Journal of Neuropathology and Experimental Neurology
Vol. 62, No. 12
December, 2003
pp. 1228–1240

Neurochemical Mechanisms Induced by High Frequency Stimulation of the Subthalamic Nucleus: Increase of Extracellular Striatal Glutamate and GABA in Normal and Hemiparkinsonian Rats

NICOLAS BRUET, MSC, FRANÇOIS WINDELS, PHD, CAROLE CARCENAC, PHD, CLAUDE FEUERSTEIN, MD, ANNE BERTRAND, BSC, ANNIE POUPARD, MSC, AND MARC SAVASTA, PHD

Abstract. High frequency stimulation (HFS) (130 Hz) of the subthalamic nucleus (STN) provides beneficial effects in patients suffering from severe parkinsonism, but the mechanisms underlying these clinical results remain to be clarified. To date, very little is known concerning the effects of STN-HFS on neurochemical transmission in the different basal ganglia nuclei and in particular the striatum. This study examines the effects of STN-HFS in intact and hemiparkinsonian rats on extracellular striatal glutamate (Glu) and GABA levels by means of intracerebral microdialysis. Unilateral STN-HFS was found to induce a significant bilateral increase of striatal Glu and GABA both in intact and in dopamine-lesioned animals. In intact rats, these increases were reversed by local administration of the D1 antagonist SCH 23390, but were potentiated by the D2 antagonist sulpiride. Potentiation was also observed after local administration of both D1 and D2 antagonists whose amplitude was similar to that measured in hemiparkinsonian rats. These data furnish the first evidence that STN-HFS influences striatal amino-acid transmission and that this influence is modulated by dopamine. They provide evidence that the effects of STN-HFS are not only restricted to the direct STN targets, but also involve adaptive changes within other structures of the basal ganglia circuitry.

Key Words: GABA; Glutamate; High performance liquid chromatography; Microdialysis; Parkinson disease; Striatum; Subthalamic nucleus.

INTRODUCTION

The subthalamic nucleus (STN) has come under focus in Parkinson disease (PD) from recent advances in the understanding of the functional organization of the basal ganglia (BG) and is now recognized as the target of choice for the neurosurgical treatment of advanced PD. Indeed, on the basis of experimental data obtained in animal models of PD, it has been proposed that the STN may become overactive in PD, through complex adaptive interactions within the BG network (1–5). Therefore, abnormal activity of the STN, which sends glutamatergic excitatory projections to the BG output structures (i.e. the substantia nigra pars reticulata [SNr] and the internal globus pallidus [Gpi]), has been thought to play a crucial role in the expression of PD symptoms. Accordingly, it has been suggested that equilibrium might be restored by lesion placement or other manipulations of STN. This hypothesis is supported by studies in animal models of PD in which neurotoxic lesions of the STN (6–8), as well as pharmacological blockade of the subthalamo-pallidal pathway (9, 10) or high frequency stimulation (HFS) of the STN (11), reduce motor impairments. Afterwards, clinical trials using STN-HFS have been initiated to treat advanced PD and a consequent alleviation of motor symptoms was obtained (12–15). The fact that STN-HFS has similar effects to STN lesion (16) has supported the position that deep brain stimulation (DBS) inhibits STN neuronal activity decreasing its pathological hyperactivity and consequently reducing its excitatory influence onto the BG output structures. However, the mechanisms of the functional impact of STN-HFS remain to be elucidated and give rise to increasing interest concerning its clinical effects (17–19). To date, very little is known about neurotransmission changes induced by DBS within direct and/or indirect STN targets and consequently within the different BG nuclei. Recently, we have reported neurochemical modifications induced by STN-HFS on its 2 main output targets in intact or hemiparkinsonian rats, suggesting that DBS may not cause simply interruption of STN outflow (20–22). In addition, we have also reported that STN-HFS influences the nigrostriatal dopaminergic system by increasing the striatal release of dopamine (DA) in both intact and partially DA-lesioned rats (23). In order to better understand these striatal neurochemical disruptions during DBS and because of complex interactions existing between DA and glutamate (Glu) or GABA in this structure, the impact of STN-HFS on striatal Glu and GABA in normal and hemiparkinsonian rats was studied by using intracerebral microdialysis. In addition, the modulatory influence exerted by DA on

From Equipe Neurochimie et Neuroplasticité Fonctionnelles, INSERM U.318—Neurosciences Pre´cliniques, Université Joseph Fourier, Pavillon de Neurologie, Grenoble, France.

Correspondence to: Dr. Marc Savasta, Equipe Neurochimie et Neuroplasticité Fonctionnelles, INSERM U.318, Université Joseph Fourier, Pavillon de Neurologie, CHU de Grenoble, 38043 Grenoble Cedex 09, France. E-mail: marc.savasta@ujf-grenoble.fr

This work was supported by grants from Institut National de la Santé et de la Recherche Médicale, European Communauty (Grant No. QLK6-1999-02173, Fifth Programme Cadre de Recherche et de Développement Technologique). N.B has been supported by a fellowship from the French Ministry of Research and Technology and the French Medical Research Foundation.

striatal Glu or GABA changes induced by STN-HFS was examined through the local action of D1 and/or D2 antagonists under STN stimulation.

MATERIALS AND METHODS

Experiments were performed on male Sprague Dawley OFA rats (IFFA CREDO, Les Oncins, L'Arbresle, France) weighing 280 to 350 g at the time of surgery. Animals were housed in a temperature-controlled environment (22 ± 2°C) with food and water ad libitum and maintained on a 12 h light-dark cycle. The experimental procedures involving their care strictly conformed to the guidelines of the French Agriculture and Forestry Ministry (authorization N° 38-R 1001). Two experimental animal groups were used for unilateral microdialysis of striatum: a control group (n = 26) and a 6-hydroxydopamine (6-OHDA) SNc-lesioned group (n = 6). The control group was divided into 4 subgroups: intact group (n = 7), SCH 23390-treated group (n = 8), sulpiride-treated group (n = 5), and SCH 23390+sulpiride-treated group (n = 6).

Lesion Procedure

Lesion procedures were performed on rats under chloral hydrate anesthesia (400 mg/kg i.p.). Six animals that had been pretreated with desipramine (25 mg/kg s.c.) to protect noradrenergic neurons received a unilateral injection of 12 μg of 6-OHDA (Sigma, St. Quentin-Fallavier, France) dissolved in 4 μl of 0.9% sterile NaCl containing 0.2% of ascorbic acid at the flow rate of 0.5 $\mu l \cdot min^{-1}$ in the left substantia nigra. The stereotaxic coordinates of the injection were as follows: anterior-posterior, 3 mm; lateral, 2 mm; dorso-ventral, −2.4 mm with the incisor bar at 3.3 mm below the interaural plane according to the stereotaxic atlas of Paxinos and Watson (24). After injections, animals were kept warm and allowed to recover from the anesthetic before being returned to the animal house for 3 weeks until the microdialysis experiments. This delay was expected to allow stabilization of the effect of the DA system degeneration induced by the neurotoxin. Control animals received an injection of the saline solution.

Microdialysis Probes and Electrode of Stimulation Implantation

Rats from the 2 experimental groups were initially anesthetized by freely breathing (1 $l \cdot min^{-1}$) 5% halothane/air mixture (air mix 22% O2, 78% N2) and mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). The dorsal skull was exposed and holes drilled in order to allow the dialysis probes to be bilaterally lowered into the striatum and the stimulation electrode to be implanted (left side) into the STN. Stereotaxic coordinates were chosen according to the atlas of Paxinos and Watson (24) and were, respectively, anterior-posterior, 1 mm; lateral, 3 mm; and dorso-ventral, 7 mm for striatum, and anterior-posterior, −3.7 mm; lateral, 2.5 mm; and dorso-ventral, −8 mm for STN from bregma. During microdialysis experiments, body temperature was maintained at 37°C with a feedback controlled heating pad (Harvard Apparatus, Edenbridge, UK) and anesthesia was maintained by freely breathing (1 $l \cdot min^{-1}$) a 1% halothane/air mixture.

Electrical Stimulation

For electrical stimulation we used a concentric stimulating bipolar electrode (SNEX 100, Rhodes Medical Instruments, Woodland Hills, CA), with outer diameter of 250 μm and distance between the 2 poles ≤1 mm (Fig. 1A). Stimuli were delivered with a World Precision Instrument (Stevenage, UK) acupulser and stimulus isolation units that gave a rectangular pulse. Stimulation parameters (130 Hz, 60 μs, 500 μA) matched those routinely used in parkinsonian patients. At the end of each experiment an electrical lesion of the STN was made via the stimulation electrode to check its histological location postmortem.

