each animal was its own control for evaluating the modification of neurotansmitter contents during HFS STN.

RESULTS

Histological Controls

Figure 1 illustrates the correct implantation of microdialysis probes in the parenchyma of the GP (Fig. 1A) or the SNr (Fig. 1B). The stimulation electrode was also correctly implanted in the STN as shown in Figure 1C.

Basal Levels of Glu and GABA in GP or SNr

The concentrations of amino acids obtained from the four fractions collected during 1 hr to appreciate basal values before the stimulation period were grouped. The average Glu and GABA concentrations corresponding to 100% (first bar in Figs. 2A,B, 3A,B) were, respectively, 0.35 ± 0.03 μM and 0.046 ± 0.005 μM for GP and and 0.55 ± 0.04 μM and 0.145 ± 0.02 μM for SNr.

Effect of Variation of Frequency (Constant Intensity 500 μA and Pulse Width 60 μsec) of Electrical STN Stimulation on Modifications of Extracellular Glu and GABA Contents in GP and SNr

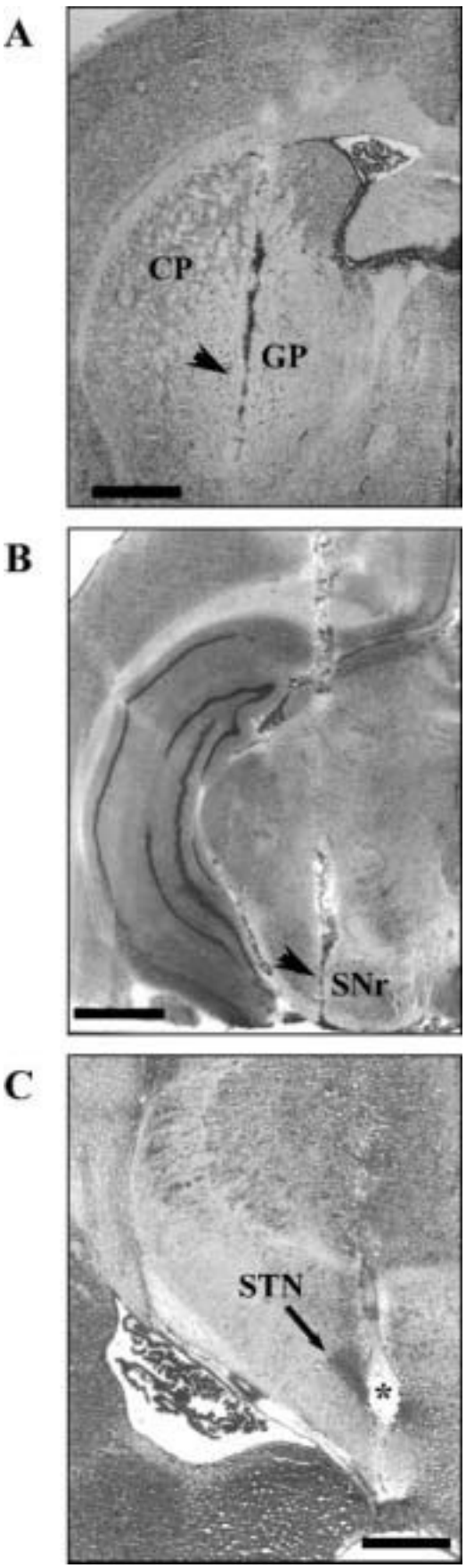
Frequency 10 Hz. During 1 hr of 10 Hz electrical stimulation of STN, no significant variations of extracellular Glu and GABA were detected in either GP or SNr. Throughout the poststimulation period, these amino acid levels remain stable, although a slight, nonsignificant upward trend was observed (Figs. 2, 3).

Frequency 60 Hz. Electrical stimulation of STN at 60 Hz provoked a significant increase of Glu contents in GP and SNr. This increase was +32% ± 14% ($P < 0.05$) and +34% ± 5% ($P < 0.05$) for GP (Fig. 2A) and SNr (Fig. 3B), respectively. This increase continued and remained significant during the poststimulation period, reaching a maximal effect at 1 hr (+124% ± 36%; $P < 0.05$) for GP (Fig. 2A) or 2 hr (+119% ± 19%; $P < 0.05$) for SNr (Fig. 3A), after the end of electrical STN stimulation.

In SNr, GABA contents were also affected by a 60 Hz electrical stimulation of STN. We observed a significant increase (+29% ± 8%; $P < 0.05$) during the stimulation period. During the poststimulation period, GABA levels continued to increase, with a maximal effect during the second hour (+106% ± 7%; $P < 0.05$) following the end of the stimulation (Fig. 3A). As illustrated in Figure 2B, 60 Hz electrical stimulation of STN did not provoke any detectable extracellular GABA variation in the GP.

Frequency 130 Hz. Electrical stimulation of STN at 130 Hz provoked a significant increase of Glu contents both in GP and in SNr. During the stimulation period, extracellular Glu measured in the GP increased significantly (+127% ± 12%; $P < 0.05$). This increase was amplified and remained significant throughout the poststimulation period, with a maximal effect (+265% ± 32%; $P < 0.05$) 1 hr after the end of stimulation (Fig. 2A). In the SNr, extracellular Glu showed a pattern of increase similar to that observed in GP during the stimulation (+74% ± 13%; $P < 0.05$) and the poststimulation (first hour: +300% ± 18%; $P < 0.05$; second hour: +311% ± 20%; $P < 0.05$) periods (Fig. 3A).

In GP, the extracellular GABA was not affected whatever the period during or after 130 Hz electrical stimulation of STN and remained comparable to basal values. In contrast, with GP, extracellular GABA in the SNr was increased during the stimulation period (+55% ±



5%; $P < 0.05$) and remained elevated during the poststimulation period (first hour: +82% ± 10%; $P < 0.05$; second hour: +65% ± 7%; $P < 0.05$).

Frequency 350 Hz. During the 1 hr 350 Hz stimulation period, collected extracellular Glu significantly increased both in GP (+49% ± 5%; $P < 0.05$) and in SNr (+30% ± 8%; $P < 0.05$; Figs. 2A, 3A), but these increases were of a signifiantly ($P < 0.05$) lower magnitude than that observed at 130 Hz both for GP and for SNr. These increased values were amplified and maintained during the first and second hours after the end of the stimulation. In GP, extracellular Glu was almost stable during the 2 hr of the poststimulation period (first hour: +107% ± 21%; $P < 0.05$; second hour: +96% ± 11%; $P < 0.05$; Fig. 2A), whereas we observed in SNr an increase between the first (+63% ± 10%; $P < 0.05$) and the second (+132% ± 35%; $P < 0.05$) hours of the poststimulation period (Fig. 3A).

In GP, as was observed with the other frequencies used (10, 60, and 130 Hz), the extracellular GABA was not affected whatever the period during or after 350 Hz electrical stimulation of STN and remained comparable to basal values. As illustrated in Figure 3B, extracellular GABA increased significantly in SNr during the stimulation period (+92% ± 17%; $P < 0.05$). This increase was the highest detected in the whole study and was of a signifiantly ($P < 0.05$) higher magnitude than that observed at 130 Hz. This GABA increase was amplified but remained stable at 1 hr (+143% ± 26%; $P < 0.05$) and 2 hr (+145% ± 29%; $P < 0.05$) during the poststimulation period.

DISCUSSION

The main result of our study conducted with normal rats is that application of increasing frequency during electrical stimulation of STN induces increases of GABA contents in SNr, while they remain stable in GP. In addition, the frequency of 130 Hz provokes the maximum increase of Glu contents both in GP and in SNr, whereas 60 or 350 Hz induced less effect and 10 Hz no effect. To our knowledge, the neurochemical mechanisms underlying changes evoked by variation of frequency during electrical stimulation of STN have not yet been documented. These data may shed some light on functional aspects of the basal ganglia network involved in the reduction of motor impairments in parkinsonian patients receiving this type of electrical stimulation.