Microdialysis

The homemade microdialysis probes were prepared and used as previously described (23) (Fig. 1B). Briefly, they consisted of a concentric arrangement of a stainless steel tube with outer diameter 0.457 mm (Hamilton, Bonaduz, Switzerland), polyethylene tubing with outer diameter 1.09 mm (Merck, Darmstadt, Germany), and silica tubing with 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 membrane (Hospal, Lyon, France) sealed at the bottom with epoxy glue. The length of the dialysis membrane was adapted according to the rat striatum size and was 3 mm in the present experiments. The perfusion liquid flowed out of the distal end of the steel tube passing proximally between the tube and the membrane (25). The probes were perfused with artificial cerebrospinal fluid (NaCl, 149 mM; KCl, 2.8 mM; MgCl2, 1.2 mM; CaCl2, 1.2 mM; glucose, 5.4 mM; pH 7.3) at a flow rate of 1 $\mu l \cdot min^{-1}$. Before implantation, each probe was tested in vitro in a standard amino acid solution for determination of recovery of Glu and GABA (25). After implantation, the dialysate fractions were collected at 15-min intervals. The first 6 fractions were discarded to avoid effects of parenchymal disturbance so that an approximately steady state level was reached. The following 6 fractions (1 h 30 min) were then collected to appreciate basal values and 4 other dialysates were collected during 1 h of STN-HFS (fractions 7–10). Post-stimulation effects were evaluated by collecting the next 8 fractions (2 h). Dialysates were automatically collected with a refrigerated autosampler (Univentor, Zejton, Malta) and stored at −80°C until analyzed.

Pharmacological Studies Using D1 (SCH 23390) and/or D2 (Sulpiride) Antagonists

The evaluation of the modulatory influence of DA on the modifications of striatal Glu and GABA levels induced by STN-HFS was performed on the SCH 23390-treated group (n = 8), the sulpiride-treated group (n = 5), and the SULP+SCH-treated group (n = 6). For each rat, the last collection during STN-HFS (fourth fraction) was performed by perfusing an artificial cerebrospinal fluid containing SCH 23390, sulpiride, or SCH 23390+sulpiride at concentrations of 10^{-7} M. These DA compounds were purchased from Sigma.

Glutamate and GABA Assay

The concentrations of Glu and GABA in the dialysate fractions were determined by using high performance liquid chromatography (HPLC) with fluorometric detection as previously

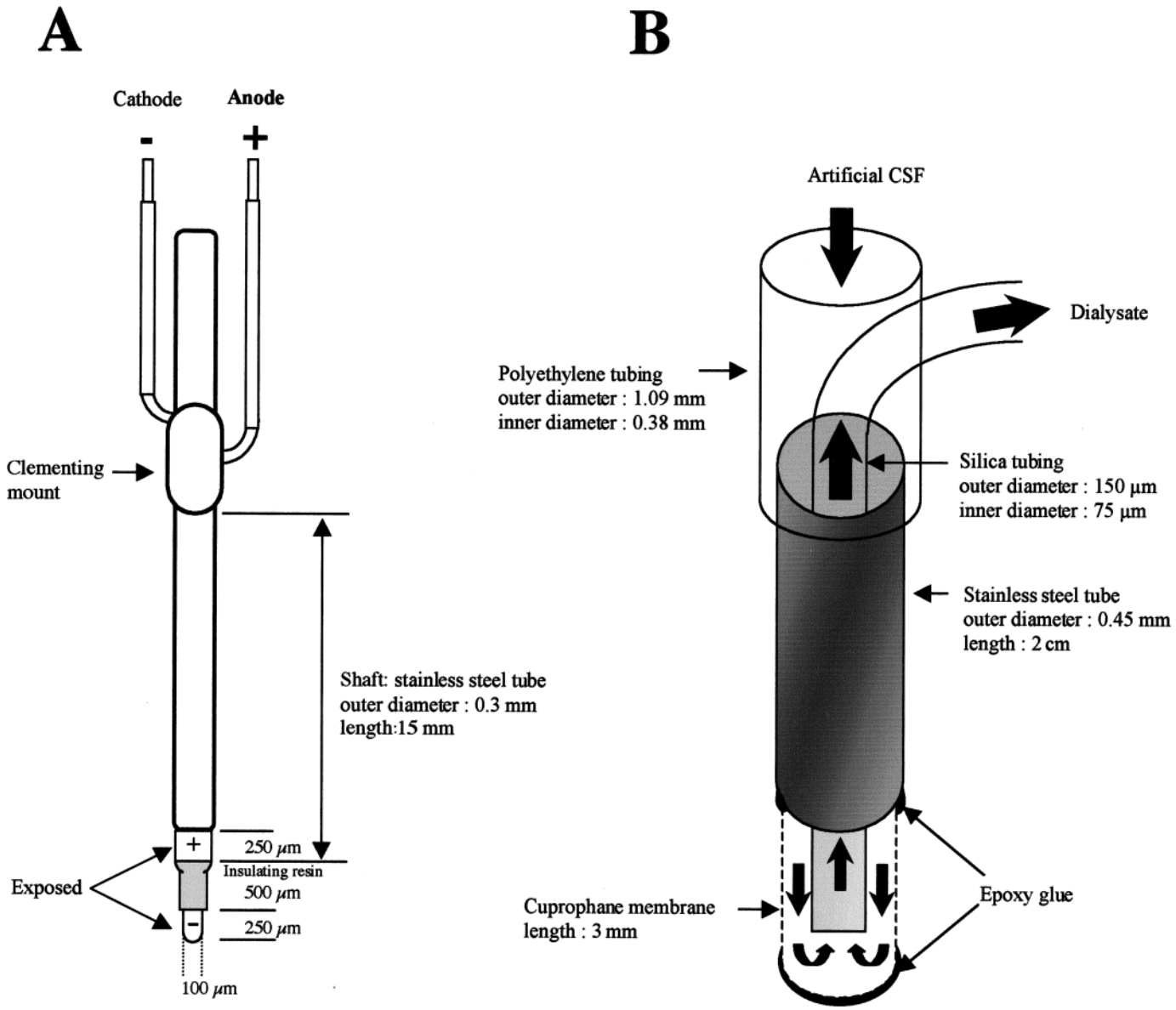


Fig. 1. Schematic drawings of the concentric bipolar electrode SNEX 100 **(A)** (Rhodes Medical Instruments, Woodland Hills, CA) was used for the electrical stimulation of the STN and of the microdialysis probe **(B)** used for collection of dialysates.

described (20, 26). Briefly, samples and standards were derivatized with o-phtaldialdehyde. The injection was automatically processed by a refrigerated autoinjector (SIL 10 AXL, Shimadzu, Kyoto, Japan) into a 3 μm C18 reversed-phase column (100 × 4.6 mm). The mobile phase consisted of NaH_2PO_4 (0.05 M, pH 5.8) with 12% of acetonitrile and the flow rate was 1.2 $mL \cdot min^{-1}$ delivered by an 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 microdialysis experiment, all animals were perfused transcardially under chloral hydrate anesthesia with 20 ml phosphate-buffered saline (PBS: 2.6 mM KCl, 1.4 mM KH_2PO_4, 136 mM NaCl, 8 mM NaH_2PO4), pH 7.2, containing heparin (5×10^4 IU/ml) followed by 350 ml of cold fixative consisting of 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.3). Brains were quickly removed and immersed overnight at 4°C in 25% sucrose solution in phosphate buffer (0.1 M, pH 7.5), frozen in cooled (−30°C) isopentane, and then stored at −20°C until cresyl violet staining and/or tyrosine hydroxylase (TH) immunohistochemistry. Serial frontal sections (30-μm thick) were cut using a microtome cryostat (Microm HM 500, Heildelberg, Germany) and collected in Tris-buffered saline (0.1 M, pH 7.4) (TBS). In order to check the correct location of the microdialysis probes and stimulation electrode, several tissue sections were cut at the striatal and subthalamic levels according to the atlas of Paxinos and Watson (24) and processed

for classical cresyl violet staining. These histological controls were systematically performed in all animals of both experimental groups. In addition, all animals presenting internal bleeding around the dialysis probes or electrodes were eliminated (to avoid contamination in microdialysates), as well as animals with misplaced microdialysis probes or stimulation electrode. In order to evaluate the DA nigrostriatal denervation induced by the 6-OHDA injection in the lesioned rats, several tissue sections were cut at the striatal and nigral levels and collected for TH immunohistochemistry. In this case, free-floating sections were washed extensively with TBS, preincubated for 1 h in TBS containing 0.3% Triton X-100 (TBST) and 3% normal rabbit serum (Miles Inc., Pittsburgh, PA). Sections were then incubated under agitation at 4°C for 48 h in primary antisera diluted in TBST containing 1% normal rabbit serum and 1% sodium azide. Dilution of antiserum was 1:100 for TH (mouse monoclonal; Boehringer Mannheim, Meylan, France). Revelation was done using the avidin biotin peroxidase complex. Diaminobenzidine (0.05%, Sigma) was used as chromagen and 0.01% H_2O_2 (Merck, Rahway, NJ) applied for 2 to 5 min, as previously described (27). Sections were then dehydrated and coverslipped with Eukitt.