Fig. 1. Photographs of cresyl violet-stained coronal sections at pallidal (**A**), nigral (**B**), and subthalamic (**C**) levels. Arrows indicate the implantation of microdialysis probes in GP (A) or in SNr (B). Note also the adequate localization of the stimulation electrode inside the subthalamic nuleus (C). Asterisk, stimulation point. Scale bars = 1.5 mm in A,B, 0.5 mm in C.

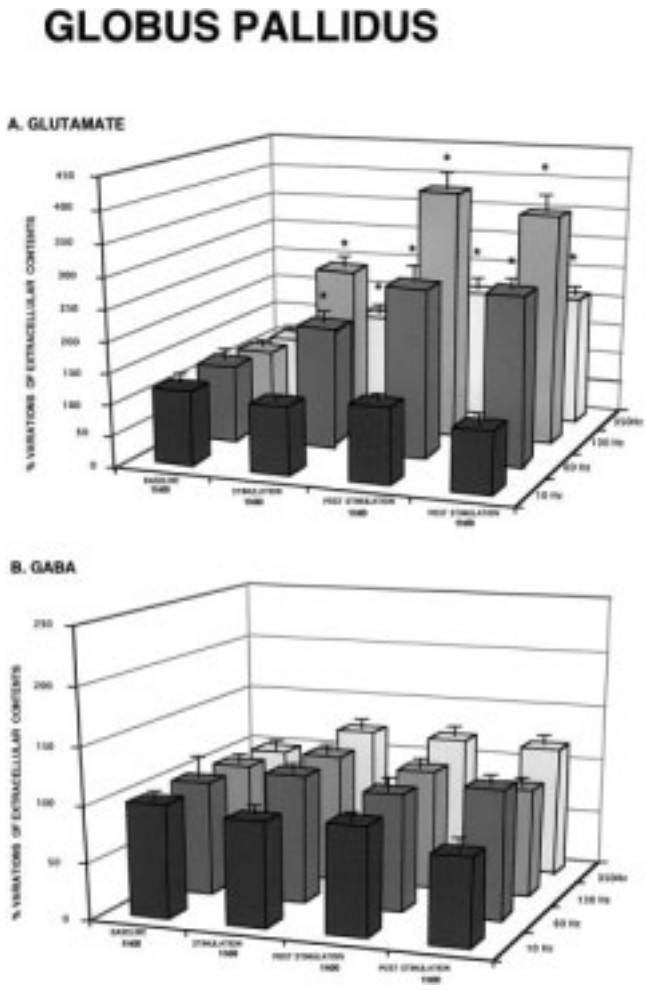


Fig. 2. Histograms showing the extracellular Glu (**A**) and GABA (**B**) changes in GP during DBS of STN according to the different frequencies applied. The prestimulation (1 hr) and stimulation (1 hr) periods each concern four dialysates (collected at 15 min intervals), which have been grouped. The poststimulation period (2 hr) concerns eight dialysates, which have been separated in two histograms (1 hr + 1 hr). The mean ± SEM of values obtained in the four dialysates collected before HFS of the STN was used as the baseline. Results are expressed as a percentage of variation of this baseline value. For each frequency, each histogram represents the mean variations ± SEM calculated from the four to six animals of a given experimental group. $\star P < 0.05$. Note the increase of extracellular Glu induced by DBS of the STN at frequencies up to 60 Hz, while GABA is not significantly affected.