Data Analysis

The mean value obtained in the 6 dialysates collected before STN-HFS was used as the baseline and corresponded to 100%. Results are expressed as a percent of variation from this baseline value. For each determination of Glu and GABA concentration values and each animal group studied, differences were assessed using the Mann-Whitney *U*-test. Each animal was its own control for appreciating the modification of neurotransmitter levels during STN-HFS. The absence of any asymmetry in baseline values was confirmed by comparing the extracellular concentrations obtained in the right and left striatum.

RESULTS

Histological Controls of the Electrode and Microdialysis Probe Locations and of the Extent of Dopamine Lesion

Three weeks after injection, the animals that received a unilateral injection of 6-OHDA showed a massive loss of TH immunolabeling in the SNc (Fig. 2A) and in the striatum (caudate-putamen nucleus) (Fig. 2C) on the lesioned side. Dense TH immunolabeling was detected throughout the striatum, the nucleus accumbens and olfactory tubercles on the intact side in 6-OHDA rats (Fig. 2B), as well as in controls (data not shown).

Figure 2D illustrates the correct implantation of the stimulation electrode in the STN. No major tissue damage was observed in the structure. Figure 2F shows the implantation of microdialysis probes in the parenchyma of the striatum in control rats. The symmetrical location of microdialysis probes allowed us to compare data obtained from homologous anatomical areas.

Effect of STN-HFS on Extracellular Glu Levels in the Striatum of Normal and Hemiparkinsonian Animals

In intact rats, the average of basal Glu striatal concentration (mean value of left and right sides) corresponding to 100% was 610 ± 98 nM. During the 1-h HFS period, Glu levels in the 4 samples (fractions 7–10) collected from the ipsilateral striatum were significantly increased and reached between +161 ± 22% and +168 ± 23% of the mean basal value (Fig. 3A). In the post-stimulation period, this increase remained significant during the following collection (fraction 11): +140 ± 21% ($p < 0.01$) and then rapidly declined towards baseline values. Interestingly, similar modifications of Glu were also observed in the contralateral striatum while only 1 side had been stimulated (Fig. 3A).

In hemiparkinsonian rats, the average of basal Glu striatal concentration (mean values of left and right sides) corresponding to 100% was 950 ± 170 nM. This basal value obtained in 6-OHDA-lesioned rats corresponded to an increase of +55.7% ($p < 0.01$) of striatal Glu levels when compared to basal values detected in normal rats (610 ± 98 nM). As illustrated in Figure 3B, STN-HFS performed on the lesioned side induces an increase of ipsilateral striatal Glu levels, reaching an average of +220 ± 28% (fractions 7–10), a highly significant increase ($p < 0.01$) when compared to basal values. The mean increase was significantly higher (+45%, $p < 0.01$) during stimulation in the lesioned rats compared to the STN-HFS induced-increase measured in normal rats. These increased values were maintained throughout the stimulation period. During the post-stimulation period, Glu levels immediately decreased towards a baseline value after turning off STN-HFS. Interestingly, a similar increase in Glu levels was also observed in the contralateral striatum of 6-OHDA rats, although only 1 side had been stimulated (Fig. 3B).

Effect of STN-HFS on Extracellular GABA Levels in the Striatum of Normal and Hemiparkinsonian Animals

In intact rats, the average of basal GABA striatal level (mean value of left and right sides) corresponding to 100% was 45 ± 15 nM. STN-HFS significantly increased extracellular GABA in the ipsilateral striatum (Fig. 4A). This increase was progressive and reached its maximum level (+166 ± 21% and 165 ± 20%, $p < 0.01$) in the last 2 fractions collected corresponding to the effect of 30 to 60 min of STN-HFS. During the post-stimulation period, GABA levels remained significantly high (+144 ± 21%) in the following collection (fraction 11) and then decreased towards values not significantly different from baseline values until the end of the experiment. Interestingly, similar modifications of GABA were also observed in the contralateral striatum although only 1 side had been stimulated (Fig. 4A).

In hemiparkisonian rats, the average of basal GABA striatal concentration (mean values of left and right sides) corresponding to 100% in 6-0HDA injected rats

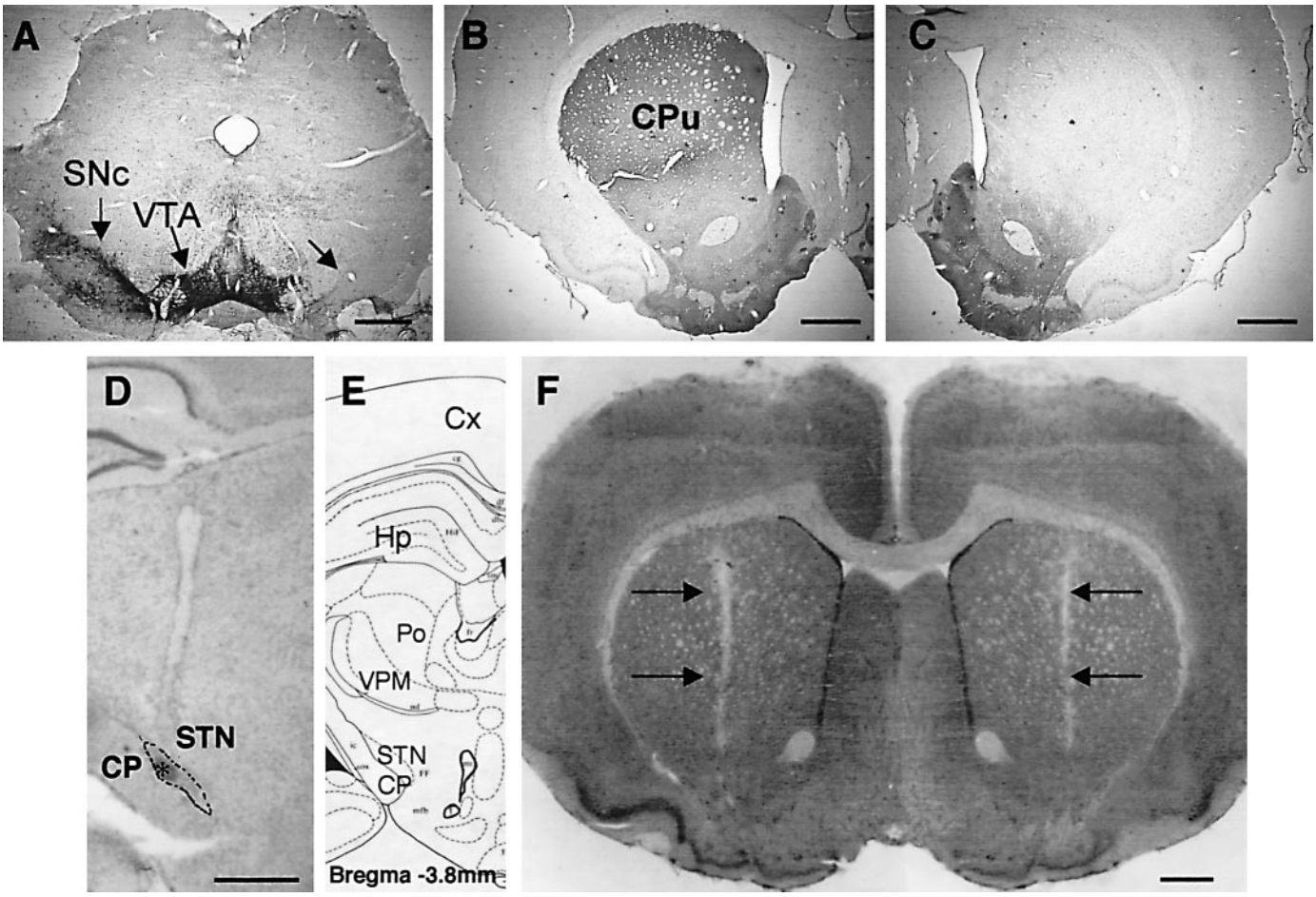


Fig. 2. Photographs of coronal rat brain sections at nigral **(A)**, striatal **(B, C, F)**, and subthalamic **(D)** levels and schematic diagram **(E)** (adapted from the stereotaxic atlas of Paxinos and Watson, 24). **A–C:** TH immunostaining at nigral and striatal levels in 6-OHDA-lesioned rats at a post-lesion delay of 1 month. Note the DA cell loss in the SNc **(A)** and the DA terminal loss in the striatum **(C)**. Photographs of cresyl violet-stained coronal sections at STN **(D)** and striatal **(F)** levels in normal rat. Note the adequate localization of the stimulation electrode inside STN **(D)**. *Stimulation point. Arrows indicate the bilateral implantation of microdialysis probes in the striatum **(F)**. CP: cerebral peduncle; CPu: caudate putamen nucleus; Cx: Cortex; Hp: hippocampus; Po: posterior thalamic nuclear group; SNc: substantia nigra pars compacta; STN: subthalamic nucleus; VPM: ventral posteromedial thalamic nucleus; VTA: ventral tegmental area. Scale bar = 1 mm.