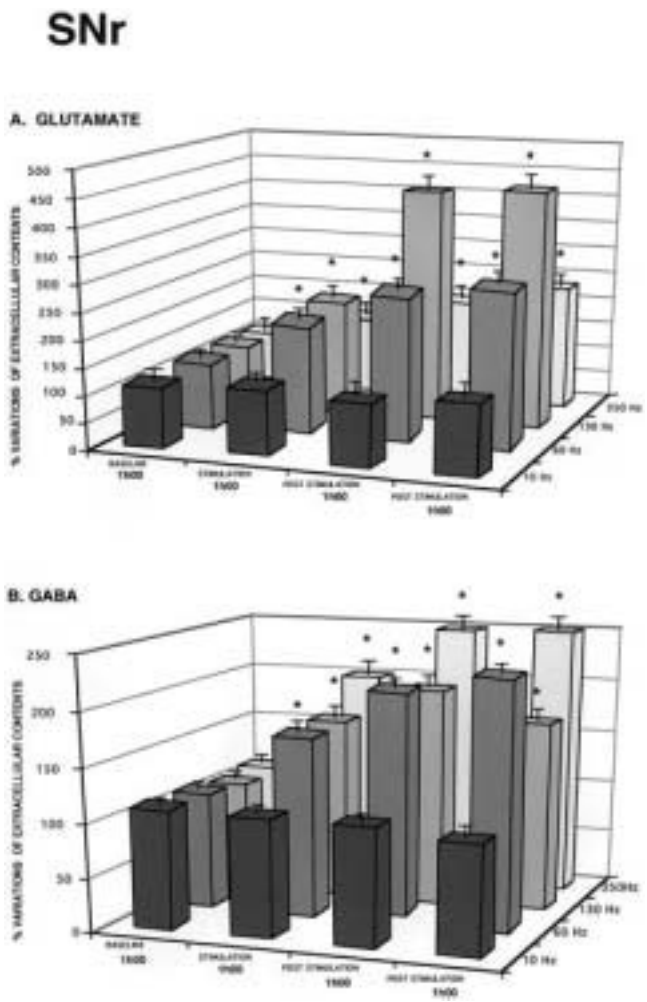


Fig. 3. Histograms showing the extracellular Glu (**A**) and GABA (**B**) changes in SNr during DBS of STN according to the different frequencies applied. The prestimulation (1 hr) and stimulation (1 hr) periods each concern four dialysates (collected at 15 min intervals), which have been grouped. The poststimulation period (2 hr) concerns eight dialysates, which have been separated in two histograms (1 hr + 1 hr). The mean ± SEM of values obtained in the four dialysates collected before HFS of the STN was used as the baseline. Results are expressed as a percentage of variation of this baseline value. For each frequency, each histogram represents the mean variations ± SEM calculated from the three to seven animals of a given experimental group. $\star P < 0.05$. Note the increase of extracellular Glu induced by DBS of the STN at frequencies up to 60 Hz, while GABA is progressively increased paralel to the amplitude of the frequency applied.

Effects of Variation of Frequency on Glu Contents in GP and SNr During Electrical STN Stimulation

We have studied different frequency values during STN electrical stimulation to examine directly whether the modification of frequency corresponds to extracellular Glu changes in both STN targets, GP and SNr. These changes may reflect modification of STN neurons activity, studied here through neurochemical measurements.

The lack of modifications of Glu contents in both GP and SNr during 10 Hz electrical STN stimulation suggests that low-frequency stimulation does not significantly induce modification of the neurochemical activity of STN neurons. In support of this, it has been reported that the firing in STN, under halothane anesthesia conditions, peaked at a frequency of about 8.5 Hz (Kreiss et al., 1996), suggesting that stimulation at 10 Hz does substantially change the activity. However, we cannot assume that

the consequences of a 10 Hz stimulation are equivalent to those related to the spontaneous activity. It can be presumed that each stimulation induces an activation, producing at least one action potential. Then, this evoked activity is added to the spontaneous activity unless this spontaneous activity has been abolished during the stimulation. In addition, we cannot exclude that very weak effects have been underestimated resulting from the limits of the microdialysis techniques in detecting minor changes of extracellular amio acids and interindividual variations.

Frequencies ≥60 Hz induce significant increase of extracellular Glu both in GP and in SNr. These results are consistent with the organization of STN projections, showing that subthalamopallidal and subthalamonigral pathways originate from the same subthalamic neurons (Van der Kooy and Hattori, 1980; Bevan and Bolam, 1995). Previous microdialysis studies showing that most of the extracellular Glu detected in substantia nigra originates from STN (Rosales et al., 1997) also confirm our present data.

These increases of Glu may correspond to stimulation-induced activation of subthalamic glutamatergic fibers and/or cells that project to these target structures. The selective increase of extracellular Glu in the GP without changes in GABA is in accordance with previous electrophysiological studies showing that STN high-frequency DBS increases GP neuronal activity (Kita and Kitai; 1987; Benazzouz et al., 1995). It has also been reported that chemical stimulation of subthalamic neurons with the $GABA_A$ antagonist bicuculline produces rapid activation of GP neurons (Robledo and Feger, 1990; Soltis et al., 1994) by blocking at least partially the normal GABA input from the GP (Smith and Grace, 1992; Hassani et al., 1996). These electrophysiological results are in relatively good agreement with the neurochemical modifications we observed in the GP.

Recent work in parkinsonian patients suggests that DBS may exert its beneficial effect by reestablishing normal patterns of temporal activity within the basal ganglia (Brown et al., 2001). Electrophysiological recordings of extracellular field potentials in GPi and STN revealed that the coherence between firing in these nuclei was strongly dependent on the state of treatment. In fact, with patients off medication, coherence between firing in GPi and firing in STN peaked at a frequency of less than 30 Hz. However, levodopa treatment resulted in a peak coherence at about 70 Hz suggesting that synchronization of activity between GPi and STN is critical for normal motor function. Interestingly, DBS begins to be efficient at 70 Hz and appears most efficient at 130–140 Hz, the first harmonic of 70 Hz (Linousin et al., 1995, 1998; Rizzone et al., 2001; Moro et al., 2002). These clinical observations could be well correlated to our neurochemical data in rats showing that nigral or pallidal extracellular Glu starts to increase under DBS at 60 Hz, and this increase is maximal at 130 Hz. Thus, the effectiveness of DBS may be in part dependent on activation and/or noninhibition of subthalamic neurons and reestablishment of appropriate synchronization. Recently, we reported that, in rats with massive dopamine depletion, extracellular Glu levels in both SNr and GP were markedly increased, which fits with an overactive STN function. However, 130 Hz electrical STN stimulation in these animals did not significantly modify the dopamine lesion-induced increase in extracellular Glu, but Glu remains high in both STN target structures (Savasta et al., 2002). These neurochemical data strongly question the current view that STN high-frequency DBS acts by inhibiting STN neurons, which has been proposed as the possible mechanism of DBS (Benazzouz et al., 1995; Hayase et al., 1996).

In addition, 350 Hz electrical STN stimulation also induced a significant increase of Glu in GP and SNr, but this effect is of lower magnitude than that observed at 130 Hz. This confirms that STN neurons are able to fire at much higher frequencies than 130 Hz, as recently reported in response to intracellular current injections (Bevan and Wilson, 1999). However, the weaker effect observed at 350 Hz on Glu contents remains difficult to explain. We have to mention that the optimal benefits in parkinsonian patients are observed at about 130–180 Hz, and it is interesting to note that our glutamate changes were maximal at 130 Hz. However, it is difficult to confirm whether there is a cause-effect relationship between these two observations.

On the other hand, increases of Glu continued in GP or in SNr after the end of the stimulation whatever the efficient frequency applied (60, 130, or 350 Hz). These Glu modifications correspond to poststimulation conditions, a situation very different from the chronic therapeutic use of DBS in parkinsonian patients. It is possible that the termination of STN electrical stimulation produces a rebound phenomenon, which in turn leads to the amplified increase of Glu. It is also possible that this continued and amplified response can be attributed not to a poststimulation effect but rather to the lengthening of the stimulation-induced effect itself via metabolic pathways. However, it is true that the time course of the changes in extracellular Glu and GABA observed here does not completely fit with the clinical effects of DBS of STN, insofar as these effects do not persist after cessation of stimualtion. Nevertheless, our neurochemical results match with the persistent benificial effects on some motor symptoms in parkinsonian patients, such as akinesia and gait impairment, which can be observed after STN stimulation has been turned off (Temperli et al., 2003). Thus, our results showing increased extracellular Glu during STN electrical stimulation at frequencies ≥60 Hz suggest that it is unlikely that nonspecific blocking of neuronal activity in target structures is the mechanism of action of DBS.