was 75 ± 12 nM. This basal value corresponded to an increase of 66.6% ($p < 0.01$) of striatal GABA levels when compared to basal values measured in normal rats (45 ± 15 nM). As illustrated in Figure 4B, STN-HFS significantly increases ($p < 0.01$) the ipsilateral striatal GABA reaching an average of +199 ± 20% for GABA (fractions 7–10). These increased values were maintained throughout the stimulation period. The mean GABA increase observed in 6-OHDA-lesioned animals was significantly higher (+41%, $p < 0.01$) compared to intact rats. Interestingly, and as observed for Glu, similar increases of GABA levels were also observed in the contralateral striatum of 6-OHDA rats, although only 1 side had been stimulated. However, this contralateral effect was slightly smaller compared to the ipsilateral side (Fig. 4B).

Effect of Local Action of D1 or D2 or D1 + D2 Antagonists on Striatal Glu and GABA Modifications Induced by STN-HFS in Normal Animals

The perfusion of an artificial cerebral liquid containing a D1 antagonist (SCH-23390) during the last fraction collected under STN-HFS (fraction 10) reversed the significant increase of striatal Glu and GABA (an average of +149 ± 24% for Glu and +160 ± 16% for GABA, $p < 0.01$) in the first 3 fractions evoked by STN stimulation, inducing a dramatic decrease of their striatal levels (−41 ± 14% and −27 ± 6%, $p < 0.01$, for Glu and GABA, respectively) when compared to control values (Fig. 5A). During the post-stimulation period, Glu and GABA immediately returned towards a baseline value.

Contrary to that observed under SCH 23390, the increase of striatal Glu and GABA provoked by STN-HFS

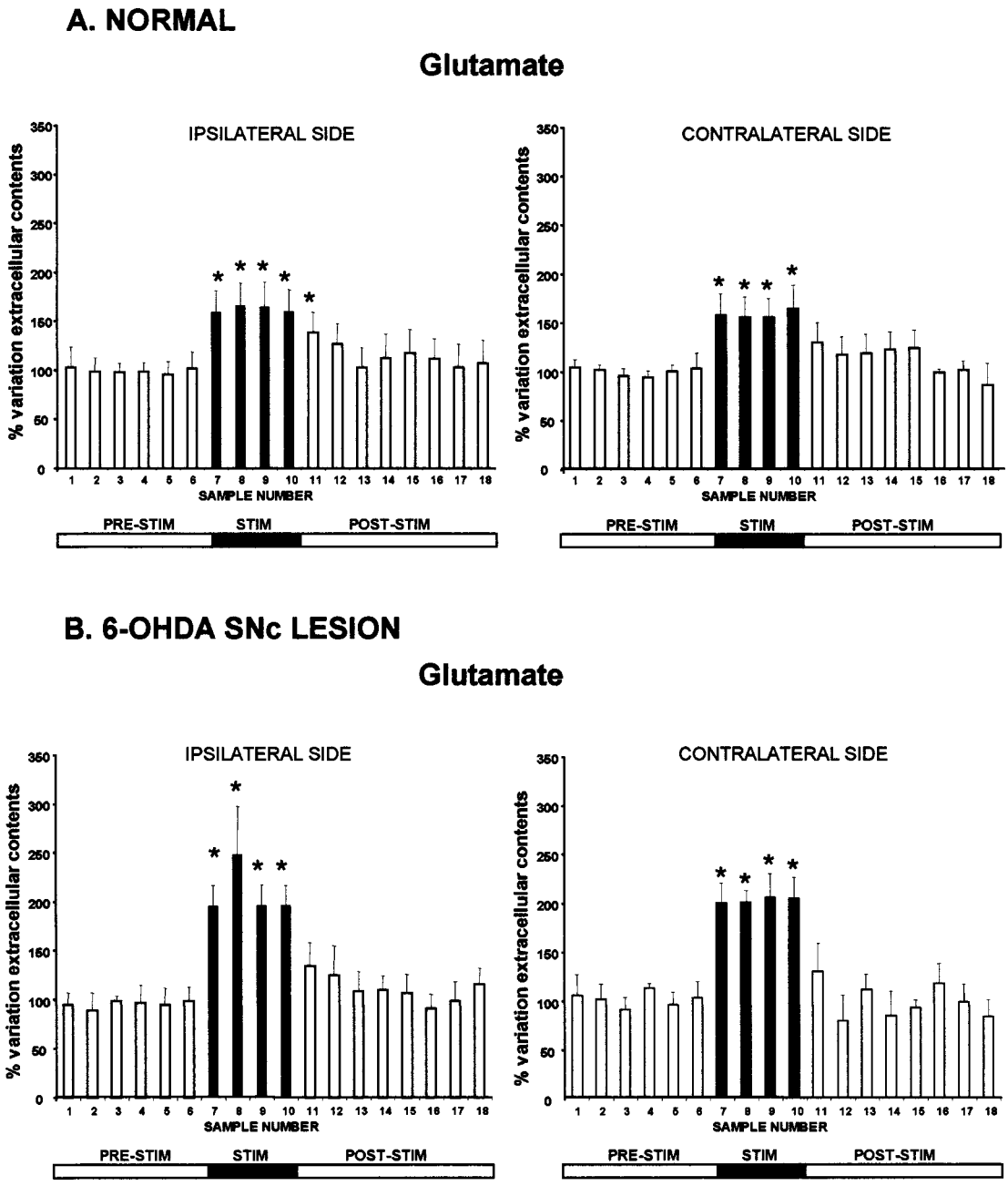


Fig. 3. Extracellular glutamate levels collected at 15-min intervals in striatum ipsi- or contralateral to the stimulation in both normal **(A)** and 6-OHDA SNc-lesioned rats **(B)**. The pre-stimulation period (fractions 1–6) consisted of 6 dialysates while stimulation (fractions 7–10) and post-stimulation period (fractions 11–18) consisted of 4 and 8 dialysates, respectively. The mean ±SEM of the 6 dialysates collected before STN-HFS was used as the baseline. Results are expressed as a percentage of variation of this baseline value. Each percentage represents the mean variation ±SEM calculated from the animals of the 2 experimental groups. Note the significant (*$p < 0.01$) increase of extracellular Glu induced by STN-HFS on both sides (stimulated and nonstimulated) in both normal **(A)** and hemiparkinsonian rats **(B)**.

in the first 3 fractions (an average of +162 ± 26% for Glu and +156 ± 14% for GABA, $p < 0.01$) was potentiated in the fourth dialysate collected by the presence of sulpiride in the artificial cerebral liquid. This effect was dramatic for Glu, reaching +407 ± 75% while it reached +200 ± 21% for GABA, $p < 0.01$ (Fig. 5B), corresponding to a significant increase ($p < 0.01$) of +241% and +44% for Glu and GABA, respectively, when compared to concentration values measured under STN-HFS. During the post-stimulation period, Glu and GABA rapidly