Effects of Variation of Frequency on GABA Contents in GP and SNr During Electrical STN Stimulation

In consideration of the functional organization of basal ganglia (Alexander and Crutcher, 1990), our observation that electrical stimulation of glutamatergic STN

neurons do not induce variations of GABA contents in GP is not surprising. Nevertheless, the modifications of GABA detected in SNr during electrical STN stimulation whatever the frequency used, except at 10 Hz, led us to consider different possible local and/or indirect origins. Since the SNr receives a GABAergic input from GP (Smith and Bolam, 1989), it could logically be related to the consequent activation of the pallidonigral neurons, as suggested above from the increase of extracellular Glu in GP. This observation is also in line with the reported increased firing activity of GP neurons described after high-frequency DBS of STN (Benazzouz et al., 1995). DBS of STN could likely activate descending GABAergic fibres from the striatum and/or GP travelling in the fiber bundle surrounding the STN (Parent et al., 2001), as evidenced by electrophysiological approaches (Nakanishi et al., 1987; Smith and Grace, 1992).

One of the interesting results obtained here concerns the increase of extracellular GABA in SNr in parallel with the use of increasing frequency values, except that of 10 Hz. This frequency-response curve cannot be explained only by the increase of Glu in GP activating the inhibitory pallidonigral pathway, because this Glu increase is maximal at 130 Hz. We suggest that the increasing effect on extracellular GABA in SNr is probably also due to an increasing recruitment of GABA fibers close to STN. In DBS, electrodes are placed in a complex volume conductor in close proximity to cells, dendrites, and pre- and postsynaptic axons. It is not well known which elements of which neurons are most susceptible to stimulation under different conditions. However, it has been recently suggested, from use of a three-dimensional cable model of a thalamocortical relay neuron with an extracellular electrode positioned adjacent to the soma, that action potentials are initiated in the axon during DBS and then propagated antidromically to invade the soma and dendrites (Grill and McIntyre, 2001). Previous in vitro studies of cortical neuron stimulation have also shown that action potential initiation by extracellular stimulation occurs in the axon, suggesting that axons are more sensitive than soma (Novak and Bullier, 1998a,b) and so can be independently excited by soma depolarization (Rattay, 1999). Thus, according to our neurochemical results, it is likely that the effectiveness of DBS may be dependent on activation of GABAergic tracts close to STN and that increased extracellular GABA in SNr could be a future key to understanding the inhibition of SNr activity (Windels et al., 2002).

In conclusion, this study demonstrates the impact of frequency on neurochemical modifications in STN targets during electrical STN stimulation and suggests that frequency is a crucial parameter for parkinsonian motor improvement. Nevertheless, the relationship between clinical effects and frequency remains complex. However, it is interesting to note that changes of extracellular Glu and GABA occur at frequencies above 60 Hz, which correspond to the efficient frequency for the beginning of improvement in rigidity, bradykinesia, and tremor in stimulated parkinsonian patients. Our results provide new neurochemical data favoring the involvement of STN target structures in the action of DBS and highlight the view that, in addition to its impact on STN neuron activity and excitatory glutamatergic outflow, DBS of STN interferes with GABA transmission in the SNr through mechanisms probably involving the GP.

ACKNOWLEDGMENTS

We thank Prof. Pierre Pollak and Drs. Paul Krack, Fiona Hemming, and Carole Carcenac for their critical comments on the manuscript. F.W. was the recipient of fellowships from Conseil Régional Rhône-Alpes through its Current Neuroscience Research supporting program and from the French Medical Research Foundation.

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