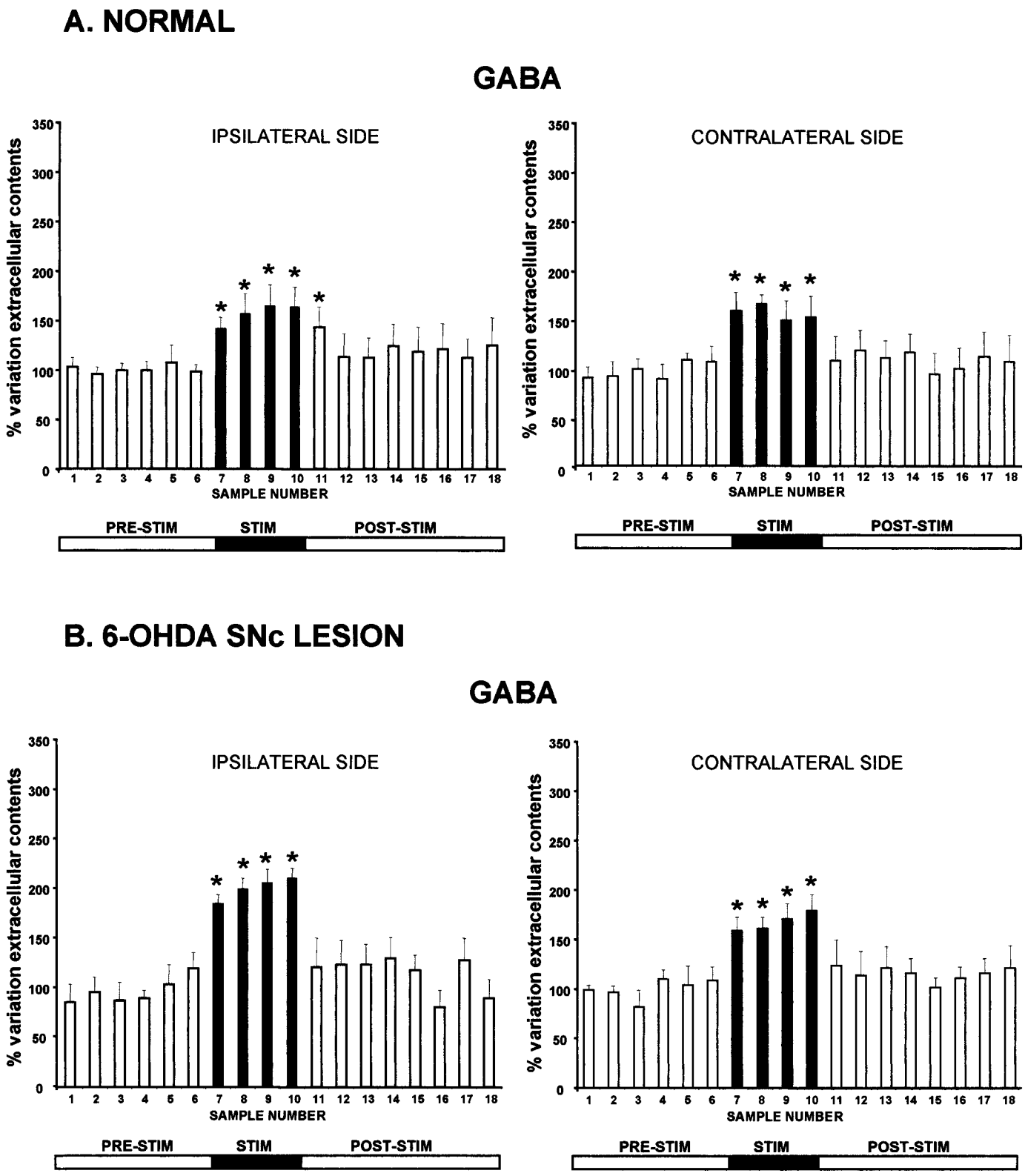


Fig. 4. Extracellular GABA levels collected at 15-min intervals in striatum ipsi- or contralateral to the stimulation in both normal **(A)** and 6-OHDA SNc-lesioned rats **(B)**. The pre-stimulation period (fractions 1–6) consisted of 6 dialysates while stimulation (fractions 7–10) and post-stimulation period (fractions 11–18) consisted of 4 and 8 dialysates, respectively. The mean ±SEM of the 6 dialysates collected before STN-HFS was used as the baseline. Results are expressed as a percentage of variation of this baseline value. Each percentage represents the mean variation ±SEM calculated from the animals of the 2 experimental groups. Note the significant (*$p < 0.01$) increase of extracellular GABA induced by STN-HFS on both sides (stimulated and nonstimulated) in both normal **(A)** and hemiparkinsonian rats **(B)**.

returned to values not significantly different from baseline values. Only the first collection after the end of the STN stimulation remained significantly different. Interestingly, during local infusion of both SCH 23390+sulpiride in the last fraction collected under STN-HFS (fraction 10), the increase of striatal Glu and GABA (an average of +165 ± 14% for Glu and +159 ± 23% for GABA, $p < 0.01$) measured in the first 3 fractions during STN stimulation, as described above, was also potentiated and reached +203 ± 23% for Glu and +252 ± 33% for GABA, $p < 0.01$. This potentiation induced by SCH 23390+sulpiride corresponded to a significant increase ($p < 0.01$) of +38% for Glu and +93% for GABA when compared to concentration values measured during STN-HFS (Fig. 5C). Interestingly, the amplitude of the increase induced by the local action of both SCH 23390+sulpiride under STN-HFS approximately corresponded to that observed in the 6-OHDA-lesioned rats during the stimulation period, although it was weaker for Glu. During the post-stimulation period, Glu and GABA levels immediately returned to values not significantly different from baseline values and remained almost stable until the end of the experiment.

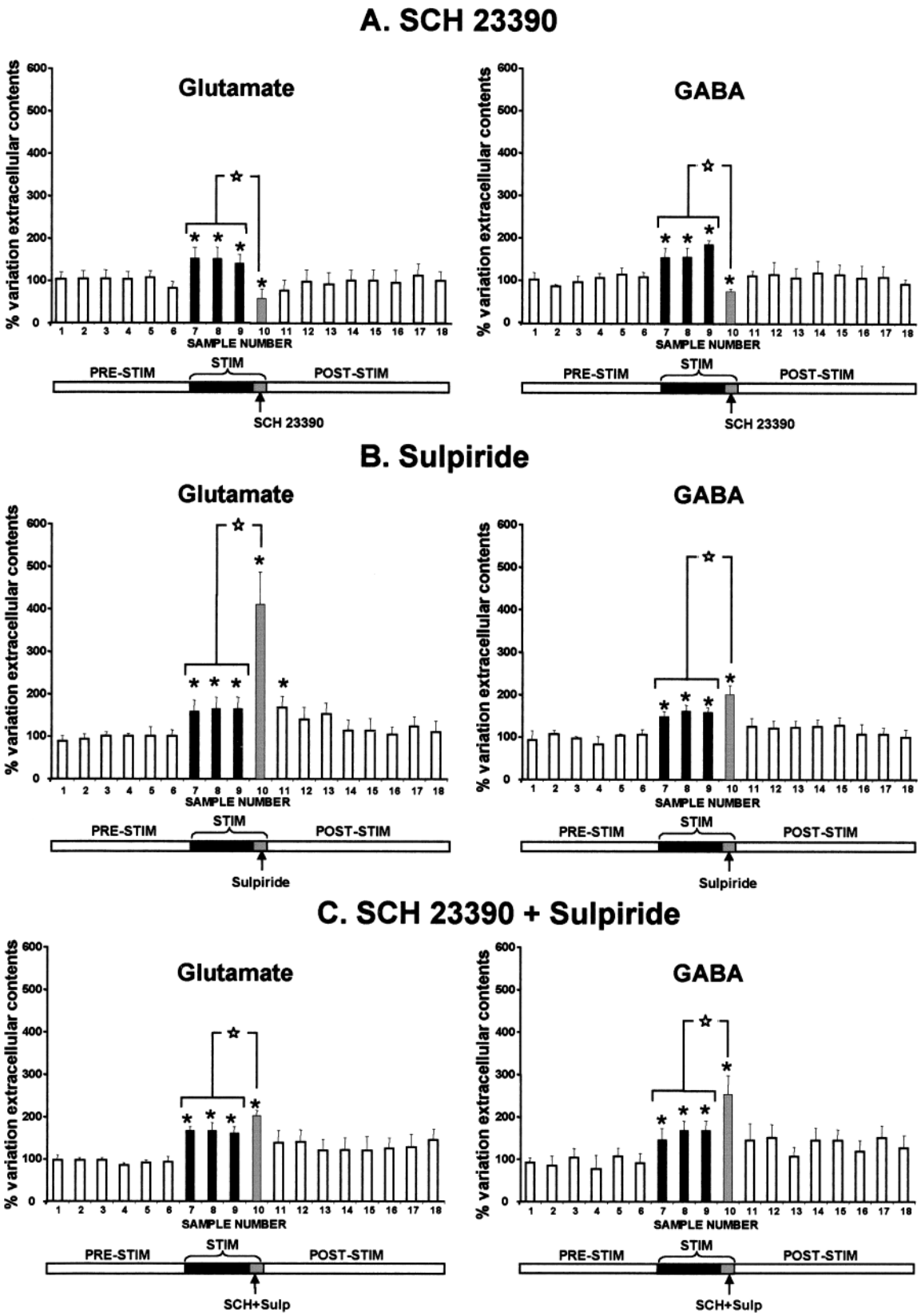
DISCUSSION

This study provides the first neurochemical evidence that STN-HFS for short duration affects striatal amino-acid transmission and that this influence is locally modulated by DA. Since the striatum receives only scarce projections from STN, these data support the view that the impact of STN-HFS on the pathophysiological functioning of BG in parkinsonism is not simply due to changes in STN outflow, but involves more complex mechanisms within the BG circuitry. Since 1993, STN-HFS has been successfully used to alleviate the motor symptoms in parkinsonian patients (12–14), but there remains considerable debate concerning the mechanisms underlying its beneficial effect (17–19). The fact that STN-HFS mostly reproduces the STN lesion effects suggests that DBS acts to suppress STN neuronal activity, leading to decreased outputs from the stimulated structure, then inducing a subsequent disinhibition of the thalamo-cortical pathway that activates the cerebral cortex (28). Nevertheless, it remains unclear whether the inhibition of activity in the BG output structures is due to a direct inhibition of STN neurons (29) and/or inhibition dependent on synaptic transmission, for example, the activation of GABAergic pallidal neurons (30, 31). Recently, we reported that STN-DBS may not simply cause interruption of STN outflow, supporting the possible role of GABA in the inhibition of the BG outputs, which may contribute to the therapeutical efficacy of STN-HFS in PD (20–22, 32). The resulting functional STN-HFS effect in the striatum is illustrated here by an increase of striatal Glu and GABA in both normal and hemiparkinsonian animals, suggesting that the consequences of STN-HFS are not restricted to the direct STN targets but involve widespread adaptive changes within the BG.

The increase in striatal Glu measured during STN-HFS in normal and hemiparkinsonian rats could be explained by an increase of activity of striatal excitatory glutamatergic afferents. Indeed, the possible inhibition of the activity of the BG outputs induced by STN-HFS (33, 34) could provoke a disinhibition of the thalamo-cortical pathway (35), activating bilateral cortico-striatal projections (36) via cortical collaterals and probably also the thalamo-striatal pathway. This disinhibitory effect is supported by our recent data showing that STN-HFS increases extracellular GABA in SNr, strongly suggesting its implication in the inhibition of the BG output structures projecting to the thalamus (20–22, 32).

However, we cannot also exclude that STN stimulation increases output from the stimulated structure. Indeed, several recent experimental results (37, 38) suggest that STN stimulation is unlikely to lead to an increase of the thalamo-cortical activity. In the light of these other studies our data would seemingly suggest another explanation, which could be an antidromic activation of the cortico-subthalamic projections induced by STN stimulation, thereby provoking an activation of the cortico-striatal projections. The contralateral Glu effect revealed here, both in normal and hemiparkinsonian animals, could therefore be explained through these cortical interactions. On the other hand, a bilateral regulation of striatal Glu by midbrain DA neurons has been described (39), suggesting Glu-DA interactions through crossed nigro-thalamo-cortical projections and some contralateral balance effects have also been reported for the dopaminergic system in lesioned animals (40). Recently, we reported that STN-HFS influences the nigrostriatal dopaminergic system by bilaterally increasing the striatal DA release in rats (23). However, recent PET studies of the D2/D3 ligand raclopride did not provide evidence for increased striatal dopamine concentration under effective DBS in patients with advanced PD (41). Therefore, modulation of dopaminergic activity may not be a main mechanism of STN HFS action on parkinsonian symptoms, at least during the late stage of the disease when the number of intact dopamine neurons is presumably too small to provide clinically relevant increase in striatal dopamine. However, it is interesting to note that patients in Hilker's study had been stimulated during a long period, at least several months. A possible adaptation process to chronic STN stimulation, in terms of plasticity of the dopaminergic transmission system, may be involved.

One interesting question addressed by the present work was whether the increase of striatal DA evoked by STN-HFS could be responsible for and/or could modulate the striatal Glu changes during the stimulation. Our results provide evidence that the Glu changes are not strictly



DA-dependent since they remain detectable in hemiparkinsonian animals. In these rats the Glu increase evoked by STN-HFS is higher than in intact rats, confirming that in physiological conditions DA modulates striatal Glu by inhibiting its release. This is in keeping with several studies describing presynaptic interactions between DA- and Glu-containing terminals in the striatum through D2 DA receptors located on glutamatergic terminals and/or through N-methyl-D-aspartate (NMDA) or non-NMDA glutamatergic receptors located on DA afferents (42–50).

It is interesting to note that baselines of striatal Glu are higher in DA-lesioned rats than in normal animals. This observation is consistent with studies showing that in PD striatum Glu levels are higher than normal (51, 52), and also confirms that SNc lesion in rats induces rearrangements of glutamatergic synapses within the striatum involving changes in the activity of the thalamocortico-striatal loop (53). Indeed, motor dysfunction induced by too low striatal intrasynaptic dopamine levels is associated with alterations in the sensitivity of glutamatergic receptor, including those of the NMDA subtype (54). Functional characteristics of these ionotropic receptors are regulated by their phosphorylation state. Lesions of the nigrostriatal DA system of rats induce parkinsonian signs and increase the phosphorylation of striatal NMDA receptor subunits on serine and tyrosine residues (54). The intrastriatal administration of certain inhibitors of the kinases capable of phosphorylating NMDA receptors produces a dopaminomimetic motor response in these animals. Similarly, in clinical studies, dextrorphan, dextromethorphan, and amantadine have been found to be effective against motor complications (54, 55). This suggests that changes of glutamatergic transmission secondary to DA denervation could aggravate motor symptoms. The basal Glu increase following 6-OHDA could be responsible for an increase in spontaneous activity of striatal neurons due to the elimination of DA acting on striatal GABA neurons and/or an increase in striatal Glu synaptic transmission (43, 53, 55, 57). However, even if our results suggest that Glu changes induced by STN-HFS are not strictly DA-dependent, they suggest that these changes are DA-modulated. This observation led us to examine which DA receptor subtypes could be involved in this modulatory action. Striatal injection of D1 antagonist was found to reverse the Glu increase induced by STN-HFS in normal rats while that of D2 antagonist dramatically potentiated it. This confirms that DA modulates the Glu increase by opposite effects of D1 and D2 DA receptors, suggesting that DA needs to act on both receptors simultaneously to tonically control the striatal outputs (58, 59). It is noteworthy that when striatal D1 and D2 receptors were simultaneously blocked by SCH 23390+sulpiride, a slight potentiation of Glu levels is detectable compared to values measured in the first 3 dialysates collected under STN-HFS, suggesting a prevalent D2 blockade effect. This is in keeping with data suggesting that D2 receptors are involved in a negative control of striatal Glu levels (45). Moreover, the amplitude of Glu increase observed with SCH 23390+sulpiride is approximately in the same range as that observed in hemiparkinsonian rats.

Concerning striatal GABA changes evoked by STN-HFS, our data clearly show that they are not strictly DA-dependent since they are similarly affected in 6-OHDA-treated rats. Striatal GABA increase induced by STN-HFS could thus be related to the parallel Glu increase provoked by the stimulation. The interaction between Glu and GABA is supported by a microdialysis study by Segovia et al (60) showing that endogenous Glu increases striatal GABA from possible stimulation of GABA interneurons. On the other hand, as discussed above for Glu, our results suggest that striatal GABA, basal, or STN-HFS is modulated by DA since the amplitude of GABA changes is higher in DA-lesioned rats than in normal animals (61). A direct synaptic connection between DA terminals and GABA neurons has been reported in the striatum (46, 62). Our data show that D2 or mixed D1 + D2 antagonists potentiate the striatal GABA increase induced by STN-HFS, confirming previous studies describing interaction between D1 and D2 DA receptors on striatal GABA neurons or terminals. Indeed, it has been suggested that nigrostriatal DA release

←

Fig. 5. Extracellular Glutamate and GABA levels collected at 15 min intervals in striatum ipsilateral to the STN-HFS in normal rats treated with SCH 23390 **(A)**, sulpiride **(B)**, and SCH 23390+sulpiride **(C)**. The pre-stimulation period (fractions 1–6) consisted of 6 dialysates while stimulation (fractions 7–10) and post-stimulation period (fractions 11–18) consisted of 4 and 8 dialysates, respectively. The pharmacological drugs were perfused just before the last fraction collected during STN-HFS (fraction 10). The mean ±SEM of the 6 dialysates collected before STN-HFS was used as the baseline. Results are expressed as a percentage of variation of this baseline value. Each percentage represents the mean variation ±SEM calculated from the animals of the 3 experimental groups. Note the significant (*$p < 0.01$) increase of extracellular Glu and GABA induced by STN-HFS in the first 3 fractions under stimulation **(A–C)** and that SCH 23390 significantly reverses (☆$p < 0.01$) the increase of Glu and GABA induced by STN-HFS **(A)** while sulpiride potentiates it significantly (☆$p < 0.01$) **(B)** when compared to values detected during the stimulation period without drugs. Note also the potentiated values for Glu and GABA induced by the perfusion of SCH 23390+sulpiride compared to values measured in the first 3 fractions under stimulation without drugs. The amplitude of this increase approximately corresponded to that observed in 6-OHDA-lesioned rats during the stimulation period although it was weaker for Glu.

may inhibit striatal GABA release (63) and that when D2 receptors are blocked, the action exerted by DA through activation of D1 receptors increases both striatal GABA neuronal activity (64) and levels (66, 66). This is in accordance with our experiments showing that SCH 23390 reverses the GABA increase evoked by STN-HFS. Whatever the nature of interaction between DA/GABA and Glu/GABA, this study clearly demonstrates that STN-HFS interferes with striatal GABA transmission, thus modulating the outputs of this structure.

Finally, one may wonder whether both amino acids present in the microdialysates are of neuronal and/or glial origin (67, 68). Modifications of extracellular Glu found here most probably originate from release changes at nerve endings, since experiments with potassium suggest that at least 65% of striatal extracellular Glu is of neuronal origin (50, data not shown). Wherever these amino acids come from, the resulting functional effect in the striatum could be the same (69). However, the changes of Glu and GABA found in microdialysates may be delayed compared to the synaptic events resulting from limited access to the dialysis membrane, reuptake, saturation, and/or metabolic processes in the vicinity of the probe. In addition, these data have been obtained under halothane anesthesia; we cannot totally exclude any putative interference of halothane on the reactivity of STN neurons to HFS. However the actual neurochemical observations need to be confirmed in freely moving rats.

In conclusion, our study shows that STN-HFS induces striatal Glu and GABA transmission changes that are not DA-dependent but DA-modulated, as suggested by the effect of D1 and/or D2 antagonists. These data reflect complex functional interactions within the BG circuitry providing a neurochemical support to the beneficial effects of the STN-HFS on parkinsonian motor symptoms.

ACKNOWLEDGMENTS

We thank Drs. Lydia Kerkerian-Le Goff, Anne Marie Thiery, Guy Chouvet for helpful discussion and Fiona Hemming for English corrections.

REFERENCES

1. Miller WC, DeLong MR. Altered tonic activity of neurons in the globus pallidus and subthalamic nucleus in the primate MPTP model of parkinsonism. In: Carpenter MB, Jayaraman A, eds. The basal ganglia II. New-York: Plenum, 1987:415–27
2. DeLong MR. Primate models of movement disorders of basal ganglia origin. Trends Neurosci 1990;13:281–85
3. Hollerman JR, Grace AA. Subthalamic nucleus cell firing in the 6-OHDA-treated rat: Basal activity and response to haloperidol. Brain Res 1992;590:291–99
4. Bergman H, Wichmann T, Karmon B, DeLong MR. The primate subthalamic nucleus. II. Neuronal activity in the MPTP model of parkinsonism. J Neurophysiol 1994;72:507–20
5. Hassani OK, Mouroux M, Feger J. Increased subthalamic neuronal activity after nigral dopaminergic lesion independent of disinhibition via the globus pallidus. Neuroscience 1996;72:105–15
6. Bergman H, Wichmann T, DeLong MR. Reversal of experimental parkinsonism by lesions of the subthalamic nucleus. Science 1990; 249:1436–38
7. Aziz TZ, Peggs D, Sambrook MA, Crossman AR. Lesion of the subthalamic nucleus for the alleviation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism in the primate. Mov Disord 1991;6:288–92
8. Guridi J, Herrero MT. Subthalamotomy in parkinsonian monkeys. Behavioural and biochemical analysis. Brain 1996;119:1717–27
9. Graham WC, Robertson RG, Sambrook MA, Crossman AR. Injection of excitatory amino acid antagonists into the medial pallidal segment of a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated primate reverses motor symptoms of parkinsonism. Life Sci 1990;47:PL91–97
10. Brotchie JM, Mitchell IJ, Sambrook MA, Crossman AR. Alleviation of parkinsonism by antagonism of excitatory amino acid transmission in the medial segment of the globus pallidus in rat and primate. Mov Disord 1991;6:133–38
11. Benazzouz A, Gross C, Feger J, Boraud T, Bioulac B. Reversal of rigidity and improvement in motor performance by subthalamic high-frequency stimulation in MPTP-treated monkeys. Eur J Neurosci 1993;5:382–89
12. Limousin P, Pollak P, Benazzouz A, et al. Effect on parkinsonian signs and symptoms of bilateral subthalamic nucleus stimulation. Lancet 1995;345:91–95
13. Limousin P, Krack P, Pollak P, et al. Electrical stimulation of the subthalamic nucleus in advanced Parkinson's disease. N Engl J Med 1998;339:1105–11
14. Krause M, Fogel W, Heck A, et al. Deep brain stimulation for the treatment of Parkinson's disease: Subthalamic nucleus versus globus pallidus internus. J Neurol Neurosurg Psychiatry 2001;70:464–70
15. Pollak P, Fraix V, Krack P, et al. Treatment results: Parkinson's disease. Mov Disord 2002;17:S75–S83
16. Alvarez L, Macias R, Guridi J, et al. Dorsal subthalamotomy for Parkinson's disease. Mov Disord 2001;16:72–78
17. Benabid AL, Benazzouz A, Pollak P. Mechanisms of deep brain stimulation. Mov Disord 2002;17:S73–74
18. Dostrovsky JO, Lozano AM. Mechanisms of deep brain stimulation. Mov Disord 2002;17:S63–S68
19. Vitek JL. Mechanisms of deep brain stimulation: Excitation or inhibition. Mov Disord 2002;17:S69–72
20. Windels F, Bruet N, Poupard A, et al. Effects of high frequency stimulation of subthalamic nucleus on extracellular glutamate and GABA in substantia nigra and globus pallidus in the normal rat. Eur J Neurosci 2000;12:4141–46
21. Windels F, Bruet N, Poupard A, Feuerstein C, Bertrand A, Savasta M. Influence of the frequency parameter on extracellular glutamate and GABA in substantia nigra and Globus Pallidus during electrical stimulation of subthalamic nucleus in rats. J Neurosci Res 2003; 72:259–67
22. Savasta M, Windels F, Bruet N, Bertrand A, Poupard A. Neurochemical modifications induced by high frequency stimulation of subthalamic nucleus in rats. In: Nicholsson LFB, Faull RLM, eds. The basal ganglia VII. New York: Kluwer Academic, Plenum, 2002:581–90
23. Bruet N, Windels F, Bertrand A, Feuerstein C, Poupard A, Savasta M. High frequency stimulation of the subthalamic nucleus increases the extracellular levels of striatal dopamine in normal and partially dopaminergic denervated rats. J Neuropathol and Exp Neurol 2001; 60:15–24
24. Paxinos G, Watson C. The rat brain in stereotaxic coordinates. London: Academic Press Inc., 1982
25. Tossman U, Ungerstedt U. Microdialysis in the study of extracellular levels of amino acids in the rat brain. Acta Physiol Scand 1986;128:9–14

26. Donzanti BA, Yamamoto BK. An improved and rapid HPLC/EC method for the isocratic separation of amino acid neurotransmitters from brain tisues and microdialysis perfusates. Life Sci 1988;43: 913–22
27. Gesdon JL, Ternynck T, Avrameas S. The use of avidin-biotin interaction in immunoenzymatic techniques. J Neurochem 1979;27: 1131–39
28. Limousin P, Greene J, Pollak P, Rothwell J, Benabid AL, Frackowiack R. Changes in cerebral activity pattern due to subthalamic nucleus or internal pallidum stimulation in P arkinson's disease. Annal Neurol 1997;42:283–91
29. Beurrier C, Bioulac B, Audin J, Hammond C. High Frequency stimulation produces a transient blockade of voltage-gated currents in subthalamic neurons. J Neurophysiol 2001;85:1351–56
30. Ashby P, Kim YJ, Kumar R, Lang AE, Lozano AM. Neurophysiological effects of stimulation through electrodes in the human subthalamic nucleus. Brain 1999;122:1919–31
31. Grill WM, McIntyre CC. Extracellular excitation of central neurons: Implications for the mechanisms of deep brain stimulation. Thalamus and Related Systems 2001;1:269–77
32. Windels F, Bruet N, Poupard A, Feuerstein C, Bertrand A, Savasta M. Evidence for a possible role of GABA in the therapeutical efficacy of high frequency stimulation of the subthalamic nucleus in Parkinson's disease: A microdialysis study in rats. (Abstract) Soc Neurosci 2001;966:10
33. Benazzouz A, Piallat B, Pollak P, Benabid AL. Responses of substantia nigra pars reticulata and globus pallidus complex to high frequency stimulation of the subthalamic nucleus in rats: Electrophysiological data. Neurosci Lett 1995;189:77–80
34. Salin P, Manrique C, Forni C, Kerkerian-Le Goff L. High-frequency stimulation of the subthalamic nucleus selectively reverses dopamine denervation-induced cellular defects in the output structures of the basal ganglia in the rat. J Neurosci 2002;15:5137–48
35. Benazzouz A, Gao DM, Ni ZG, Piallat B, Bouali-Benazzouz R, Benabid AL. Effect of high-frequency stimulation of the subthalamic nucleus on the neuronal activities of the substantia nigra pars reticulata and ventrolateral nucleus of the thalamus in the rat. Neuroscience 2000;99:289–95
36. McGeorge AJ, Faull RL. The organization of the projection from the cerebral cortex to the striatum in the rat. Neuroscience 1989; 29:503–37
37. Anderson ME, Postupna N, Ruffo M. Effects of high-frequency stimulation in the internal globus pallidus on the activity of thalamic neurons in the awake monkey. J Neurophysiol 2003;89:1150–60
38. Hashimoto T, Elder CM, Okun MS, Patrick SK, Vitek JL. Stimulation of the subthalamic nucleus changes the firing pattern of pallidal neurons. J Neurosci 2003;23:1916–23
39. Lindefors N, Understedt U. Bilateral regulation of glutamate tissue and extracellular levels in caudate-putamen by midbrain dopamine neurons. Neurosci Lett 1990;115:248–52
40. Nieoullon A, Cheramy A, Glowinski J. Interdependence of the nigrostriatal dopaminergic systems on the two sides of the brain in the cat. Science 1977;198:416–68
41. Hilker R, Voges J, Ghaemi M, et al. Deep brain stimulation of the subthalamic nucleus does not increase the striatal dopamine concentration in parkinsonian humans. Mov Disord 2003;18:41–48
42. Maura G, Giardi A, Raiteri M. Release-regulating D-2 dopamine receptors are located on striatal glutamatergic nerve terminals. J Pharmacol Exp Ther 1988;247:680–84
43. Nieoullon A, Kerkerian-Le Goff L. Cellular interactions in the striatum involving neuronal systems using ''classical'' neurotransmitters: Possible functional implications. Mov Disord 1992;7:311–25
44. Exposito I, Porras A, Sanz B, Mora F. Effects of apomorphine and L-methionine sulphoximine on the release of excitatory amino acid neurotransmitters and glutamine in the neostriatum of the conscious rat. Eur J Neurosci 1994;1:287–91
45. Yamamoto BK, Davy S. Dopaminergic modulation of glutamate release in striatum as measured by microdialysis. J Neurochem 1992;58:1736–42
46. Yung KKL, Bolam JP. Localization of D1 and D2 receptors in the rat neostriatum: Synaptic interaction with glutamate- and GABA-containing axonal terminals. Synapse 2000;38:413–20
47. Smith Y, Charara A, Paquet M, et al. Ionotropic and metabotropic GABA and glutamate receptors in primate basal ganglia. J Chem Neuroanat 2001;22:13–42
48. Garcia-Munoz M, Young SJ, Groves PM. Terminal excitability of the corticostriatal pathway. I. Regulation by dopamine receptor stimulation. Brain Res 1991;14:195–206
49. Garcia-Munoz M, Young SJ, Groves PM. Terminal excitability of the corticostriatal pathway. II. Regulation by glutamate receptor stimulation. Brain Res 1991;14:207–15
50. Bert L, Parrot S, Robert F, Desvignes C, Denoroy L, Suaud-Chagny MF, Renaud B. In vivo temporal sequence of rat striatal glutamate, aspartate and dopamine efflux during apomorphine, nomifensine, NMDA and PDC in situ administration. Neuropharmacol 2002;43: 825–35
51. Kish SJ, Rajput A, Gilbert J, et al. Elevated γ-aminobutyric acid level in striatal but not extrastriatal brain regions in Parkinson's disease: Correlation with striatal dopamine loss. Ann Neurol 1986; 20:26–31
52. Hornykiewicz O. Chemical neuroanatomy of the basal ganglia—normal and in Parkinson's disease. J Chem Neuroanat 2001;22:3–12
53. Meshul CK, Emre N, Nakamura CM, Allen C, Donohue MK, Buckman JF. Time-dependent changes in striatal glutamate synapses following a 6-hydroxydopamine lesion. Neuroscience 1999;88: 1–16
54. Chase T, Oh JD, Konistsiotis S. Antiparkinsonian and antidyskinetic activity of drugs targeting central glutamatergic mechanisms. J Neurol 2000;247:36–42
55. Oh JD, Chase T. Glutamate-mediated striatal dysregulation and the pathogenesis of motor response complications in Parkinson's disease. Amino Acids 2002;23:133–39
56. Calabresi P, Pisani A, Mercuri NB, Bernardi G. Electrophysiology of dopamine-denervated striatal neurons. Brain 1993;116:433–52
57. Chang WY, Webster RA. Effect of L-dopa alone and with benserazide on the spontaneous activity of striatal neurones in normal and 6-hydroxydopamine-lesioned rats. Br J Pharmacol 1997;121:331–37
58. Bertorello AM, Hopfield JF, Aperia A, Greengard P. Inhibition by dopamine of Na(+)+K(+) ATPase activity in neostriatal neurons through D1 and D2 dopamine receptor synergism. Nature 1990;27: 386–38
59. Hu XT, White F. Dopamine enhances glutamate-induced excitation of striatal neurons by cooperative activation of D1 and D2 class receptors. Neurosci Lett 1997;224:61–65
60. Segovia G, Del Arco A, Mora F. Endogenous glutamate increases extracellular concentrations of dopamine, GABA, and taurine through NMDA and AMPA/kainate receptors in striatum of the freely moving rat: a microdialysis study. J Neurochem 1997;69: 1476–83
61. Lindefors N, Brodin E, Tossman U., Segovia J, Ungerstedt U. Tissue levels and in vivo release of tachykinins and GABA in striatum and substantia nigra of rat brain after unilateral striatal denervation. Expl Nrain Res 1989;74:527–34
62. Smith Y, Bolam JP. The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurones. Trends Neurosci 1990;13:259–65
63. Reid MS, O'Connor WT, Herrera-Marschitz M, Ungerstedt U. The effects of intranigral GABA and dynorphin A injections on striatal dopamine and GABA release: Evidence that dopamine provides inhibitory regulation of striatal GABA neurons via D2 receptors. Brain Res 1990;519:255–60

64. Harsing LG Jr,Zigmond MJ. Influence of dopamine on GABA release in striatum: Evidence for D1–D2 interactions and non-synaptic influences. Neuroscience 1997;77:419–29
65. Girault JA, Spampinato U, Glowinski J, Besson MJ. In vivo release of [3H]gamma-aminobutyric acid in the rat neostriatum-II. Opposing effects of D1 and D2 dopamine receptor stimulation in the dorsal caudate putamen. Neuroscience 1986;19:1109–17
66. Girault JA, Halpain S, Greengard P. Excitatory amino acid antagonists and Parkinson's disease. Trends Neurosci 1990;13:325–27
67. Westerink BH, Damsma G, De Vries JB. Effect of ouabain applied by intrastriatal microdialysis on the in vivo release of dopamine, acetylcholine, and amino acids in the brain of conscious rats. J Neurochem 1989;52:705–12
68. Herrera-Marschitz M, Goiny M, Meana JJ, et al. On the origin of extracellular glutamate levels monitored in basal ganglia of the rat by in vivo microdialysis. J Neurochem 1996;66:1726–35
69. Baker DA, Xi ZX, Shen H, Swanson CJ, Kalivas PW. The origin and neuronal function of in vivo nonsynaptic glutamate. J Neurosci 2002;15:9134–41

Received April 14, 2003
Revision received July 21, 2003
Accepted August 21, 2003